



Microbiological Advances in Biohydrometallurgy

Helen Watling

CSIRO Mineral Resources, Australian Minerals Research Centre, P.O. Box 7229, Karawara, WA 6152, Australia; Helen.Watling@csiro.au; Tel.: +61-8-9334-8034

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Abstract: The most exciting advances in biohydrometallurgy are occurring in the field of microbiology. The two main technologies employed in biohydrometallurgy, agitated tanks for the processing of refractory concentrates and heaps and dumps for the processing of low-grade ores, are technologically sound and widely practised at commercial scale, but their development began at a time when very little was known of the microorganisms that assisted metals extraction from sulfide ores. During and subsequent to those developments it has been shown that microbial communities in metals extraction are more diverse than originally thought, and extremely robust and adaptable to different and variable environments. Recent advances in genomics and proteomics, exploiting hugely increased computing power and speed, have made it possible to describe not only which microorganisms are present in bioleaching systems, but also what physiological functions are being exercised. The body of knowledge being acquired through the application of molecular biology methods will be used increasingly to monitor microbial behaviour, optimise conditions for more appropriate microbiological activity and/or infer the "microbiological health" of bioreactors (tanks and heaps).

Keywords: biomining; microbial communities; acidophiles; heap leaching; sulfide concentrates; sulfide ores; iron(II)-oxidation; sulfur-oxidation

1. Introduction

Biohydrometallurgy (biomining or mineral bioprocessing) is a sub-discipline of hydrometallurgy that exploits some attributes of micro-organisms (bio-catalysts) to facilitate and/or enhance the separation of elements from their ores or other materials. Traditional biohydrometallurgy involves the aqueous, inorganic chemistry of acidic sulfate solutions contacted with sulfide concentrates or ores containing valuable metals. As a method of extracting elements, biohydrometallurgy is an alternative to some intense chemical leaching methods [1-4]. However, its application to the processing of mineral concentrates compares unfavourably with pyrometallurgical processing because it is slower and because, usually, the infrastructure required for pyrometallurgical processing is already in place. According to Holmes and Debus [5], biological processing (of concentrates) would need to demonstrate a greater than 20% advantage over conventional pyrometallurgical processing to interest the mining industry. Similarly, Poulter et al. [6] remarked that industry reluctance to embrace the technology is partly due to the "inherent process and economic advantage of modern smelting technologies" and the "real and perceived risks associated with the introduction of a novel technology". On the other hand, biohydrometallurgy has achieved acceptance for the processing of low-grade, secondary copper sulfide ores on a very large scale, particularly in Chile, and accounts for approximately 20% of annual global copper production [7,8]. Likewise, pre-treatment of refractory gold concentrates in agitated tanks at commercial scale has a long and successful history from Fairview, South Africa [9] to Runro, the Philippines [10]. Looking to the future, this suite of well-developed and reliable biohydrometallurgical technologies can be applied, for example, to small deposits in remote locations perhaps with unfavourable terrain, to complex ores that are difficult to process, or to concentrates containing impurities that attract smelter penalties.

Man has benefited from the bio-assisted extraction of metals for much longer than the underlying microbiological foundation of the technology has been understood. Accounts from almost 2000 years ago describe the recovery of copper from mine water by cementation (Table 1) but the 'technology' could pre-date these accounts by many centuries, possibly since man started to mine ores and extract metals.

Table 1. Historical biohydrometallurgy	Approximately 2000 years of anecdotes and evidence up to
the discovery of Acidithiobacillus (At.) features	rooxidans (collated from [11–28]).

Chronology	Observation—Report		
25–220 A.D.	The Roman Gaius Plinius Secundus (Pliny the Elder, 23–79 A.D.) wrote to the effect that chrysocolla is also artificially produced by slowly passing water through the mine during the winter until the month of June; subsequently the water is evaporated in June and July.		
	Notes in old Chinese texts on leaching of rocks and the formation of gall (CuSO ₄) springs; copper recovery was by cementation on iron.		
	The scientist Galen described <i>in situ</i> leaching at a mine in Cyprus.		
1086 A.D.	The Gall (CuSO ₄)—copper process described: copper leaching and recovery using cementation on iron as a commercial process; the copper was used for coinage.		
1500s	Copper leached from some ores (<i>in situ</i>) and mines (Spain, Hungary, Germany) and copper recovered by cementation on iron. A wood cut illustration in Agricola's <i>De re metallica</i> represents the manual collection of copper solutions in wooden 'vats' and transfer to evaporative ponds to concentrate the solutions.		
1600s	The extraction of copper from mine water (Peru) described by Alvaro Alonso Barba de Garfias (priest and metallurgist); he was granted a patent to recover copper from mine water.		
1800s	Large-scale copper heap leaching (Spain): Heaps of low-grade ore were left for 1–3 years to undergo <i>natural decomposition</i> .		
1900–1920	Commercial copper production by leaching and copper cementation (Butte, MT, USA); in place (stope) leaching (Cananea, Sonora, Mexico); recovery of copper from underground mine water (Bisbee, AZ, USA).		
1921–1940	The oxidation of sulfur by soil microorganisms described; <i>At. thiooxidans</i> (previously <i>Thiobacillus</i>) isolated and described.		
	The concept of the 'biosphere' in which living organisms shaped the environment (biogeochemistry) expounded by Vladimir Vernadskii (1863–1945).		
	Biological production of organic acids reported.		
	Biooxidation of pyrite first reported and the suggested commercial application of biohydrometallurgy for zinc sulfide.		
	Large scale dump leaching of open pit waste rock with recirculation of leach solutions (Bingham Canyon, UT, USA).		
1941–1951	Roles of microorganisms in rock-weathering processes recognised.		
	<i>Acidithiobacillus ferrooxidans</i> isolated from acid drainage of a coal mine and its role in oxidising Fe(II) and reduced inorganic sulfur compounds (RISC) in acid mine drainage (AMD) described.		

Not surprisingly, metal extraction dominated commercial developments before the discovery that microorganisms were involved in the process. However, that discovery created a broad new research field within which the biogeochemical roles of acidophilic microorganisms were explored in relation to acidic drainage at mine sites and geothermally-heated sulfur-rich environments. Naturally, the greater understanding of microbial attributes was incorporated into established or new technologies. For the purposes of this targeted review of microbiological advances in biohydrometallurgy, it is convenient to discuss separately those advances relating to discoveries about the microorganisms, responses to processing environments, and diversity in managed heaps, columns and agitated tanks.

2. Microbiological Discovery

The discovery and description of *At. ferrooxidans* prompted a rapid increase in microbiological studies related to the oxidation of sulfide minerals. Arguably, *At. ferrooxidans* is the most studied

acidophilic bacterium known, with more than 9000 papers in Web of Science, far more than any other acidophile. However, the perceived importance of *At. ferrooxidans* in bioleaching environments was largely a consequence of the relative ease of its enrichment and isolation, and its rapid growth in acidic iron(II) media, by which it out-competed other members of its microbial community. In the early years (post-1950), source materials of microorganisms tended to be spoils dumps at coal mines [29] or waste dumps at base metals mines [30]. Since then, many acidophiles have been isolated and described and many more are anticipated (Figure 1), constrained only by the number of research groups interested in acidophiles. Nevertheless, iron(II)- and sulfur-oxidising acidophiles are relatively few in number, especially those isolated from, or detected in, heap- or agitated-tank bioleaching reactors for the processing of sulfide minerals (refer to discussions and collations of microorganisms in [8,31–35].



Figure 1. Trend with time of the numbers of acidophilic microorganisms (*Bacteria* and *Archaea*) isolated from environments relevant to biohydrometallurgy since the discovery of *At. thiooxidans*. They are grouped according to whether they are chemolithotrophs or mixotrophs that utilise iron(II) (Fe OX), both iron(II) and sulfur (Fe + S OX), sulfur but not iron(II) (S OX) or are heterotrophs (HET). Data to February 2016.

More general overviews have been focused on the broader range of microorganisms and microbial interventions associated with acid mine drainage (AMD) environments including collations of acidophilic prokaryotic micro-organisms on the basis of their physiological characteristics [36,37]. These microorganisms are of direct interest in the context of the microbial communities that can inhabit mineral sulfide heaps. Another group of acidophiles of strong interest are the hyperthermophilic archaea found in sulfur-rich geothermal regions (solfataras, volcanoes, hot springs or combusting coal dumps) [38–41] that might be used to enhance metal extraction from mineral sulfide concentrates in higher-temperature processes.

Before leaving microbiological discovery, it is worth recapping how biomining organisms assist metals extraction. The microbial capabilities of ferrous ion oxidation and RISC oxidation are the two key functions in the most commonly practised form of biohydrometallurgy, ferric sulfate—sulfuric acid leaching in agitated tanks and heaps/dumps. Microbial ferrous-ion oxidation regenerates the oxidant (ferric ion, Reaction (1)) required for sulfide mineral dissolution (e.g., covellite, Reaction (2)). At pH < 4, microbial ferrous ion oxidation is faster than chemical oxidation (e.g., Johnson [36] and references therein). RISC oxidation to sulfuric acid contributes to the acidic environment required for leaching (Reaction (3)).

$$4Fe^{2+} + 4H^+ + O_2 + \text{microbial catalysts} \rightarrow 4Fe^{3+} + 2H_2O$$
(1)

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} + S^0$$
 (2)

$$2S^{0} + 3O_{2} + 2H_{2}O + \text{microbial catalysts} \rightarrow 2H_{2}SO_{4}$$
(3)

However, the geochemistry and biochemistry of sulfide mineral dissolution is more complex than is conveyed by Reactions (1) to (3). It encompasses the electrochemical reactions that take place at the mineral surface during sulfide dissolution as well as physiological processes that control the passage of compounds or ions across the microbial cell membrane and within the cell during growth. These multi-faceted topics are beyond the scope of the review but are discussed comprehensively [42] as well as in some focused reviews on individual species (e.g., *At. ferrooxidans* [43]), genera (e.g., *Sulfolobus* spp. [44]), or particular extreme conditions (e.g., microbial mechanisms of coping with high copper concentrations [34], living in acidic environments [39], or the adaptability required to thrive in biomining environments [45]).

3. Microbial Responses to Extreme and Variable Habitats

The two main processes employed in bio-assisted extraction of metals, heap leaching of low-grade sulfide ores and agitated-tank leaching of sulfide concentrates, present very different habitats for the microbial communities that colonise them. Heaps of low-grade ore particles with a broad size distribution (up to 25 mm) are constructed on a large scale and are essentially dynamic, heterogeneous systems in terms of ore mineralogy and grade. Heat generation is a consequence of microbially-catalysed oxidation of sulfide minerals and heat management requires a suitable balance between heap aeration and irrigation [46]. In contrast, agitated tanks are closer to homogeneous in terms of concentrate grade, solution acidity, provision of O_2 and CO_2 and a selected and controllable temperature for the process. In tanks, there is an abundance of substrate (the sulfide minerals, ferrous ions and sulfur) that diminishes with passage through the train of tanks. Ideally, microorganisms are not exposed to severe fluctuations in conditions.

Much of our understanding of biomining microorganisms, their functions and capabilities, rests upon the collection of acidophiles from mine-impacted sites or geothermal regions that have been isolated and characterised. They form the foundation upon which subsequent studies of the microbial ecology of new sites can be described, monitored and expanded. In particular, the availability of "type strains" deposited in commercial culture collections has facilitated numerous research studies focused on microbial responses to different conditions relevant to bioleaching heap or agitated-tank processes. The four main parameters affecting microbial growth and activity are temperature, acidity, and cations and anions in process water, assuming a substrate is available. In the discussion that follows, unless otherwise noted, it can be assumed that studies are focused on the impact of a particular condition or contaminant on microbial ferrous-ion oxidation rates, this being the key reaction for successful sulfide dissolution and the concomitant extraction of the target metals.

3.1. Temperature

Primarily, microorganisms whether or not they are acidophiles tend to be "classified" according to the temperature range within which they grow, for example mesophiles with temperature optima of 20–40 °C; moderate thermophiles (40–60 °C) or extreme thermophiles (60–80 °C) [36]. Variable temperature is more likely to impact microorganisms in heaps than in agitated tanks. A number of temperature-related examples of advances in understanding taken from laboratory-, pilot- and commercial-scale studies of columns and/or heaps are reported in the literature. Rapid temperature increases and their impacts on microbial communities can be problematic in heap leaching, especially at start up when the sulfide content is at its greatest [47–49]. For example, the heat generated during the bioleaching of an ore in a dynamically-controlled, insulated column simulated heat generation in a 5000 tonne test heap of the same ore [47,50]. The catastrophic effect on the microbial population in that simulation as a consequence of the rapid heating to more than 70 °C is of particular interest (Figure 2). The difference in the rates of heat generation is attributed to the initial cell density in the column (5 × 10⁹ cells per kg ore) being higher than that in the test heap (estimated to be 5×10^5 cells per kg ore).



Figure 2. Heat generation profiles (solid lines) for (**a**) a 5000 tonne test heap of Ni-Cu-FeS ore and (**b**) the same ore, acid-agglomerated, inoculated and leached in an aerated, dynamically-controlled, insulated column; (**c**) estimates of cell numbers (broken line) in column effluent representing the microbial response to increased ore temperature.

The determination of preferred temperatures for growth (ranges and optima) of some single strains of acidophiles, based on their iron(II)- or RISC-oxidation rates, illustrates the rapid decline in the activities of microorganisms at temperatures greater than their respective optima [51,52]. It is generally held that hyperthermophiles, which grow well in sulfur-rich, persistently high-temperature environments such as solfataras and hot springs, would not naturally colonise heaped ores of low-sulfide content [35,51]. The results from a series of columns subjected to different temperature regimes and inoculated with mesophilic, moderately thermophilic and hyperthermophiles microorganisms as appropriate, showed a small benefit from inoculation with hyperthermophiles [53,54]. Recently "patches" of *Sulfolobus* spp. were found in the extremely large Escondida run-of-mine dump, indicative of the existence of persistent high-temperature regions in the dump [55], suggesting that thermophiles may colonise large sulfide dumps and heaps that generate and sustain high temperatures over a long period, removing the need for an inoculation strategy.

3.2. Acidity

In general, known acidophiles associated with AMD, solfataras and/or managed bioleaching reactors, while widely distributed across the pH range at temperatures favourable to their growth (Figure 3), tend to have a relatively narrow 'pH window' below which their activity (iron(II)- or RISC-oxidation) is greatly reduced [56]. However, differences in acid tolerance occur between species/strains. For example, in laboratory flask and reactor tests, a sudden drop in acidity from pH 1.5 to pH 1 caused the cessation of pyrite oxidation by *At. ferrooxidans* but did not affect the activity of a *Sulfobacillus*-like strain [57]. Very few acidophiles prefer or are active in habitats poised below pH 1. This was demonstrated in column tests where solution ORP fell, indicative of lower iron(II)-biooxidation activity, when the recycled, pH 1.7 solution, was acidified further to pH < 1 [58]. Similarly, the addition of acid to raffinate prior to recycle to a heap also caused decreased iron(II)- and RISC-oxidising activity in a heap microbial population [59]. Nevertheless, some acidophiles can adapt to very low-pH conditions; *Acidiplasma (Ap.) cupricumulans* (formerly *Ferroplasma cupricumulans*) grows in the range pH 0.4–1.8, *Ap. aeolicum* in the range pH 0–4 and *Picrophilus (P.) oshimae* and *P. torridus* can both grow at about pH 0 [60–62]. *Acidianus sulfidivorans* is another extremely acidophilic archaeon with preferred pH range 0.8–1.4 for growth [63].



Figure 3. Preferred solution pH of microorganisms associated with AMD sites, geothermal regions and managed leaching heap or tank bioreactors, referenced against their preferred growth temperatures. Data obtained from their descriptions or from the recommended growth conditions obtained from commercial culture-collection database.

3.3. Cations

A series of laboratory studies have been conducted in which mainly mesophilic and moderately thermophilic microorganisms were adapted by successive culture to different metal cations, up to $(g \cdot L^{-1})$: Co, 15; Cu or Ni, 50; Zn, 98 but, overall, there are limited data on natural adaptation [64]. However, an important point to note is that, while tolerance to particular metals concentrations may increase the viability of a microbial population in a leaching operation, it does not necessarily result in increased metals extraction [65]. Indeed, slightly lower copper extractions were achieved in 40 days by the more Cu-tolerant strains of *S. thermosulfidooxidans* isolated from a commercial copper bioleaching heap (Figure 4).



Figure 4. Bioleaching of chalcopyrite concentrate by isolates of *S. thermosulfidooxidans* exhibiting different copper tolerances. Tests were conducted with 3 wt % concentrate in basal salts medium (initial pH 1.8) in a shaking incubator (45 °C, 165 rpm). Initial cell density 5×10^6 cells mL⁻¹.

While data from laboratory studies provide a helpful guide to microbial metals tolerances, more robust data can be obtained from long-term biological processing such as continuous agitated-tank or heap-leach processing. For example, two-years into the continuous pilot-scale operation of a biooxidation plant for the treatment of arsenopyrite gold concentrates, the retention time had reduced from 12 to 3.5 days, the mesophilic microbial population being active in solutions of 13 g· L⁻¹ As [9]. Microbial adaptation to base metals in pilot- and demonstration agitated-tank plants is less well documented but microbial populations have been developed that tolerate >5 g· L⁻¹ Co, 23 g· L⁻¹ Ni with 38 g· L⁻¹ Fe, or 36 g· L⁻¹ Cu in cobalt, nickel and copper continuous plants, respectively [66–69]. From these data it can be inferred that adaptation has taken place, given that the concentrations are much higher than those encountered in most metal-rich acidic environments.

3.4. Anions

High concentrations of anions can be present in process waters associated with both heap and agitated-tank bioleaching, but in tank leaching sulfate will dominate. Ore heaps are more likely to contain mineral species enriched in chloride, nitrate or fluoride, which are known to inhibit microbial activity.

In bioleaching heaps, especially in Chile, the mineral atacamite $(Cu_2Cl(OH)_3)$ presents a problem because of the ease with which it dissolves in acidic solutions and releases chloride ion to the circulating process water. In addition, Chile is an arid region with a scarcity of freshwater, leading to the use of seawater at some mines, a factor that has led to strong interest in the development of bacterial cultures active in seawater media. However, efforts to discover or develop acidophilic iron-oxidising strains or adapted cultures that tolerate chloride concentrations equivalent to seawater have had limited success. A survey of microorganisms from saline and acidic drains, lakes and sediments of Western Australia (52 samples) resulted in only five enrichment cultures that could tolerate between 26–41 g· L⁻¹ Cl⁻ in growth media (pH 2) [70]. The authors commented that cultures that oxidised sulfur generally tolerated higher concentrations of salts than those that oxidised iron(II), consistent with previous data [71–73].

Overall, known biomining acidophiles do not tolerate chloride concentrations equivalent to seawater (approximately equivalent to 29 g L^{-1} chloride salts) regardless of the conditions of growth. In several studies, mesophiles tolerated 2–20 g \cdot L⁻¹ NaCl, moderate thermophiles tolerated 10–30 g· L⁻¹ NaCl and a mixed culture of thermophilic archaea tolerated up to 15 g· L⁻¹ NaCl [70–72,74]. Data from descriptions of bacteria and archaea for salt tolerance referenced against their preferred pH for growth (Figure 5) indicated that acidophilic iron-oxidising strains with preferred pH 1–2 have limited tolerance to chloride. Strains with the dual capability of iron- and RISC-oxidation, some within slightly less acidic environments, exhibited greater chloride tolerance, particularly Acidihalobacter prosperus (formerly Thiobacillus prosperus [75]) and some Alicyclobacillus-like strains obtained from marine environments. In Figure 5, known biomining microorganisms mainly lie below the dotted line (equivalent chloride content to seawater). Halophilic bacteria, including the RISC-oxidising Halothiobacillus spp [76], and archaea that grow in environments at least 1.5 M NaCl, well above the chloride content of seawater, generally prefer to grow in environments with neutral pH or higher, above the extended acidophile or acid-tolerant range shown in Figure 5 and of lower acidity than would be useful in bio-assisted metals extraction. The discovery or development through adaptation of halo-tolerant acidophiles with iron(II)- and/or RISC-oxidising capability appears to be a major challenge that must be overcome if seawater is to become widely used in heap (bio)leaching.



Figure 5. Maximum salt tolerance of acidophiles referenced against their preferred solution pH. The dotted line represents the concentration of chloride salts in seawater. (Data obtained from published descriptions of species or from the recommended growth conditions obtained from the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH database).

Concentrations of fluoride higher than $0.5 \text{ g} \cdot \text{L}^{-1}$ are detrimental to microbial growth [69], potentially making fluoride in heap process water a greater challenge than chloride. A case in point was a chalcocite heap for which the failure to reach design recoveries was partly attributed to the failure to test the solubility of fluoride-containing minerals in the ore [77]. In that study, concentrations of iron-oxidizing microorganisms were lower than is typical for sulfide heap leaching, diminishing from the critical concentration of $10^5 \text{ cells} \cdot \text{mL}^{-1}$ [78] to $10^3 \text{ cells} \cdot \text{mL}^{-1}$ within a year. In an earlier but related study, finely ground particles of a variety of minerals inhibited iron(II) oxidation by *At. ferrooxidans* but fluorapatite (Ca₅(PO₄)₃F) particles (<74 µm) caused an anomalously severe response in suspensions < 2 wt % [79].

In a study of sulfur oxidation using *At. thiooxidans*, the toxicity of fluoride was shown to be pH dependent. It was strongly inhibitory at pH 2.3, where undissociated HF dominates the speciation, and is known to penetrate cell membranes [80,81]. Fluoride was less inhibitory at pH 4.5, where only 10% is present as undissociated HF, and not toxic at pH 7 where the undissociated MF molecule is dominant (M = Li, Na or K) [80]. Fluoride toxicity differs between bacterial species. In a recent comparison of iron(II)- and/or RISC-oxidation by five known bioleaching bacteria, the rank order of fluoride tolerance in media pH 1.5–2 was *At. ferrooxidans* > *At. thiooxidans* > *Leptospirillum ferrooxidans* > *At. caldus* > *Sulfobacillus* (*S.) thermosulfidooxidans* [82]. The fluoride tolerance of bacterial cultures can be increased substantially, in one case from 100 to 850 mg· L⁻¹ through adaptive protocols [81,83].

The impact of the bioleach solution composition on the concentration of the HF species was investigated at 65 °C using *Sulfolobus metallicus* growing on pyrite (initial solution pH 1.5) and monitoring changes in ORP as a surrogate measure of microbial activity and growth [84]. The tests varied in the concentration of aluminium added to the growth medium, in the knowledge that Al(III) (and Fe(III)) form Al- or Fe-fluoride complexes [85–87]. A similar strategy of aluminium addition counteracted fluoride toxicity during the bioleaching of a low-grade, fluoride-containing ore using *S. thermosulfidooxidans* (50 °C, growth media in the range pH 1.4–2.1) [88], demonstrating that, if fluoride solubilisation is accompanied by strong aluminium or iron(III) solubilisation from gangue minerals, then the effects on the microorganisms may be substantially mitigated due to the formation of aluminium- or iron(III)-fluoro complexes.

As well as being a copper province, Chile is also a nitrate province. The very large Chilean deposits of salitre (saltpetre), mixtures of sodium and potassium nitrate, are a valuable export commodity. It is not surprising therefore that nitrate minerals are sometimes components of sulfide ores and that nitrate in process water may impact on microbial activity. Studies using *At. ferrooxidans* as the test species showed that nitrate was more inhibitory to iron(II) oxidation than to RISC oxidation [89,90]. However, in a recent comparison of the effects of nitrate ion on substrate utilisation by bacteria and archaea, it was found that the bacteria adapted to the presence of nitrate in growth media and resumed iron(II)-oxidation within a nine-week period in bioleaching tests [91]. Thus nitrate in ores may have only a transient impact on metals extraction in heaps. In contrast, in the same study, it was found that archaea did not adapt to the presence of nitrate. Based on this result, it was proposed that nitrate may be a useful means of controlling ORP during the higher-temperature oxidation of chalcopyrite in an agitated-tank process [91].

Surprisingly, the concentrations of sulfate ion, by far the predominant anion in most bioleaching process waters, are seldom reported and the effect of sulfate on microbial activity seems not to be of great concern. Sulfate concentrations can be more than 100 g· L⁻¹ in agitated tanks [68,69] or up to 150 g· L⁻¹ in the process water circulating through heaps [92,93], concentrations which are much higher than the 40 g· L⁻¹ sulfate that caused an adapted mesophilic culture in iron(II) growth medium to halve its replication rate [94] or *At. caldus* in tetrathionate growth medium to increase the lag time two- to three-fold [74]. During a column bioleaching study (low-grade copper ore), planktonic cells in process water exiting a column declined rapidly by two orders of magnitude when concentrations of ferric and sulfate ions exceeded 30 and 75 g· L⁻¹, respectively [95], and sulfur-oxidising bioactivity

in the Escondida heap was reduced by 50% when sulfate concentrations in process water exceeded 120 g·L⁻¹ [96].

4. Biodiversity in Bioleaching Environments

The ease with which the microbial diversity of environmental samples can be examined has led to many recent descriptions of the orders, genera and sometimes species inhabiting bioleaching reactors (heaps, dumps, tanks) and/or AMD impacted environments. The collation of example data in Figure 6 illustrates the increased knowledge of bioreactor diversity as well as some effects of variable environments on that diversity, for example changes in community structure with time (H1, days 30 to 400; and T1, days 0 to 8), with depth in the heap (H2, depth 1 *cf.* 3 m) and with operating pH of the process water (H2 and H3, T2). In agitated tanks, the population of attached microorganisms differs from the planktonic community (T3, attached *vs.* planktonic) and the community structure differs with different solids loading (T3, 10% *cf.* 20%).



Figure 6. Microbiological diversity to genus level in heaps (H), and agitated tanks (T); A, attached; P, planktonic cells; % solids loading. Data collated from [97–102].

In general, abundances of *Acidithiobacillus* spp. in heaps including *At. ferrooxidans* and the sulfur-oxidising species *At. caldus, At. thiooxidans* and *At. albertensis* are greater early in leaching when there is a greater sulfur content [99,103]. *Leptospirillum* and *Ferroplasma* are more abundant later in the leach when the iron concentrations are higher and possibly inhibitory to *Acidithiobacillus* and other species [99,104,105]. Microbial communities change with time in both heaps and tanks, influenced by parameters such as substrate content and availability, solution composition and acidity, solids loadings in tanks, and temperature [92,102–104,106–108]. However, comparisons between studies tend to reveal a variety of effects as the communities respond to a combination of environmental parameters and, in many instances, to adapt to them with time [64].

It is in the nature of sulfide heaps that sulfur contents tend to be low and the heat generated within the bed during sulfide oxidation is transient [49], essentially because heap-management strategies are employed to maintain temperatures in the range suited to bacterial growth. In general, extreme thermoacidophiles with iron(II)- and sulfur-oxidising capabilities colonise habitats that are persistently hot and sulfurous [40,109,110]. As a consequence, it has been assumed that thermophiles such as *Acidianus, Metallosphaera* and *Sulfolobus* spp [111–114] would not naturally colonise sulfide heap operations and that thermophiles would need to be introduced to managed "hot heaps" to exploit increased metal extraction kinetics [49,54]. However, some recent studies on the biodiversity of sulfide heaps and mine-waste dumps included the putative identification of *Archaea* and some thermophiles have been detected, including *Acidianus, Acidiplasma, Ferroplasma, Metallosphaera, Sulfolobus* and *Thermoplasma* spp. [49,115–119].

The advances in both DNA sequencing and protein extraction, accompanied by huge advances in computing power and speed, launched the age of genomics and metagenomics, proteomics and meta-proteomics and metabolomics (Table 2).

Term	Definition		
Genomics	The branch of molecular biology concerned with the structure, function, evolution and mapping of genomes (the complete set of genes present in an organism).		
Metagenomics	The study of the collected genetic material (genomes) from a mixed community of organisms.		
Proteomics	The analysis of the structure, function and interactions of the proteins produced by the genes of a particular cell, tissue or organism; a proteome is the total complement of proteins present in a cell.		
Metaproteomics	Metaproteomics The study of all protein samples recovered directly from environmental sources also called community proteomics or environmental proteomics.		
Metabolomics	Systematic study of the unique chemical fingerprints that specific cellular processes leave behind; the study of their small-molecule metabolite profiles.		

Table 2.	Molecular	biology	terminology.
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Not surprisingly, the first genome sequence of a bioleaching microorganism was that of *At. ferrooxidans* [120]. Since then, the number of genomes of microorganisms of direct interest in bioleaching has increased significantly, largely due to a general interest in acidophiles and their activities in AMD environments. The strong overlap with bioleaching is understandable if bioleaching is viewed as an exemplar of extreme but controlled AMD. In a 2010 review [121], it was reported that there were 30 bacterial genomes and 26 archaeal genomes for extremely acidophilic microorganisms, eight metagenome projects of extremely acidic environments (four associated with the AMD at Iron Mountain, CA) and complete sequences for 38 plasmids and 29 viruses from acidic environments. At the same time, it was concluded that these data were not sufficient "to provide a reasonably complete description of the genomic complexity and, by inference, of the full metabolic potential present in bioleaching operations" because (i) there are many gaps regarding the metabolic data; (ii) the microbial diversity of bioleaching and AMD environments is considerably greater than was previously appreciated; and (iii) often genomes were obtained using laboratory-maintained strains that may have accumulated genetic modifications.

Nevertheless, despite the body of research required to address the gaps and the greater diversity in natural microbial communities, the applications of the complementary techniques (Table 2) are already augmenting our understanding of the microbial processes involved in extracting metals from minerals. Numerous data on microbial functions and the conditions under which they are exercised best are being gathered. For example, their applications to AMD and bioleaching systems are resulting in better understanding of microbial physiology for individual organisms [40,122–125]. Bacterial, archaeal and eukaryotic diversity and their metabolic networks in AMD habitats have been described [126–128] and the ways in which metabolic activity levels and the partitioning of function are affected by community composition and the physico-chemical environment have been explored [129,130]. The extent to which each of the individual participants contributes to the process and how they evolve in time to keep the conglomerate healthy and therefore efficient during the entire process of bioleaching is being investigated [131]. Finally, the hypothesis that advanced knowledge of microbial processes can lead to better process design, control and optimization for metals extraction from minerals is supported by the interest shown by some mining companies in transcriptomic analysis of the genomic sequences of newly-isolated (commercially-sensitive) microorganisms with superior ability to extract copper from sulfides [132].

A valuable output from genomic studies is the generation of genomic sequences for microarray analysis [132]. The development of microarray technology over a number of years [133,134] has

the potential to revolutionise the study of biodiversity in environmental samples. A review on the development of functional gene arrays was introduced as follows: "the recent advance of metagenomic technologies such as high throughput sequencing and functional gene arrays provides powerful high throughput tools for analysing microbial communities" [135]. A variety of microarrays have been constructed to contain probes for the genes involved in key microbial functions for specific applications [135]. For example, a microarray was developed based on most of the genes associated with acidophiles, including genes for carbon, nitrogen, sulfur and iron metabolism, DNA replication and repair, and metal resistance [136]. A suite of GeoChip microarrays, of which GeoChip 4 is the most comprehensive [137], have been used to characterize microbial communities in terms of functional diversity, composition, structure and metabolic activity/capability from a variety of habitats (e.g., soils, aquatic, contaminated sites, bioreactors).

Two modes of microarray application have been described. The use of microarrays based on the complete genomes of the members of a microbial community enables a nearly complete view of gene expression under different bioleaching conditions but it is time-consuming and expensive for the study of organisms isolated from the environment. Examples of specific studies based on complete genome sequences in combination with physiological and biochemical studies include for bacteria Acidithiobacillus spp [138–140] and for archaea, Metallosphaera sedula [141,142]. The shotgun DNA microarray, is a general approach by which gene expression can be studied in environmental isolates and can be applied to any organism regardless of how much of its genome is sequenced [132,143,144]. Applications for acidophiles include bacteria, *Leptospirillum* spp. [145,146] and Acidithiobacillus spp. [147] and archaea, Acidianus [148] and Ferroplasma [149]. Applications of microarray analysis of bioleaching and/or AMD environments include the study of bacterial population dynamics at a uranium-contaminated site [150], the monitoring of acidophile activity in AMD systems [151,152] and in the Escondida copper heap [99,153]. As no public domain research on heap or tank microbial populations funded by industry was found in this survey, it is assumed that such applications of advanced molecular-microbiological techniques for the discovery of superior bioleaching microorganisms are commercially sensitive.

5. Summary

Until recently, the success of the two, widely-practised technologies employed in biohydrometallurgy, the pre-treatment of refractory gold concentrates in agitated tanks and the heap and dump leaching of low-grade, copper sulfide ores, was thought to depend on a few acidophilic microorganisms that oxidised iron(II) or sulfur compounds (chemolithotrophs). The technological concern was to optimise processing conditions to suit the microorganisms in the hope of faster and greater metals production.

In the late 20th century, the research emphasis was focused in two areas, discovering microorganisms with 'superior' metal-extraction capabilities and gaining a better understanding of how adaptable and resilient they were to extreme and variable mineral processing environments. The numbers of new species from natural and mine-impacted acidic environments increased steadily, with particular value being placed on extremely acidophilic, thermophilic and/or halophilic species with the requisite iron(II) and sulfur oxidising capabilities. Once characterised, their ready availability from commercial culture collections promoted an impressive number of studies on their responses to acid, metals and other soluble components, high ionic strength, high temperature and other processing parameters, depending on whether the research interest was rapid metals extraction from ores/concentrates or remediation of acid-drainage systems. The result is that what were once thought to be environments with low biodiversity have been shown to host diverse, robust, adaptable communities of acidophiles.

Now, in the 21st century, more than 50 genomes of acidophilic microorganisms have been published and analysed, leading to greatly increased knowledge of their physiological capabilities. Functional-gene microarrays are providing snapshots of microbial diversity in AMD environments and

managed bioleaching reactors in terms of community structure and metabolic activity. The increasing capability of microarrays to describe not only what acidophiles are present but also what active contributions the acidophiles are making to metals extraction, is set to revolutionise both biodiversity studies of operating heaps and bioreactors as well as laboratory-based studies of the impacts of changing growth conditions on microbial activities. The body of knowledge being acquired through such studies can be used to predict microbial behaviour under varied conditions and from those data, to modify conditions for better metal extraction or for more appropriate microbial activity. Conversely, it should become possible to infer "microbial health" particularly in heaps based on measured physicochemical parameters and microbial diversity in process water, without having to disturb a production heap by sampling the solids.

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