Extremophiles: Acidic Environments

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Glossary

autotroph An organism that obtains its carbon by fixing carbon dioxide, bicarbonate, or other C_1 compound.

chemolithotroph A prokaryote that uses an inorganic energy source and fixes carbon dioxide.

FISH (fluorescent* in situ *hybridization) A technique for identifying individual cells using molecular (RNA) probes.

heterotroph An organism that obtains both its carbon and energy from an organic source.

lentic waters Nonflowing continental waters, such as ponds and lakes.

lotic waters Flowing continental waters, such as streams and rivers.

mixotroph A prokaryote that uses an inorganic energy source and an organic carbon source.

RISCs (reduced inorganic sulfur compounds) Oxysulfur anions other than sulfate.

snotites Small gelatinous growths of microorganisms that grow suspended from the roofs of underground mines and caves.

sulfidogen An organism that generates (hydrogen) sulfide.

Abbreviations

AMD	acid mine drainage
DGGE	denaturing gradient gel electrophoresis
FISH	fluorescent *in situ* hybridization
PGM	platinum group metal
RISC	reduced inorganic sulfur compound
SRB	sulfate-reducing bacteria

Defining Statement

This article gives an overview of the nature of extremely acidic environments and of the biodiversity of microorganisms found within them. Ways in which acidophiles interact with each other in both positive and negative fashions are described. Finally, the microbial ecology of some of the most widely studied extremely acidic environments on our planet is discussed.

Nature and Origin of Extremely Acidic Environments

Oceanic waters, which constitute the largest biome on planet Earth, are uniformly moderately alkaline (pH 8.2–8.4). In contrast, some lentic and lotic waters, soils, and anthropogenic environments are moderately acidic (pH 3–5) or extremely acidic ($pH < 3$). In some rare cases, environments that have recorded negative pH values have been documented. Living organisms that are active in extremely acidic environments are now known to be far more diverse than was recognized even a couple of decades ago. As with other extremophiles, acidophiles tend to be specialized life-forms, in that many are unable to grow in neutral pH environments. The majority of acidophiles are prokaryotic microorganisms, and these comprise a large variety of phylogenetically diverse Bacteria and Archaea, though some single-celled and multicellular eukaryotes are known to grow in highly acidic ponds and streams.

Extremely acidic environments may be formed by processes that are entirely natural, though human activities have became increasingly important in generating such sites. While the scale of human impact has paralleled global industrialization, small-scale anthropogenic generation of acidic, metal-polluted environments probably began in the Bronze Age. Overall, the majority of extremely acidic sites that now exist on planet Earth are associated with one particular human activity – the mining of metals and coal.

There are a number of important (in terms of their scale) microbial activities that can generate acidity. Among the most important of these is the formation of organic acids as waste products in either anaerobic (fermentative) or aerobic metabolisms. However, the generation of strong inorganic acids by aerobic microorganisms gives rise to the most acidic environments on our planet. Nitrification (the formation of nitric acid from ammonium) is potentially one of these, though the process is self-limiting in poorly buffered environments, as the majority of nitrifying bacteria are highly sensitive to even mild acidity. In contrast, prokaryotes that oxidize sulfur (in many of the large variety of reduced forms of this element that exist) include species that grow in neutral pH and moderately alkaline environments, as well as those that grow optimally in acidic environments. Indeed, some of the most extremely acidophilic life-forms known are those that obtain energy by oxidizing reduced sulfur to sulfuric acid.

Geothermal Areas

Elemental sulfur may occur in geothermal areas (e.g., around the margins of fumaroles) where it can form by the condensation of sulfur dioxide and hydrogen sulfide, two common volcanic gases (eqn [1]):

$$SO_2 + 2H_2S \rightarrow 2H_2O + 3S^0 \qquad (1)$$

Oxidation of sulfur by acidophilic bacteria and archaea generates sulfuric acid (eqn [2]):

$$S^0 + H_2O + 1.5O_2 \rightarrow H_2SO_4 \qquad (2)$$

This can result in severe acidification of environments both on the micro- (i.e., microbial habitats) and on the macro-scale. Oxidation of sulfide minerals (see 'Mine-impacted environments') may also contribute to acid genesis in these locations. Whether or not specific sites develop net acidity depends on how effectively acid generation is counterbalanced by the dissolution of basic minerals, such as carbonates. Geothermal, sulfur-rich, acidic sites are known as solfatara (**Figure 1**); water temperatures in solfatara fields approach boiling point (~85–100 °C, depending on altitude) but tend to cool rapidly as the water flows from the source of the geothermal spring. These sites may therefore be colonized by a variety of acidophilic microorganisms that have different temperature optima, and are therefore very fertile locations for isolating novel acidophilic microorganisms.

High-temperature environments that host solfatara and acid streams occur in zones of volcanism and in areas where the earth's crust is relatively thin. Examples of terrestrial and shallow marine locations include Yellowstone National Park (USA); Whakarewarewa (New Zealand); Krisuvik (Iceland); the Kamchatka Peninsula (Russia); Sao Michel (Azores); Volcano, Naples, and Ischia (all Italy); Djibouti (Africa); and some Caribbean islands, such as Montserrat and St. Lucia. Related to these are deep and abyssal submarine hydrothermal systems, such as the Mid-Atlantic Ridge, the East Pacific Rise, the Guaymas Basin, and active seamounts (e.g., around Tahiti). In contrast to many terrestrial sites, submarine hydrothermal systems are generally in the range pH 3–8, and saline, due to the high buffering capacity of seawater.

Mine-Impacted Environments

Many of the most important base metals (such as copper, lead, and zinc) used by humankind are sourced mostly from sulfide minerals. In addition, many precious metals including gold, silver, and PGMs (platinum group metals) are

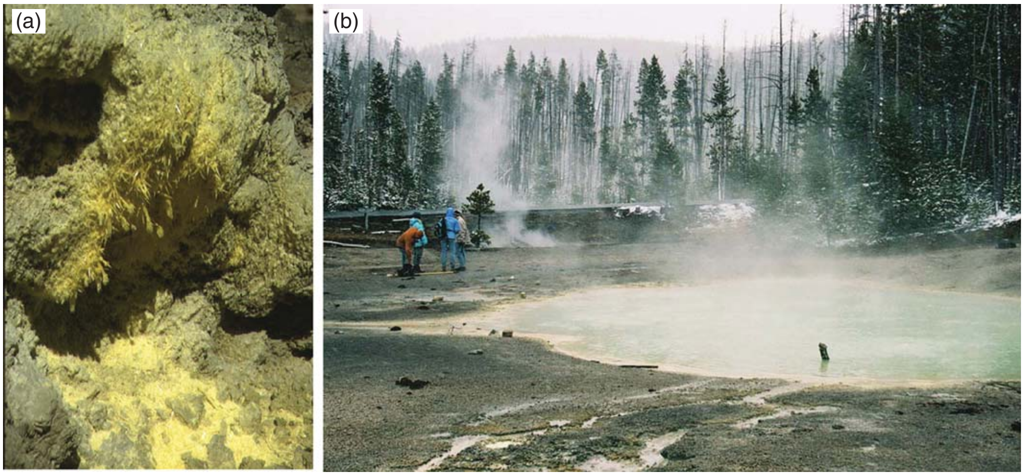

Figure 1 (a) Elemental sulfur forming from gases venting the Soufriere Hills volcano on Montserrat, West Indies; (b) an acidic geothermal pool (Frying Pan Hot Spring) in Yellowstone National Park, Wyoming.

often found in association with sulfidic ores. Mining of metallic ores has, in the past, involved smelting whole rocks, though the advent of concentration techniques (mostly involving froth flotation and separation of target minerals) and nonpyrometallurgical techniques (such as pressure oxidation and biological processing) have had, and continue to have, a major impact on the mining industry. Production of mineral concentrates results in the generation of large quantities of waste minerals that, because of the intensive rock grinding involved, are fine grain. These waste minerals (referred to as tailings) are usually disposed of in large lagoons, which may, in time, become drained thereby allowing ingress of oxygen and dissolution of the minerals, a process often paralleled by intensive acidification. Apart from this, waste rocks from mines and the abandoned mines themselves can serve as source points for the generation of metal-rich, acidic wastewaters.

The mining of copper ores, which is carried out in many different parts of the world, is one potential source of acid pollution. Copper exists in a variety of sulfide minerals, of which the most important (quantitatively) is the mixed copper–iron sulfide chalcopyrite (generally notated as $CuFeS_2$, though a more accurate mineral formulation is probably $CuFeS_{1.5}$, as both copper and iron occur in their more reduced ionic forms). Other significant copper minerals include single-metal sulfides (chalcocite, Cu_2S; and covellite, CuS) and mixed-metal sulfides bornite (Cu_5FeS_4) and enargite (Cu_3AsS_4). The sulfide moiety in these minerals represents a source of energy that can be utilized by some lithotrophic (literally, rock-eating) prokaryotes, many of which are obligate acidophiles (**Figure 2**). In addition, the ferrous iron present in chalcopyrite and bornite is a second potential energy source for mineral-oxidizing Bacteria and Archaea. These microorganisms require both oxygen and water (though little else) to facilitate their attack on the minerals, which is why mine wastes may be safely stored in environments that are either totally dry or anoxic. In moist, aerated environments, however, the minerals are prone to oxidative dissolution, resulting in the release and potential solubilization of their component metals.

Acid genesis, however, is relatively limited when copper sulfides are (biologically) dissolved. In contrast, the iron disulfide mineral pyrite (FeS_2; ‘fool’s gold’), which is the most abundant sulfide mineral in the lithosphere and which is invariably associated (often as the dominant mineral) with copper and other metal sulfide ores, generates significant levels of acidity due to its greater sulfur content. Pyrite has served as the model mineral for most studies of microbial attack on sulfide minerals and, although there have been various schemes proposed, that described by Wolfgang Sand of Duisburg–Essen University is generally regarded as the most accurate. In this, the initial attack on the hard, dense mineral is by ferric iron, which is a powerful oxidizing agent in acidic liquors. Ferric iron oxidizes the sulfur moiety of the mineral to thiosulfate, and in so doing is reduced to ferrous iron (eqn [3]):

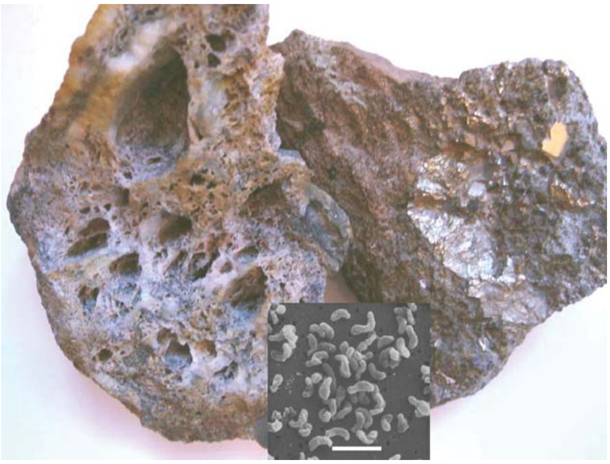

Figure 2 Partial dissolution of a sulfidic rock by chemolithotrophic bacteria. The rock on the right is freshly exposed and contains grain of pyrite (fool’s gold) and other metal sulfides. That on the left has been exposed to attack by chemolithotrophic acidophiles, and the sulfide minerals have been effectively dissolved, leaving a porous remnant composed of inert minerals. Inset: the mineral-oxidizing acidophile *Leptospirillum ferrooxidans* (the scale bar represents 2 μm).

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow 7Fe^{2+} + S_2O_3{}^{2-} + 6H^+ \tag{3}$$

The ferrous iron formed is reoxidized to ferric iron by a variety of iron-oxidizing acidophilic bacteria and archaea in an oxygen-consuming reaction (eqn [4]):

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \tag{4}$$

Thiosulfate is unstable in acidic liquors (particularly when ferric iron is present) and oxidizes to form a variety of other reduced inorganic sulfur compounds (RISCs) such as trithionate ($S_3O_6{}^{2-}$) and tetrathionate ($S_4O_6{}^{2-}$), as well as elemental sulfur (S^0). The latter can all serve as substrates for sulfur-oxidizing Bacteria and Archaea and are oxidized, when oxygen is available, to sulfuric acid, thereby generating the extreme acidity that helps maintain a suitable pH required by the mineral-oxidizing microorganisms.

A second mechanism has been proposed for minerals, such as sphalerite (ZnS) and galena (PbS), that are soluble in sulfuric acid. In this scenario (the polysulfide mechanism) the metal–sulfur bond is broken by proton attack and hydrogen sulfide (H_2S) is liberated. If ferric iron is also present, concomitant attack by iron and protons results in the proposed formation of H_2S^+, which dimerizes to form free disulfide (H_2S_2), and is further oxidized forming, ultimately, elemental sulfur. In the absence of sulfur-oxidizing prokaryotes, this sulfur accumulates, and though in their presence it is oxidized to sulfuric acid.

Dissolution of sulfidic ores not only produces acidity, but also generates liquors that contain concentrations of base metals (copper, zinc, manganese, etc.) and aluminum that are far greater than those found in most surface waters. The two reasons for this are (1) the occurrence of these metals in sulfide minerals and others (many aluminosilicates) that spontaneously degrade at low pH, and (2) the far greater solubility of these metals in low pH than in circumneutral pH solutions. Exceptions to the latter are metals, such as molybdenum and vanadium, that occur mostly as oxyanions, rather than cations. Metalloids, most significantly arsenic, can also be present at highly elevated concentrations in sulfide ore leach liquors; arsenopyrite (FeAsS) and realgar (As_4S_4) are two other relatively common sulfide minerals. Waters percolating through fissures in worked-out underground mines, as well as those draining stockpiled mine waste rock dumps and mine tailings, become enriched in these soluble metals and metalloids. At their point of discharge from underground mines or tailings ponds, mine waters are frequently devoid of oxygen and appear untainted. However, flowing waters become increasingly aerated, facilitating the oxidation of uncolored ferrous iron (usually the dominant dissolved metal found in mine drainage waters) to highly colored (yellow-red) ferric iron. This is why mine water-impacted waters are, in the main, very obvious sites of water pollution. Depending on pH, the ferric iron formed will either remain in solution or hydrolyze (react with water) to produce a variety of solid phase minerals (e.g., schwertmannite ($Fe_8O_8(OH)_6SO_4$), ferrihydrite ($5Fe_2O_3.9H_2O$), and amorphous ferric hydroxide ($Fe(OH)_3$)):

$$Fe^{3+} + H_2O \rightarrow Fe(OH)_3 + 3H^+ \qquad (5)$$

This has two important consequences: First, the reaction generates protons, as illustrated in eqn [5], thereby helping to maintain the acidity of water. Aluminum and manganese also behave similarly, but these metals are generally less abundant than iron in mine waters. Second, the precipitates that form sink to the bottom of the stream, forming a dense coating (known in Europe as ochre and in the USA as yellow boy) that can seriously impact benthic life. In the most extremely acidic mine waters (pH < 2.5), ferric iron remains in solution and the resulting red water color is often reflected in the names given to these streams or rivers, most famously, the Rio Tinto in Spain (**Figure 3**).

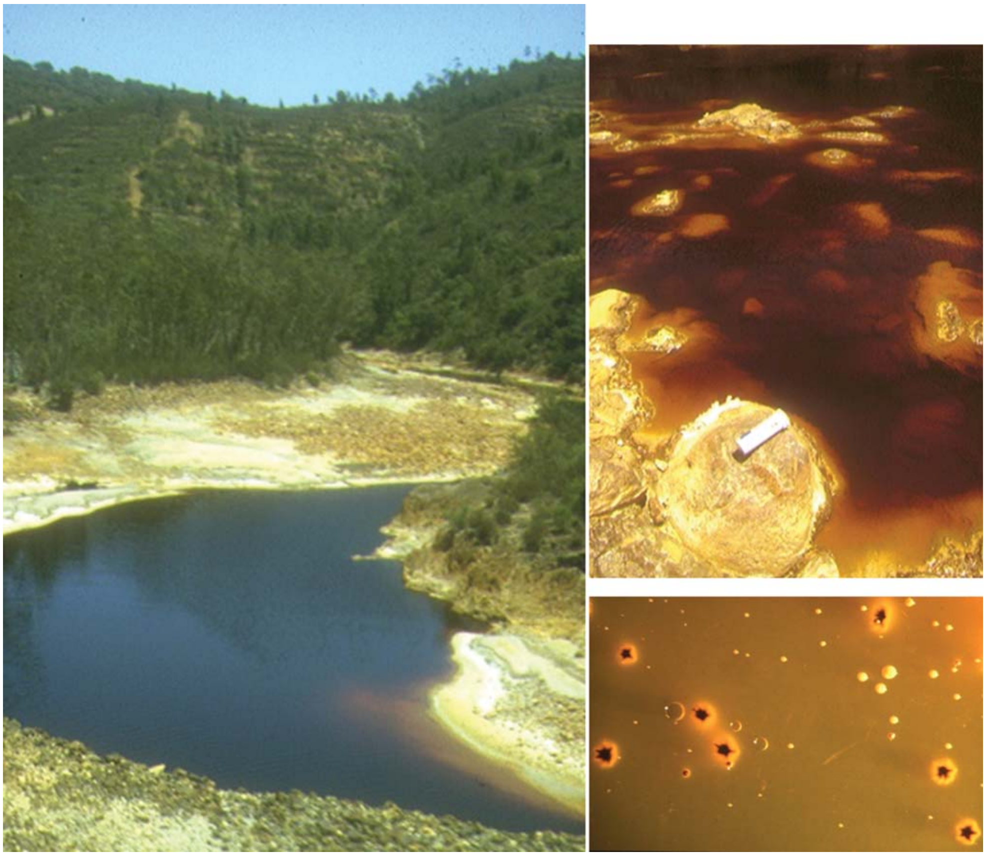

Figure 3 The Rio Tinto, an iron-rich extremely acidic river that flows though southwest Spain (left); the deep red-colored water of the Rio Tinto, due to the presence of elevated concentrations of soluble ferric iron (top right); colonies of rust-colored iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans*) and heterotrophic acidophiles (*Acidiphilium* spp.) isolated from the Rio Tinto (bottom right).

Apart from their characteristic low pH and elevated metal contents, the chemistries of mine waters are highly variable, as described in the section titled ‘Acid mine streams and lakes’. However, concentrations of inorganic nitrogen (generally exclusively ammonium, except where there is input of nitrate from rock blasting at working mines), phosphate, and dissolved organic carbon all tend to be relatively small.

Biodiversity of Extreme Acidophiles

Extremely acidophilic organisms are exclusively microbial and include both prokaryotes and eukaryotes. The axiom that as an environmental parameter (in this case, acidity) becomes more extreme biodiversity declines holds true for both groups. Although some angiosperms have been observed to grow in highly acidic lakes, their root systems grow in sediments in which the pH is usually much higher than the water body itself. Many eukaryotic microorganisms that have been observed in extremely low-pH environments are acid-tolerant rather than truly acidophilic, and may grow equally well, or better, in circum-neutral pH environments.

Primary Producers in Acidic Environments

The first extremely acidophilic microorganism to be isolated and characterized was the sulfur-oxidizing bacterium, *Acidithiobacillus* (*At.*) *thiooxidans* (then referred to as *Thiobacillus thiooxidans*) by Waksman and Joffe in 1921. Some years later, another sulfur-oxidizing bacterium was isolated from water draining a coal mine that had the unique trait (at the time) of also being able to oxidize ferrous iron to ferric. (*Acidi*)*thiobacillus ferrooxidans* has subsequently become the most well studied of all acidophilic microorganisms. Both of these early isolates are autotrophic chemolithotrophs, that is, they use inorganic electron donors and fixed carbon dioxide. Such a metabolic lifestyle is highly appropriate in extremely acidic environments, which, as noted previously, tend to contain elevated concentrations of potential inorganic energy sources (ferrous iron and reduced sulfur) but often low concentrations of dissolved organic carbon. While more recent studies have led to the isolation of a number of other genera and species of chemolitho-autotrophic acidophiles, a large number of highly biodiverse microorganisms that have very different metabolic lifestyles (e.g., phototrophic microalgae, heterotrophic bacteria and yeasts, and phagotrophic protozoa) have also been shown to be obligate acidophiles (see ‘Acidophilic eukaryotic microorganisms’).

Primary production (net assimilation of carbon) in extremely acidic environments is carried out by two main groups of microorganisms, the relative importance of which varies from site to site. Chemolitho-autotrophic acidophiles are CO_2 fixers that use either ferrous iron or reduced sulfur (or, in some cases, both) as energy sources. Sulfide (e.g., in minerals), elemental sulfur, and RISCs are far more energetic substrates than ferrous iron (**Table 1**) though interestingly at least one bacterium (*At. ferrooxidans*) that can use both ferrous iron and sulfur as substrates appears to opt for the former when both are available. Some acidophiles have also been shown to use hydrogen as an electron donor. The significance of this is unknown, though hydrogen may form in these environments from reactions between protons and various minerals in contact with acidic liquors. Other chemolitho-autotrophic metabolisms (e.g., nitrification) have not been observed in extremely acidic environments. Photoautotrophy (the use of solar energy to fuel carbon dioxide fixation) may be the dominant mechanism of primary production in acidic ecosystems, as in most others, though in underground sites (acidic caves and mine caverns) primary production is exclusively mediated by chemolithotrophs. All acidophilic phototrophic microorganisms that have been identified are eukaryotic microalgae. No truly acidophilic phototrophic bacteria (aerobic cyanobacteria or anaerobic purple/green S-bacteria) have been described, though clones of anaerobic photosynthetic green sulfur and purple nonsulfur bacteria have been obtained from an acidic geothermal site in New Zealand (see ‘Geothermal areas’).

Many acidophilic microorganisms that fix carbon dioxide are obligate autotrophs. Some, however, can switch to assimilating organic carbon if and when this

Table 1 Comparison of free energy changes associated with the oxidation of inorganic substrates used by chemolithotrophic acidophiles

Reaction	*Free energy change ΔG^o (kJ mole substrate^{-1})*
Ferrous iron oxidation	
$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$	–30 (at pH 2.0)
Hydrogen oxidation	
$H_2 + 0.5O_2 \rightarrow H_2O$	–237
Elemental sulfur oxidation	
$S^0 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$	–507
Hydrogen sulfide oxidation	
$H_2S + 2O_2 \rightarrow H_2SO_4$	–714
RISC oxidation	
(1) $S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+$	–739
(2) $S_4O_6^{2-} + 3.5O_2 + 3H_2O \rightarrow 4SO_4^{2-} + 6H^+$	–1225

Source: Data from Kelly DP (1978) Bioenergetics of chemolithotrophic bacteria. In: Bull AT and Meadows PM (eds.) *Companion to Microbiology*, pp. 363–386. London: Longman, and Kelly DP (1999) Thermodynamic aspects of energy conservation by chemolithotrophic bacteria in relation to the sulfur oxidation pathways. *Archives of Microbiology* 171: 219–229.

becomes available. The metabolic logic for this is obvious, as CO_2 fixation is a highly energy-consuming process (e.g., *At. ferrooxidans* has been estimated to utilize most of the energy it obtains by oxidizing iron on this single process), and using prefixed carbon, assuming that it is readily incorporated and metabolized, avoids this expenditure of energy. Various terms have been used to describe such microorganisms, which include some eukaryotic algae as well as some prokaryotic acidophiles, though the most appropriate (and least ambiguous) is to refer to them as facultative autotrophs. Whether such acidophiles are net contributors to total primary production depends not only on the presence of metabolizable organic carbon, but also (in the case of phototrophs such as *Cyanidium caldarium*) on the availability of solar energy.

Heterotrophic Acidophiles

Unusually for microbial ecology, the first obligately heterotrophic acidophilic bacteria (*Acidiphilium* spp.) were isolated some 70 years after the first chemolithotrophic acidophile, though a heterotrophic acidophilic archaeon (*Thermoplasma* (*Tp.*) *acidophilum*) was actually described a decade before the first *Acidiphilium* sp. (*Acidiphilium cryptum*). There are now a large number of characterized species of acidophilic bacteria and archaea that are known to use organic compounds as sources of both carbon and energy. Some of these are able to supplement their energy budgets by oxidizing inorganic substrates (ferrous iron or reduced sulfur) when these are also available. In the case of truly mixotrophic acidophiles, such as the iron-oxidizing heterotroph *Ferrimicrobium* (*Fm.*) *acidiphilum*, the inorganic substrate can serve as the sole source of energy and the organic moiety only to meet the carbon requirements of the bacterium.

Bacteria, in particular, are renowned as a collective group of microorganisms for their abilities to degrade a multitude of small and large molecular weight organic compounds, including many synthetic materials. Acidophilic prokaryotes, on the other hand, appear to use a far more restricted range of monomeric organic substrates and few polymeric materials. Simple sugars and alcohols are utilized by many heterotrophic acidophiles, but aliphatic acids (such as acetic acid) tend to be lethal to acidophiles when present in only micromolar concentrations. The reason for this relates to the fact that many small molecular weight organic acids exist as undissociated, lipophilic molecules in low-pH liquors. These can freely permeate microbial membranes and accumulate in the circum-neutral pH cell interiors where they dissociate and cause intracellular acidification of the cytoplasm. Di- and tricarboxylic organic acids, such as citric acid, are not so toxic and are actually used as substrates by many heterotrophic acidophiles. Some organic acids, most notably glutamic acid, also serve as appropriate substrates for many acidophiles, though others (e.g., glycine) do not. Complex, nitrogen-rich organic substrates, such as yeast extract and tryptone, are also suitable substrates for isolating and cultivating many heterotrophic acidophiles and supplementing defined organic growth media with, for example, yeast extract often promotes growth of heterotrophic acidophilic bacteria and archaea. One of the few known examples of an acidophile being able to grow on an organic polymer is the archaeon *Acidilobus aceticus*, which grows anaerobically on starch, forming acetate as the main metabolic product.

Aerobic and Anaerobic Acidophiles

The majority of known acidophilic prokaryotes have been classed as obligate aerobes. More detailed examination has revealed that, in a number of cases, they can also grow in the absence of oxygen and are therefore facultative anaerobes. Of the various options that microorganisms use for living in the absence of oxygen, by far the most widespread among acidophiles appears to be ferric iron respiration. This is understandable since iron, as both ferrous and ferric, is usually abundant in extremely acidic environments, particularly those originating from the oxidative dissolution of sulfide minerals. There is also a thermodynamic advantage to be gained from using ferric iron in that the redox potential (E_h value) of the ferrous/ferric couple at low pH is about +770 mV, a value which is not much below that of the oxygen/water couple (+840 mV) and considerably more positive than alternative inorganic electron acceptors such nitrate and sulfate. Most Bacteria (and the Euryarchaeote *Ferroplasma* (*Fp.*) *acidiphilum*) that can oxidize ferrous iron in the presence of molecular oxygen can also reduce it when oxygen is absent. Notable exceptions are species of *Leptospirillum*, though this is explained by the fact that these highly specialized bacteria have not been found to use an electron donor other than ferrous iron. Other acidophilic bacteria that also reduce ferric iron to ferrous are obligate heterotrophs, one of which *Fm. acidiphilum* is an iron oxidizer, while others (all species of *Acidiphilium*, as well as many *Acidocella* and *Acidobacterium* spp.) are not. This trait is not universal among acidophilic heterotrophic bacteria, however, as illustrated by the fact that *Acidisphaera rubrifaciens* and closely related isolates do not appear to reduce ferric iron. The earlier claim that the sulfur-oxidizing bacterium *At. thiooxidans* can reduce ferric iron was later challenged as probably being an artifact resulting from chemical reduction by RISCs that are produced during sulfur metabolism. The thermotolerant sulfur oxidizer *Acidithiobacillus caldus* also does not appear to reduce ferric iron.

Sulfur respiration (the use of elemental sulfur as electron acceptor) is not uncommon among acidophilic archaea: *Acidianus, Stygiolobus, Sulfurisphaera* (all thermoacidophilic crenarchaeotes), and *Thermoplasma* (a moderately thermoacidophilic euryarchaeote) can all grow anaerobically by reducing sulfur to hydrogen sulfide. *Acidianus* spp. and *Sulfurisphaera ohwakuensis* are both facultative anaerobes that couple the oxidation of hydrogen to the reduction of sulfur in anoxic environments, while *Stygiolobus azoricus* is an obligately anaerobic thermoacidophile that can do the same. In contrast, both classified species of *Thermoplasma* (*Tp. acidophilum* and *Tp. volcanium*) are facultative anaerobes that couple the oxidation of organic carbon to the reduction of elemental sulfur. No extremely acidophilic sulfur- or sulfate-reducing bacteria (SRB), or sulfate-reducing archaea, have yet been isolated and characterized, though there is evidence that sulfidogens are both present and active in some anaerobic acidic environments. A *Desulfosporosinus*-like isolate (M1), isolated from a geothermal site on Montserrat, West Indies, has been demonstrated to grow in a mixed culture at pH 3.2 and above, but is probably acid-tolerant rather than a true acidophile. Many other apparently acid-tolerant sulfidogenic isolates and putative clones detected in acidic mine waters have also been found to be Gram-positive bacteria.

Clones of methanogenic archaea have also been identified in gene libraries constructed from DNA extracted from some extremely acidic environments, but no extremely acidophilic methanogens are known. Likewise, no acetogenic acidophiles have been isolated, though this may be explained on the biotoxicity of acetic acid in low-pH liquors, as discussed previously. The general toxicity of aliphatic acids may also help account for the apparent absence of fermentative metabolism among extreme acidophiles, with the exception of the thermophilic archaeon *A. aceticus*, which can grow by fermenting starch to acetic acid. The scarceness of nitrate in most acidic environments, apart from those in the vicinity of rock blasting, and the fact that acidophiles are more sensitive to nitrate and nitrite than most other bacteria are probably why nitrate respiration is apparently absent in these microorganisms.

Temperature and pH Characteristics of Acidophilic Microorganisms

One of the most widely used methods to categorize acidophilic prokaryotes is on the basis of their temperature characteristics, that is, their optimum temperatures for growth and the range of temperatures within which they are active (**Table 2**). Three groups of acidophiles have often been recognized in this way: (1) mesophiles, with temperature optima of 20–40 °C; (2) moderate

Table 2 Categorization of validated species and genera of extremely acidophilic prokaryotic microorganisms, based on growth temperature optima

	Carbon assimilation	*Fe^{2+} oxidation*	*Fe^{3+} reduction*	*S^0 oxidation*	*S^0 reduction*
(a) Mesophiles (temperature optima 20–40 °C)					
At. ferrooxidans	OA	+	+	+	+
L. ferrooxidans	OA	+	−	−	−
Fm. acidiphilum	OH	+	+	−	−
At. thiooxidans	OA	−	−	+	−
Thiomonas spp.	FA	+	−	+	−
Acidiphilium spp.	OH	−	+	+	−
A. acidophilum	FA	−	+	+	−
Acidocella spp.	OH	−	+	−	−
Acidobacterium spp.	OH	−	+	−	−
Fp. acidiphilum	OH	+	+	−	−
(b) Moderate thermophiles (temperature optima 40–60 °C)					
L. ferriphilum	OA	+	−	−	−
Sulfobacillus spp.	FA	+	+	+	−
Alicyclobacillus spp.[a]	OH/FA	+/−	+/−	+/−	−
Am. ferrooxidans	FA	+	+	−	−
Fx. thermotolerans	OH	+	+	−	−
Acd. organivorans	OH	−	−	+	−
At. caldus	OA	−	−	+	−
Thermoplasma spp.	OH	−	−	−	+
Picrophilus spp.	OH	−	−	−	−
(c) Extreme thermophiles (temperature optima >60 °C)					
H. acidophilum	OA	−	−	+	−
S. acidocaldarius	OH	−	−	−	−
S. solfataricus	OH	−	−	−	−
S. metallicus	OA	+	−	+	−
S. tokodaii	OH	+	−	+	−

(*Continued*)

Table 2 (Continued)

	Carbon assimilation	*Fe^{2+} oxidation*	*Fe^{3+} reduction*	*S^0 oxidation*	*S^0 reduction*
Metallosphaera spp.	FA	−	−	+	−
Sulfurococcus spp.	FA	−	−	+	−
A. infernus	OA	−	−	+	+
Ac. ambivalens	OA	−	−	+	+
Ac. brierleyi	FA	+	−	+	+
Sg. azoricus	OA	−	−	−	+
Ss. ohwakuensis	FA	−	−	−	+

[a] *Alicyclobacillus* spp. include species that are facultatively autotrophic and obligately heterotrophic, and vary in terms of their dissimilatory transformations of iron and sulfur.

Note: OA, obligate autotroph; FA, facultative autotroph; OH, obligate heterotroph.

Genera abbreviations: *At., Acidithiobacillus; L., Leptospirillum; Fm., Ferrimicrobium; A., Acidiphilum; Sb., Sulfobacillus; Fp., Ferroplasma; Am., Acidimicrobium; Fx., Ferrithrix; Acd., Acidicaldus; H., Hydrogenobaculum; S., Sulfolobus; Ac., Acidianus; Sg., Stygiolobus; Ss., Sulfurisphaera.*

thermophiles, with temperature optima of 40–60 °C; and (3) extreme thermophiles, with temperature optima of 60–80 °C. While some acidophiles (strains of *At. ferrooxidans* and *Acidiphilium*) have been demonstrated to be active at very low (<5 °C) temperatures, all of these have temperature optima well above 20 °C, and are therefore psychrotolerant rather than psychrophilic microorganisms. At the other end of the temperature spectrum, the most thermophilic extreme acidophile known is the facultatively anaerobic sulfur-metabolizing archaeon *Acidianus infernus*, which has a growth temperature optimum of 90 °C and a maximum of about 96 °C. However, relatively few hyperthermophilic acidophiles are known and the fact that the maximum temperature for growth of an acidophile is about 25 °C lower than that of the most thermophilic life-forms known (neutrophilic *Pyrolobus*-like archaea) is possibly a reflection of the difficulty that living organisms have when challenged by the dual stresses of extreme temperature and acidity. In addition, the pH of high-temperature (>100 °C) abyssal environments around submarine vents is maintained at close to neutral by the strong buffering capacity of seawater, precluding extensive colonization by acidophiles.

As with neutrophilic prokaryotes, extremely thermophilic acidophiles are mostly Archaea while mesophiles are predominantly Bacteria. The majority of moderate thermoacidophiles are also Bacteria, and mostly Gram-positives, while most known Gram-negative acidophilic bacteria grow best at below 40 °C. There are exceptions to this general trend. Indeed, the most thermophilic acidophilic bacteria known – the sulfur-oxidizing autotroph *Hydrogenobaculum acidophilum*, which grows at up to 70 °C, and the heterotroph *Acidicaldus organivorans*, which grows at up to 65 °C – are both Gram-negative.

The ability to tolerate elevated concentrations of protons (strictly speaking, hydronium ions; H_3O^+) is obviously what defines an acidophile. While there is no official cutoff pH value that delineates whether an organism is or is not an acidophile, the generally accepted view is that, as a group, these can be divided into extreme acidophiles that have pH optima for growth at pH < 3, and moderate acidophiles that have pH optima of between 3 and 5. As can be anticipated, the most extremely acidic environments have less potential biodiversity than those that are moderately acidic. The number of prokaryotes that are known to grow at pH < 1 is relatively small and includes some Gram-positive bacteria (e.g., *Sulfobacillus* spp.), Gram-negative bacteria (e.g., *Leptospirillum* spp. and *At. thiooxidans*), and Archaea (e.g., *Ferroplasma* spp.) that oxidize iron and/or sulfur. The most acidophilic of all currently known life-forms is, however, a heterotrophic archaeon, *Picrophilus*. Two species are known, *Picrophilus oshimae* and *Picrophilus torridus*, both of which have optima pH for growth of ~0.7, and grow in synthetic media poised at pH ~ 0. These 'hyper-acidophiles' are also thermophilic, with optimum temperatures for growth at ~60 °C.

Physiological Versatility in Acidophilic Prokaryotes: Specialized and Generalist Microorganisms

Acidophiles as a group are highly versatile and are able to utilize a wide variety of energy sources (solar and inorganic and organic chemicals), grow in the presence or complete absence of oxygen, and at temperatures of between 4 and 96 °C. However, individual species display very different degrees of metabolic versatility. On the one end of this spectrum are members of the genus *Leptospirillum.* Three species are known: *Leptospirillum ferrooxidans, Leptospirillum ferriphilum*, and *Leptospirillum ferrodiazotrophum.* All grow as highly motile curved rods and spirilli, and species and strains vary in temperature and pH characteristics. All three species, however, appear to use only one energy source – ferrous iron. Because of the high redox potential of the ferrous/ferric couple (see 'Aerobic and anaerobic acidophiles'), these Bacteria, by necessity, have to use molecular oxygen as an electron acceptor, restricting them to being active only in aerobic environments. All three species fix carbon dioxide (but not organic carbon) and two of the three (*L. ferrooxidans* and *L. ferrodiazotrophum*) are also able to fix molecular nitrogen. *Leptospirillum* spp. are, therefore, highly specialized acidophiles. Their metabolic limitations appear, however, to be compensated by their abilities to outcompete other iron-oxidizing bacteria in many natural and anthropogenic environments, such as stirred tank bioreactors used to bioleach or biooxidize sulfide ores. This is achieved, at least in part, by their greater affinities for ferrous iron and greater tolerance of ferric iron than most other iron oxidizers.

At. ferrooxidans is, in contrast, a more generalist bacterium. Initially it was described as an obligate aerobe that obtains energy by oxidizing ferrous iron, elemental sulfur, sulfide, and RISCs, and fixes CO_2 as its sole source of carbon. The first hint of a more extensive metabolic potential was in a report by Thomas Brock and John Gustafson in 1976 who showed that the bacterium could couple the oxidation of elemental sulfur to the reduction of ferric iron, though it was not confirmed at the time whether this could support growth of the acidophile in the absence of oxygen, though the free energy of the reaction ($\Delta G = -314\ \mathrm{kJ\ mol^{-1}}$; eqn [6]) suggested that this might be the case.

$$S + 6Fe^{3+} + 4H_2O \rightarrow HSO_4^{-} + 6Fe^{2+} + 7H^{+} \qquad (6)$$

Later, Jack Pronk and colleagues at Delft University showed conclusively that *At. ferrooxidans* is, indeed, a facultative anaerobe and can grow anaerobically by ferric iron respiration using not only sulfur as electron donor, but also formic acid (which can also be used as sole energy source under aerobic conditions). The finding that this acidophile can use formic acid, although somewhat unexpected, does not imply that it is capable of heterotrophic as well as autotrophic growth, as C_1 compounds, such as formate and methanol, are also used by other autotrophic prokaryotes. About the same time, it was discovered that some strains of *At. ferrooxidans* (including the type strain) can use hydrogen as an energy source, but that bacteria cultivated on hydrogen are less acidophilic than when grown on sulfide ores. It was shown later that hydrogen oxidation could also be coupled to ferric iron reduction by some *At. ferrooxidans* isolates.

The most generalist of all acidophiles are, however, *Sulfobacillus* spp. These Gram-positive bacteria can grow as chemolithotrophs, heterotrophs, or mixotrophs in aerobic or anaerobic environments. Although there are no reports of *Sulfobacillus* spp. using hydrogen, they can (unlike *At. ferrooxidans*) use a variety of organic compounds (such as glucose and glycerol) as carbon and energy sources, though their capacities for heterotrophic growth are more limited than *Alicyclobacillus* spp. (related acidophilic Firmicutes, some of which can also oxidize ferrous iron and sulfur).

Acidophilic Eukaryotic Microorganisms

Extremely acidophilic organisms are exclusively microbial. While some angiosperms, such as *Juncus bulbosus* and *Eriophorum angustifolium*, can grow in highly acidic ($pH < 3$) ponds and lakes, their root systems grow in sediments where the pH is usually significantly higher than the water body itself. Many eukaryotic microorganisms that may be found in extremely low pH environments are acid-tolerant rather than truly acidophilic and may grow equally well, or better, in higher pH waters.

All known phototrophic acidophiles are eukaryotic, and both mesophilic and moderately thermophilic species are known. Some photosynthetic acidophiles are also capable of heterotrophic growth in the absence of light, provided that a suitable carbon source is available. Microalgae that can live in highly acidic environments include genera of Chlorophyta, such as *Chlamydomonas acidophila* and *Dunaliella acidophila*; Chrysophyta, such as *Ochromonas* sp.; and Euglenophyta, such as *Euglena mutabilis* (**Figure 4**). Some diatoms, including several *Eunotia* spp., have also been found to colonize extremely acidic waters. A filamentous alga, identified from its morphology as *Zygnema* and confirmed from biomolecular analysis to be *Zygnema circumcarinatum*, has been found in abundance on surface streamer growths in an extremely acidic ($pH \sim 2.7$) metal-rich stream draining a mine adit in southwest Spain. Four species of thermoacidophilic Rhodophyta have been described. Of these, *Galderia* spp. (*Galderia sulfuraria* and *Galderia maxima*) can grow as heterotrophs, while *Cyanidioschyzon merolae* and the original strain of *C. caldarium* are strict autotrophs. One *C. caldarium*-like isolate has been reported to grow in synthetic media poised as low as pH 0.2. *Chlorella*-like microalgae have also been detected in acidic geothermal waters.

Many species of yeasts and fungi can tolerate moderate or even extreme acidity. Truly acidophilic fungi are, however, less common, though these include some remarkable species, such as *Acontium velatum* and *Scytalidium acidophilum*, both of which are copper-tolerant mitosporic fungi that can grow at pH values of below 0.5. Among the most commonly encountered yeasts in metal-rich acidic waters are *Rhodotorula* spp., while some *Cryptococcus* spp. and *Trichosporon dulcitum* are also acidophilic yeasts. Novel acidophilic fungal isolates (proposed name *Acomyces richmondensi*) have been isolated from warm (30–50 °C), extremely acidic (pH 0.8–1.38), and iron/zinc/copper/arsenic-contaminated waters within the Richmond mine at Iron Mountain, California.

Microscopic animal life-forms may also be found in acidic environments. The most biodiverse of these appear to be protozoa (**Figure 4**). Phagotrophic flagellates

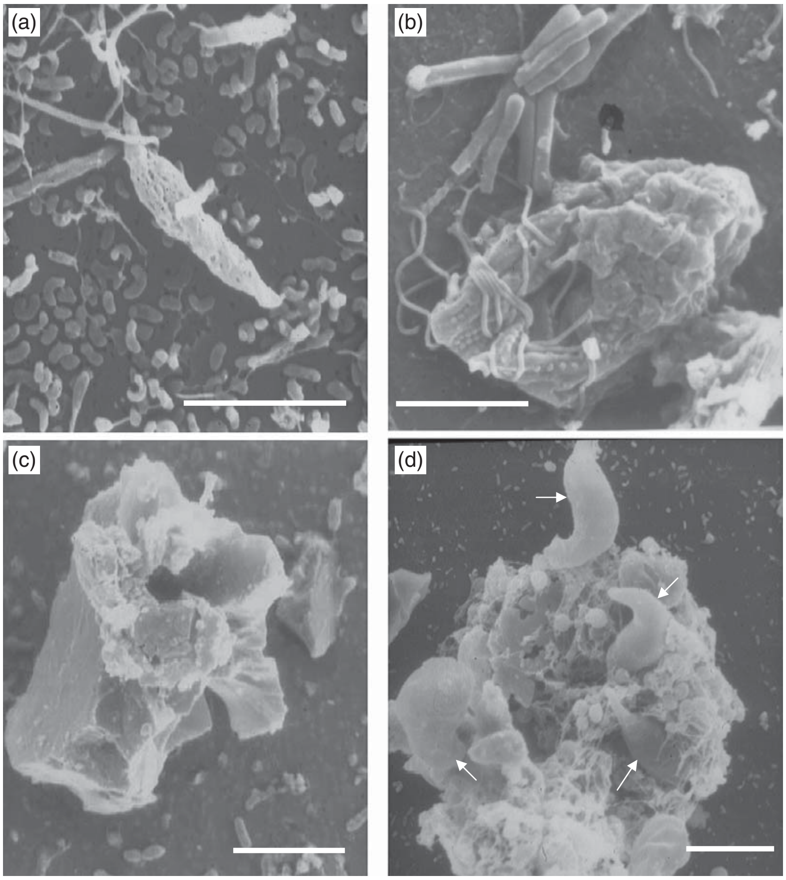

Figure 4 Scanning electron micrographs of eukaryotic acidophiles: (a) a *Eutreptia*-like flagellate protozoan, grazing on *Leptospirillum ferrooxidans*; (b) a *Cinetochilum*-like ciliate protozoan, grazing on *Acidithiobacillus ferrooxidans*; (c) a *Vahlkampfia*-like amoeboid protozoan; (d) a bundle of *Euglena mutabilis* (an acidophilic microalga) with individual cells arrowed. The scale bar represents 5 μm in micrographs (a)–(c), and 10 μm in micrograph (d).

(*Eutreptia*), ciliates (*Urotricha, Vorticella, Oxyticha*, and *Cinetochilum*), and amoeba (*Vahlkampfia*) have all been encountered in acidic mine waters, and some have also been grown in acidic media in the laboratory. Multicellular animal life-forms are relatively uncommon, though rotifers (such as *Cephalodella hoodi* and *Cephalodella gibba*) have occasionally been identified in acidic mine waters. The two most acidophilic species of known rotifers appear to be *Elosa woralii* and *Brachionus sericus*, though the latter can also grow at neutral pH *in vitro*. The pioneering crustacean *Chydorus sphaericus* has also been observed in the pelagic community of acid mine lakes in Germany, though it is acid-tolerant rather than acidophilic, with a pH range of 3.2–10.6.

Interactions Between Acidophilic Microorganisms

The study of microbial ecology involves not only understanding the impact of the environment on microorganisms (and vice versa) but also examining how microorganisms interact with each other. Along with increasing awareness of the biodiversity and complexity of life in extremely acidic environments have come fresh insights into the wide range of microbial interactions that occur within them. In some cases, such as grazing by phagotrophic protozoa on acidophilic bacteria, the interaction may be readily observed, though more often it is more clandestine.

Mutualistic Interactions

Mutualistic interactions are where both partners derive some benefit from their association. One way in which this occurs in extremely acidic environments is via redox transformations and transfer of iron and/or sulfur between prokaryotes. As noted in the section titled ‘Biodiversity of extreme acidophiles’, ferrous iron is an energy source that is widely used by acidophilic Bacteria and some acidophilic Archaea, while ferric iron can act as a highly effective alternative electron acceptor to oxygen in low pH environments. Juxtaposition of aerobic and microaerobic/anaerobic environments can lead to rapid cycling of iron between the two zones. This is aided by the fact that, in contrast to most environments, ferric iron is soluble at pH < 2.5 and is more readily utilized as an electron sink as soluble Fe^{3+} than when present in its various amorphous and crystalline forms. The importance of iron cycling has been illustrated in major acidic environments such as the Rio Tinto, and also demonstrated *in vitro*. Obviously, an extraneous energy source is required for iron cycling to perpetuate. In acidic environments, this may be organic carbon, originating as exudates and lysates from primary producers (phototrophs and chemolithotrophs) that act as electron donors for iron-reducing acidophiles. Cycling of iron may involve more than one species (e.g., the iron-oxidizer *At. ferrooxidans* and the iron-reducer *Acidiphilium*) or a single species (e.g., of *Sulfobacillus*). The situation with sulfur transformations is less clear, due in part to the relative paucity in the knowledge of bacterial sulfate/sulfur reduction in low-temperature acidic environments, and the far greater insolubility of some reduced sulfur compounds (metal sulfides and elemental sulfur) than ferric iron at extremely low pH, which limits their free diffusion. Sulfate produced by aerobic sulfur-oxidizing acidophiles (such as *Acidithiobacillus* and *Thiomonas* spp.) can diffuse into underlying sediments and act as a terminal electron acceptor for any acidophilic/acid-tolerant SRB present. These generate sulfide, which, at low pH, is present almost exclusively as gaseous H_2S. The presence in the sediments of soluble metals, such as copper, that form very insoluble sulfides, results in the rapid removal of H_2S, even at very low pH. However, if, as is often the case, the dominant soluble chalcophilic metal present is (ferrous) iron, the lower solubility of the sulfide mineral (FeS) means that it does not form until the pH has risen to ~5. If the sediment pH is <5, at least some of the H_2S can, at least in theory, diffuse into the overlying water and act as an energy source for sulfur-oxidizing acidophiles.

Other examples of mutualistic interaction between acidophilic bacteria involve the metabolism of organic compounds. Autotrophic iron- and sulfur-oxidizing acidophiles fix carbon dioxide to incorporate into cell biomass. Some of this, mostly small molecular weight material, is lost from actively metabolizing cells and has been shown to accumulate in axenic cultures of these prokaryotes grown in the laboratory. Additional organic carbon originates from dead and dying cells as cell lysates. In the environment, much of this organic carbon is metabolized by heterotrophic acidophiles, such as *Acidiphilium* spp., which are adept scavengers. Positive feedback to the autotrophs comes from the fact that many of these are sensitive to small molecular weight organic compounds in general, and aliphatic acids in particular, and the catabolism of these materials by heterotrophic acidophiles therefore reduces or eliminates this potential toxicity hazard. Iron-oxidizing acidophiles vary in their sensitivities to organic materials; *L. ferrooxidans* is, for example, much more sensitive than *At. ferrooxidans*. This is reflected in the far greater mortality rate of the former in spent (substrate-depleted) media, and is the reason why mixed cultures of *L. ferrooxidans* and *Acidiphilium* spp., grown on ferrous iron or pyrite, tend to be far more stable than pure cultures of the iron oxidizer. In practical terms, the inclusion of obligately or facultatively heterotrophic acidophiles in mineral-leaching consortia has been shown to improve metal recovery, and commercial-scale stirred tanks used to bioprocess sulfide ores have invariably been found to include organic carbon-degrading acidophiles as well as those that fix CO_2. The same rationale of using heterotrophic acidophiles to remove potentially toxic organic materials has been used to develop solid media for isolating and cultivating iron- and sulfur-oxidizing acidophiles from environmental and industrial samples.

Synergistic Interactions

Microbial interactions that result in the complimentary activities of both (or all) participants being more efficient in, for example, degrading a substrate, than the individual species working alone, are referred to as synergistic. One such example involves the oxidative dissolution of pyrite by mixed cultures of *L. ferrooxidans* and *At. thiooxidans*. *L. ferrooxidans* is an iron oxidizer that is unable to oxidize sulfur, while *At. thiooxidans* has the opposite abilities. Pyrite, being an acid-insoluble sulfide mineral (see 'Mine-impacted environments'), is oxidized by ferric iron produced by ferrous iron-oxidizing *L. ferrooxidans* in an acid-consuming reaction. The RISCs produced as a result of ferric iron attack on pyrite are oxidized to sulfuric acid by *At. thiooxidans*, thereby generating the extremely low pH conditions under which *L. ferrooxidans* thrives and mineral dissolution is accelerated. Pure cultures of *L. ferrooxidans* are, however, also able to accelerate the oxidative dissolution of pyrite, in contrast to the heterotrophic iron oxidizer *Fm. acidiphilum*, which requires a source of organic material, such as yeast extract, as carbon source. When *Fm. acidiphilum* and *At. thiooxidans* are grown in mixed culture, the interaction involves carbon transfer as well as the modulation of acidity. *Fm. acidiphilum* can obtain sufficient carbon to grow from autotrophic partners, such as *At. thiooxidans*. This facilitates ferric iron generation, producing RISCs from the degrading pyrite that serves as the energy source for *At. thiooxidans* and continued fixation of CO_2 and release of organic carbon (**Figure 5**).

Syntrophic Interactions

In syntrophic relationships, the degradation of a substrate by one species is made thermodynamically possible through the removal of an end product by another species. Neutrophilic SRB have frequently been reported as one of the partners in a syntrophic association. Hydrogen is a common end product of fermentative metabolism and the oxidation of this gas, coupled to sulfate reduction, may

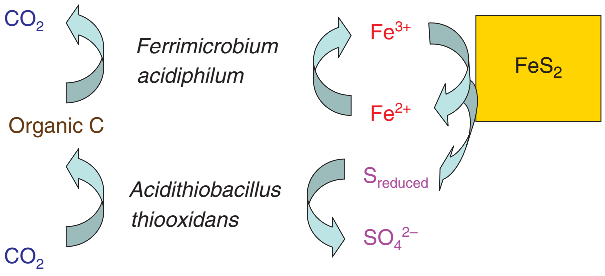

Figure 5 Dissolution of pyrite by a mixed culture of *Ferrimicrobium* (*Fm.*) *acidiphilum* and *Acidithiobacillus* (*At.*) *thiooxidans*. Though *Fm. acidiphilum* can oxidize ferrous iron to ferric iron (which is the chemical that directly attacks the pyrite mineral), it requires organic carbon to grow. *At. thiooxidans* can fix CO_2 and releases some of this as organic carbon, but it cannot access its energy source (reduced sulfur) directly from pyrite. Therefore, neither bacterium can grow in organic carbon-free pure cultures, using pyrite as an energy source, but together they form a successful synergistic consortium, via the interactions shown. Modified from Bacelar-Nicolau P and Johnson DB (1999) Leaching of pyrite by acidophilic heterotrophic iron-oxidizing bacteria in pure and mixed cultures. *Applied and Environmental Microbiology* 65: 585–590, with permission from the publisher.

result in a change in the overall free energy (ΔG) and allow an otherwise thermodynamically unfeasible reaction to proceed. A syntrophic association involving an acid-tolerant *Desulfosporosinus*-like SRB and an acetic acid-degrading *Acidocella* sp. has been proposed to account for sulfidogenesis in moderately acidic (pH 3.2 and above) media. This mixed culture grows anaerobically using glycerol as sole carbon and energy source, a substrate that the SRB can oxidize but the *Acidocella* cannot. In pure cultures of the *Desulfosporosinus*, acetic acid accumulates in equimolar proportion to the amount of glycerol oxidized, but in the presence of *Acidocella* the appearance of acetic acid is transient and more sulfide is produced. It was postulated that acetic acid is degraded to hydrogen and carbon dioxide, a reaction that is only feasible in thermodynamic terms if at least one of these products is rapidly removed. This role was fulfilled by the *Desulfosporosinus* sp., which was shown to use hydrogen as well as glycerol as an electron donor. The energetic bonus for the SRB (hydrogen), which was only available in the mixed culture, resulted in it generating more hydrogen sulfide than when it was grown in pure culture. Additional support for the hypothesis came from the observation that the mixed culture, but not axenic cultures of the *Desulfosporosinus* isolate, could use acetic acid to fuel sulfidogenesis.

Predation

The fact that some acidophilic Bacteria are predated by protozoa and rotifers has been known for many years. One of the first indicators that some protozoa could grow in highly acidic waters was a report in 1941, where it was noted that the flagellate *Polytomella caeca* could grow over a wide pH range (from 1.7 to 9.2). Early studies of acid mine drainage (AMD) frequently reported the presence of flagellates, ciliates, and amoeba, and the first laboratory study was by Henry Ehrlich at the Rensselaer Polytechnic Institute (in 1963) who found that a *Eutreptia*-like flagellate could grow in enrichment cultures prepared using mine water as an inoculum. A detailed study of another *Eutreptia*-like flagellate was described some 30 years later. This protozoan was found to be obligately acidophilic, with a pH range of 1.8–4.5 for growth. Although it was highly sensitive to some heavy metals (e.g., copper, silver, and molybdenum), it could tolerate very high concentrations of both ferrous and ferric iron. The flagellate was found to graze a wide range of acidophilic bacteria, including *At. ferrooxidans, L. ferrooxidans*, and *A. cryptum*. It was noted that the highly motile iron oxidizer *L. ferrooxidans* was less effectively grazed than the less motile acidophile *At. ferrooxidans*, leading to mixed cultures of the two bacteria being dominated by *L. ferrooxidans* when the protozoan was present. Filamentous growth by some acidophiles also appeared to give them some protection from predation by the flagellate. Detailed examination of mixed cultures of acidophilic bacteria and five different acidophilic protozoan isolates (three flagellates, and *Cinetochilum*-like ciliate, and a *Vahlkampfia*-like amoeba) showed that, in each case, population dynamics followed classic predator–prey population dynamics (e.g., **Figure 6**).

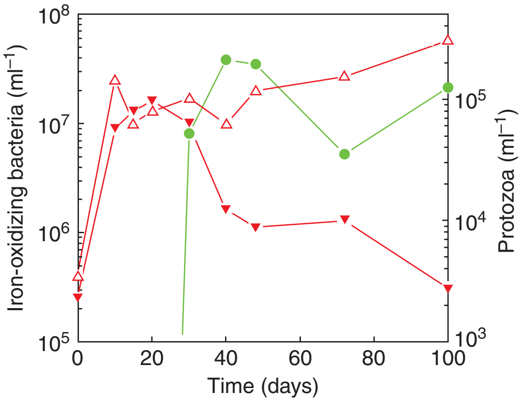

Figure 6 Grazing of acidophilic iron-oxidizing bacteria (a mixed culture of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) by a *Cinetochilum*-like acidophilic ciliate protozoan, showing a classic predator–prey relationship. Numbers of iron-oxidizing bacteria are shown in red: solid symbols show data from a ciliate-containing culture, while hollow symbols show data from a corresponding culture where protozoa were absent. Protozoan numbers are shown in green. Modified from Johnson DB and Rang L (1993) Effects of acidophilic protozoa on populations of metal-mobilizing bacteria during the leaching of pyritic coal. *Journal of General Microbiology* 139: 1417–1423, with permission from the publisher.

Early attempts to cultivate the acidophilic protozoa in media containing pyrite failed, even though large populations of iron-oxidizing and other bacteria were present. This was later shown to be due to the fine size (<61 μm) of the pyrite grains used. When coarser-grain (61–200 μm) pyrite particles were used, all five protozoa were able to grow effectively, suggesting that the phagotrophic protozoa were unable to differentiate between pyrite and bacteria, and that inadvertent ingestion of bacteria-sized pyrite grains resulted in death of the protozoa. Interestingly, dramatic reductions of numbers of iron-oxidizing bacteria due to protozoan grazing did not necessarily result in decreased rates of pyrite dissolution, possibly because of the overriding influence of mineral-oxidizing bacteria attached to the pyrite, which were not grazed.

Evidence for predation of acidophilic bacteria by other microorganisms has come mostly from microscopic observations. Rotifers, for example, have been seen to feed on acid streamer microbial communities (see 'Acidophilic eukaryotic microorganisms') using their wheel-like cilia to draw a vortex of bacterial cells into their mouths.

Competitive Interactions

As might be anticipated, competition between acidophiles for electron donors and acceptors, inorganic nutrients, and so on, is as important in acidic as in all other environments. One of the most detailed studies of this kind, given the importance of iron-oxidizing bacteria in commercial mineral processing and the genesis of AMD, has been the competition between *At. ferrooxidans* and *Leptospirillum* spp. for their communal substrate (electron donor), ferrous iron. Early assumptions that *At. ferrooxidans* was invariably the dominant iron-oxidizing bacterium in metal-rich, acidic environments have gradually been eroded, with increasing numbers of reports describing *L. ferrooxidans* (though in some cases this is probably *L. ferriphilum*) as the more abundant species. These include mine drainage waters and some (mostly stirred tank) commercial biomining operations. In general, Bacteria classified as *At. ferrooxidans* tend to grow more rapidly than *Leptospirillum* spp., and are better able to exploit acidic environments that contain relatively large concentrations of ferrous iron. In terms often used to differentiate heterotrophic bacteria, *At. ferrooxidans* is a copiotroph while *Leptospirillum* spp. are oligotrophs. This is also the reason why using ferrous iron-rich synthetic media to enrich for iron-oxidizing acidophiles favors *At. ferrooxidans* rather than *L. ferrooxidans*. On the other hand, the greater affinity for ferrous iron and the greater tolerance of ferric iron of *L. ferrooxidans* (and probably other *Leptospirillum* spp.) facilitates their dominance in stirred tank mineral leachates, where ferric iron concentrations can be many grams per liter and, conversely, in those extremely acidic environments ($pH < 2.3$) where ferrous iron concentrations are very small. In both situations, redox potentials (which are determined by the relative concentrations, rather than actual concentrations, of ferrous and ferric iron) are commonly above 750 mV, and *Leptospirillum* spp. are known to be far more efficient iron oxidizers than *At. ferrooxidans* under such highly oxidizing conditions. Other important factors that affect competition between these iron-oxidizing autotrophs are temperature and pH. *Leptospirillum* spp. in general (and *L. ferriphilum* in particular) tend to be more thermotolerant than *At. ferrooxidans*, which partly explains their greater importance within the warm interior of the Richmond mine at Iron Mountain (see 'Acid mine streams and lakes') and in stirred tanks used to bioprocess gold and cobaltiferous ores, which generally operate at around 40 °C. On the other hand, cold-tolerant iron-oxidizing acidophiles have been invariably identified as *At. ferrooxidans*-like. *Leptospirillum* spp. also tend to be more tolerant of extreme acidity (many stains grow at pH 1) than *At. ferrooxidans*, some strains of which do not grow below pH 1.8, though others, including the type strain, can grow at pH 1.5. The higher pH optima for their growth is one of the reasons why *At. ferrooxidans* is often more important in heap leaching of mineral ores, as engineered mineral heaps are generally not so acidic as stirred tanks.

In one of the few studies to describe competition between two other iron-oxidizing acidophiles, dissolution of pyrite at 45 °C by a mixed culture of the thermotolerant facultatively autotrophic bacterium *Acidimicrobium* (*Am.*) *ferrooxidans* and a thermotolerant strain of the obligate autotroph *L. ferriphilum* was examined. Numbers of the two bacteria (estimated using fluorescent *in situ* hybridization; FISH) remained very similar until the pH of the bioreactor was lowered from 1.5 to 1.2, at which point *L. ferriphilum* emerged as the dominant bacterium. However, when the thermotolerant sulfur oxidizer *At. caldus* was also included in the microbial consortium, *Am. ferrooxidans* was more abundant than *L. ferriphilum* at pH 1.5 and 1.2. The reason for this was probably the additional amount of organic carbon available for the heterotrophically inclined *Am. ferrooxidans* originating from the CO_2-fixing *At. caldus* that used the RISCs produced by ferric iron attack of the pyrite in the bioreactor.

Microbial Ecology of Extremely Acidic Environments

Geothermal Areas

Geothermal areas occur is discreet zones in various parts of the world, as noted in the section titled "Nature and origin of extremely acidic environments". Probably the most well-studied land-based geothermal area is Yellowstone National Park, Wyoming, USA. Much of the early pioneering microbiological work in Yellowstone was carried out by Thomas Brock and coworkers in the 1970s and formed the basis of later research on thermophilic microorganisms (including thermoacidophiles). One of the major breakthroughs around the time was the isolation, by James Brierley, of the first thermophilic acidophile (a *Sulfolobus*-like archaeon, though it was not recognized as such at the time) from an acid hot spring in Yellowstone. Two of the more important solfatara fields in Yellowstone are located in and around the Norris Geyser Basin, and at Sylvan Springs, though there are numerous other smaller scale, often ephemeral sites (such as around the Gibbon River) where acidic ponds and streams may be found. Moderately thermophilic and acidophilic phototrophs (*Galderia/Cyanidium*-like rhodophytes) and Gram-positive bacteria (*Sulfobacillus* and *Alicyclobacillus* spp.) have frequently been isolated from these sites. A greater biodiversity was revealed in a study reported in 2003, where strains of what turned out to be novel genera of thermophilic Gram-negative bacteria (*Acidicaldus*), and moderately thermophilic Gram-positive bacteria (*Ferrithrix*) were isolated, as well as novel strains of *Firmicutes* that have not, as yet, been formally classified. Interestingly, many of the moderately thermophilic bacteria isolated from these sites (where the acidity is derived chiefly from the oxidation of sulfur) have been shown to catalyze the oxidation or reduction (and, in some cases, both) of iron. A study of acidic geothermal springs within the Norris Geyser Basin that contained a variety of electron donors that support the growth of chemolithotrophic acidophiles (hydrogen, hydrogen sulfide, arsenic(III), and ferrous iron) revealed complex and changing microbial communities that were determined, at least in part, by changing chemical gradients, which in turn effected major geochemical transformations. It was found that (1) *Hydrogenobaculum* (a hydrogen and sulfur oxidizer) and *Stygiolobus* (a hydrogen-oxidizing anaerobe) were present in the high temperature (~79 °C) source waters; (2) *Hydrogenobaculum* and *Thiomonas* (a ferrous iron, sulfur, and arsenic(III) oxidizer) were present in zones of rapid As(III) oxidation; and (3) *Metallosphaera* (a sulfur oxidizer), *Acidimicrobium* (an iron oxidizer), and *Thiomonas* were present in areas where As(V)-rich ferric iron oxides were being generated.

In a separate study based in the Ragged Hills area of Yellowstone, the effect of increased geothermal activity on soil microbial diversity across a temperature gradient of 35–65 °C was assessed. The pH of the soil samples analyzed ranged from 3.7 to 5.1. It was found that the DNA profiles of the soil bacteria (estimated using denaturing gradient gel electrophoresis; DGGE) in heated soils were less complex than those that had not undergone geothermal heating. The majority of clones obtained belonged to *Acidobacterium*, cultivated species of which are mostly moderate acidophiles, and mesophilic. It was concluded that thermophilic and thermotolerant microbial species are probably widely distributed in soils within Yellowstone, and that localized geothermal activity selects for them. The effects of natural hydrocarbon seeps (composed almost entirely of saturated, branched C_{15} to C_{30}, straight-and branched-chain alkanes) on the microflora of acidic (pH 2.8–3.8) sulfate-rich soils in the Rainbow Springs area of Yellowstone were examined in another study. Over 75% of the clones recovered in 16S rRNA gene libraries were related to known species of heterotrophic acidophiles (*Acidiphilum* and *Acidisphaera*) though clones related to *Acidithiobacillus* spp. were also recovered. An alkane-degrading alphaproteobacterium (distantly related to *Acidicaldus* (*Acd.*) *organivorans*, a Yellowstone isolate that has been shown to grow on phenol and other organic substrates) was isolated and partially characterized.

Other geothermal areas where the distribution of acidophilic microorganisms has been studied include New Zealand, the Caribbean island of Montserrat, and northern California. One site that has been studied in New Zealand was an acidic (pH 2.5) stream water on White Island that, in addition to soluble iron and sulfate, contained significant concentrations (2000–4400 mg l^{-1}) of chloride. Among the clones identified from DNA extracted directly from the acid stream were, unusually, those closely related to a green sulfur bacterium (*Chlorobium vibrioforme*), the marine, purple nonsulfur bacterium *Rhodovulum*, and the heterotrophic bacterium *Ralstonia solanacearum*, while those obtained from enrichment cultures also included a bacterium that was closely related to the Yellowstone isolate, *Acd. organivorans*. Pure cultures of *Acidiphilium* and *C. caldarium* were also obtained from the site. Results of a large-scale survey of geothermal sites (many of which were also extremely acidic) on Montserrat, carried our shortly prior to the major eruption of the Soufriere Hills volcano in 1996, showed that temperatures of pools and streams in the volcanic southern region of the island ranged from 30 to 99 °C, and pH from 1.0 to 7.4. Most of the acidophilic bacteria that were isolated were similar to known strains, though some *Sulfobacillus*-like isolates had novel traits in being able to grow as mesophiles, or at higher maximum growth temperatures (up to 65 °C) than classified species. Clone libraries constructed from DNA extracted from acidic sites with different temperatures indicated the presence of (1) *Acidiphilium*-like bacteria and *At. caldus* (33 °C site); (2) *At. caldus*, and a putative moderately thermophilic sulfate-reducing bacterium of the *Desulfurella* group (48 °C site); (3) novel *Ferroplasma*-like and *Sulfolobus*-like archaea (78 °C site); and (4) an archaeon distantly related to *A. infernus* (98 °C site). Elsewhere, a microbiological survey of high-temperature (82–93.5 °C) acidic (pH 1.2–2.2) hot springs located in the Lassen Volcanic National Park in northern California failed to detect any bacteria, though archaea distantly related to the crenarchaeotes *S. azoricus* and *Sulfolobus solfataricus* (both extremely acidophilic thermophiles), and others more closely related to the moderately acidophilic thermophile *Vulcanisaeta distributa*, were identified in clone libraries.

Acid Mine Streams and Lakes

Waters draining abandoned mines, mine spoils, and tailings deposits are often characterized by low pH and elevated concentrations of soluble metals (particularly iron) and sulfate (**Table 3**). These are generically referred to as AMD waters (or acid rock drainage in North America). Acidity in such waters derives from the presence of soluble aluminum, manganese, and iron (mineral acidity) as well as hydronium ions. Extremely acidic lakes may develop naturally in volcanic area, for example, Lake Kawah Idjen in Indonesia, which has a pH of ~0.7. Acidic mining lakes, in contrast, are relics of opencast mining, where worked-out voids have not been backfilled, and become progressively filled with rising groundwater or river water. Where the surrounding bedrocks are rich in sulfide minerals (normally chiefly pyrite and marcasite) and contain small amounts of carbonates, the oxidative dissolution of the former can lead to the formation of extremely acidic mine lakes. Acid mine lakes are particularly abundant in central Europe, in parts of Germany, Poland, and the Czech Republic. In past times (up to the end of the twentieth century) the extensive reserves of lignite in these areas were extracted by opencast mining on enormous scales, leaving a legacy of a very large number of man-made lakes of varying sizes and chemistries. In the Lusatia district of eastern Germany alone there are an estimated 200 mining lakes of >1 ha that have pH values of <3.

The microbiology of AMD streams has been the subject of a number of reviews in books and journals. Knowledge of how biodiverse these flowing waters can be has expanded considerably since *At. ferrooxidans* was first isolated from an AMD stream draining a bituminous coal mine in the United States in 1947. The most important factors in determining which microbial species are present in AMD appear to be pH, temperature, and concentrations of dissolved metals and other solutes. At the most extreme end of the AMD spectrum, the microbiology of mine waters within the Richmond mine at Iron Mountain, California (which can have negative pH values), has been studied extensively. Within this abandoned mine, pyrite is undergoing oxidative dissolution at a rate that is sufficient to maintain air temperatures of between 30 and 46 °C, and produce mine waters containing ~200 g l^{-1} of dissolved metals. A novel iron-oxidizing archaeon, *Ferroplasma acidarmanus*, was found to be dominant in waters within the mine that had the lowest pH and highest ionic strengths, while *L. ferriphilum* and *L. ferrodiazotrophum* were also associated with exposed pyrite faces. *Sulfobacillus* spp. were more important in some of the warmer (~43 °C) waters. *At. ferrooxidans* was rarely found in sites that were in contact with the ore body, though it was found in greater abundance in the cooler, higher pH waters that were peripheral to the ore body. In contrast, a microbiological survey of much cooler and higher pH mine waters at an abandoned subarctic copper mine in Norway showed that an *At. ferrooxidans*-like isolate (closely related to a psychrotolerant strain found subsequently in a mine in Siberia) was the dominant iron oxidizer present. *L. ferrooxidans* was only detected in enrichment cultures using mine water inocula. The Norwegian AMD waters also contained significant numbers of acidophilic heterotrophs related to some

Table 3 Examples of mine water chemistries (all units are mg l^{-1}, except pH)

	pH	*$[\mathrm{Fe}_{\mathrm{total}}]$*	*$[\mathrm{Fe}^{2+}]$*	*[Al]*	*[Cu]*	*[Zn]*	*$[\mathrm{SO}_4]$*
Coal mines							
Bullhouse (UK)	5.9	61	45	1.2	<1	<1	
Ynysarwed (UK)	6.2	160	140	20			460
Oatlands (UK)	5.5	287		0.97	<0.007	0.05	146
Sverdrupbyen (Norway)	2.7	179		27.5	0.168	1.3	1077
Metal mines							
Mynydd Parys (UK)	2.5	650	650	70	60	40	3100
Roeros (Norway)	3.7	6.7		4.3	11	3.76	
Wheal Jane (UK)	3.6	130	130	50	2	130	350
Cwm Rheidol (UK)	2.6–2.7			104–128	1.2–9.35	577–978	250
Sao Domingos (Portugal)	1.7	31 000	10 000				14 850
Iron Mountain (California)	1.5	2670	2470		293	58	14 000

Source: Data are from Johnson DB (2006) Biohydrometallurgy and the environment: Intimate and important interplay. *Hydrometallurgy* 83:153–166, and Nordstrom DK, Alpers CN, Ptacek CJ, and Blowes DW (2000) Negative pH and extremely acidic minewaters from Iron Mountain, California. *Environmental Science and Technology* 34: 254–258.

species (*Acidiphilium, Acidocella*, and *Acidisphaera*) that had previously been observed in acidic environments, and one (a *Frateuria*-like bacterium) that had not.

The importance of *At. ferrooxidans*-like bacteria in cooler (<20 °C) mine waters of pH 2–3 has also been supported at sites in other parts of the world. For example, biomolecular analysis (from clone libraries) of four AMD sites at the Dexing copper mine in the Jiangxi province of China found differences in the distribution of acidophiles with water pH. In the most acidic site (pH 1.5), *Leptospirillum* spp. (*L. ferrooxidans, L. ferriphilum*, and *L. ferrodiazotrophum*) were the dominant species in the clone library, while in pH 2.0 AMD *L. ferrodiazotrophum* was the single dominant species detected. In slightly higher pH (2.2) AMD, most clones recovered were related to *At. ferrooxidans*, while in the highest pH waters (3.0) most were related to the heterotrophic moderate acidophile *Acidobacterium*. Where mine waters have pH values of above 3, however, there is increasing evidence that moderately acidophilic iron oxidizers assume a more important role than *At. ferrooxidans*. The dominant iron oxidizer in AMD flowing from an underground coal mine in south Wales was found to be a *Thiomonas*-like bacterium, and similar strains (given the novel species designation *Thiomonas arsenivorans*) were isolated from an abandoned tin mine in Cornwall, England, and a disused gold mine (Cheni) in France. Other acidophilic Bacteria isolated from the Cornish site included *Acidobacterium*-like and *Frateuria*-like isolates, and an iron oxidizer related to *Halothiobacillus neopolitanus*. Further evidence of the importance of previously uncultured acidophiles in AMD has come from a study of acidic (pH 2.7–3.4) iron- and arsenic-rich water draining mine tailings at Carnoulès in France. The dominant bacteria found in clone libraries were betaproteobacteria, many of which were related to a *Gallionella*-like sequence previously reported in a chalybeate spa in north Wales. The sole *Gallionella* sp. that has been characterized (*Gallionella ferruginea*) is a neutrophilic iron oxidizer that grows best under microaerophilic conditions, and the circumstantial evidence for the existence of an acidophilic (or acid-tolerant) species of *Gallionella* is intriguing. Researchers also found evidence of SRB distantly related to *Desulfobacterium* in AMD at Carnoulès. SRB may also be found in sediments (and microbial mats) underlying AMD, though the pH in such sediments is frequently much higher than the AMD itself.

Microbiological studies of acid mine lakes in Germany have focused on phototrophic eukaryotes as well as acidophilic bacteria and have also examined how dissimilatory microbial reductive processes may be stimulated in order to ameliorate water acidity and immobilize metals. A survey of 14 acidic lakes in Lusatia (ranging in pH from 2.14 to 3.35, and conductivities from 690 to 4460 $\mu S\,cm^{-1}$) found a positive correlation between the relative numbers of the iron-oxidizing heterotroph *Fm. acidiphilum* and concentrations of aluminum. However, it was concluded that indicator groups of bacteria, rather than single species, were better correlated with different lake chemistries. Addition of organic carbon, nitrogen, and phosphorus to enclosed water columns in a pH 2.6 mine lake was shown to induce changes in both water chemistry and microbiology. Treatment of water resulted in increased microbial diversity, and SRB (*Desulfobacter* spp.) were among the microorganisms detected in the amended water columns.

One other important extremely acidic ecosystem that has been studied extensively is the Rio Tinto, a major river, some 92 km in length, located in southwest Spain (**Figure 3**). The source of the river is the Peña de Hierro (Iron Mountain) in the Iberian Pyrite Belt, and from there it flows though a large and historic area of copper mining (the Riotinto mines), eventually reaching the Atlantic Ocean at Huelva. Interestingly, even above the Riotinto mines, the river is acidic and enriched with metals, but this is very much accentuated as it flows through the (now abandoned) mining district. The river has a mean pH of about 2.2 and its distinctive red coloration derives from its soluble ferric iron content ($\sim 2\,g\,l^{-1}$). Primary production in the river is carried out by both photosynthetic and chemoautotrophic acidophiles. A study of the indigenous prokaryotes showed that >80% were Bacteria, and that Archaea accounted for only a relatively small proportion of cells. A variety of different iron oxidizers (*At. ferrooxidans, Leptospirillum* spp., *Fm. acidiphilum*, and *Fp. acidiphilum*) as well as the iron-reducing heterotroph *Acidiphilium* were identified. A geomicrobiological model involving cyclical oxidation of ferrous iron and reduction of ferric iron has been proposed to account for the remarkable chemical stability of the river ecosystem.

Acid Streamers, Mats, and Slimes

The most obvious and dramatic manifestations of microbial life in extremely acidic environments are macroscopic growths, referred to as acid streamers, slimes, and mats. Streamers may occur in flowing AMD streams inside and outside of abandoned mines; these have distinct filamentous morphologies and each filament may be more than a meter in length. Acid mats are denser in texture and are often found below growths of acid streamers. Acid slimes are thick, macroscopic biofilms that grow on moist surfaces of exposed rock faces. In addition, macroscopic filaments (or pipes) composed of acidophilic microorganisms may attach to, and suspend from, mine roofs and pit props. Where these are small-scale, they have been referred to as snotites, though larger structures have been described as microbial stalactites. Because of the macroscopic nature of acid streamer growths, they were among the first life-forms to be reported in extremely acidic environments (in 1938). Most of the early reports described acid steamers as being composed of bacteria embedded in a gelatinous matrix. The first attempts, in the 1970s, to characterize their component microorganisms used classical microbiological (cultivation-based) techniques. In the main, neutrophilic (chiefly spore-forming) *Bacillus* spp. were isolated from the acid streamers examined, leading to the erroneous conclusion that acid streamers were composed of neutrophilic heterotrophic bacteria that maintained circum-neutral pH within the macroscopic growths. In contrast, other researchers noted that streamers found in an iron/sulfur mine in Japan were able to catalyze the oxidation of both ferrous iron and sulfur, though some subsamples of streamers have very limited capacity to oxidize iron. *At. ferrooxidans*-like bacteria were isolated from these growths, leading the researchers to conclude that acid streamers were a mass of *At. ferrooxidans* embedded in a gelatinous matrix. However, it is only after biomolecular tools have been used to analyze acid streamers and related macroscopic growths that their true nature has been elucidated. This approach, coupled with the major advances made in the past two decades in techniques for isolating and cultivating acidophiles in the laboratory, has shown that streamer communities are both highly complex, and vary from site to site. Superficial similarities in their gross morphologies may mask completely contrasting microbial diversities.

One of the first intensive biomolecular examinations of acid slime (~1 cm thick) and snotite growths was carried out with materials collected from the Richmond mine at Iron Mountain, California. Microscopic examination showed that both growth forms were composed mostly of spirillum-shaped cells embedded in an extracellular polymeric matrix. Phylogenetic analysis based on 16S rRNA genes showed that most of the recovered sequences were novel, but related to known iron-oxidizing acidophiles. The single most dominant sequence recovered from slime growths was a novel strain of *Leptospirillum* (subsequently named *L. ferrodiazotrophum*). *L. ferriphilum*-related clone sequences were also identified. Other iron-oxidizing Bacteria identified were related to the Gram-positive actinobacteria, *Acidimicrobium*, and *Ferrimicrobium*, while sequences that affiliated with deltaproteobacteria (which includes anaerobic sulfate and iron reducers) were also detected, suggesting that microzones of low redox potential existed within the macroscopic growths. Archaeal genes were also amplified, and sequences related to *Fp. acidarmanus* were identified. Other archaeal sequences were, however, only distantly related to known Archaea.

In contrast to the Richmond mine slimes, acid streamer growths in less extremely acidic and cooler sites in north Wales (one an AMD stream at an abandoned copper mine and the other water in a chalybeate spa) were both found to be composed predominantly of betaproteobacteria. At the copper mine site, a single novel bacterium was the dominant prokaryote present, while this and a second betaproteobacterium accounted for 90% of bacteria (determined by the quantitative FISH technique) in the spa water streamers. A modified solid medium was developed to isolate the unknown bacterium from the copper mine streamers, and the betaproteobacterium isolate was shown to be the first representative of a novel genus (proposed name, *Ferrovum*) of iron-oxidizing chemoautotrophic acidophiles. The second (spa water) unknown species was identified as being most closely related to *G. ferruginea* (and a clone obtained from the Carnoulès mine; see 'Acid mine streams and lakes') but was not isolated. Although known species of acidophilic bacteria (*At. ferrooxidans, Acidiphilium, Acidocella, Thiomonas, Ferrimicrobium*, and *Acidobacterium*) were also isolated from the streamers, these were shown to be present in only relatively small numbers.

Another underground site that has been the focus of intense study is an abandoned pyrite mine (Cae Coch) located in northwest Wales. This mine is home to the most extensive and diverse macroscopic acidophile growths yet reported, with an estimated acid streamer biovolume of >100 m^3 alone, in addition to extensive slime biofilms and mats, microbial stalactites, and snotites. Although the temperature in the mine shows very little seasonal fluctuation (8.5 ± 1°C), other physico-chemical factors, including pH, dissolved oxygen, and concentrations of dissolved metals and other solutes, vary from site to site within the mine. This, together with the fact that the underground mine has been largely undisturbed for 90 years, has facilitated the colonization of different niches by different streamer/slime acidophilic communities. Using a combination of biomolecular and cultivation-based techniques, the Cae Coch streamer microflora has been shown to be very different to those of the AMD streams and ponds in which they bathe. All of the macroscopic growths were found to be composed of acidophilic bacteria (though protozoa and rotifers were also found in some locations), and the novel iron oxidizer *Ferrovum* was found to be the most abundant single organism present overall. Many of the Bacteria identified were well-known acidophiles (*At. ferrooxidans, Leptospirillum, Ferrimicrobium, Acidiphilium*, and *Acidocella*) while others (including *Frateuria*- and *Ralstonia*-like bacteria) were not. Included in the latter group was a *Sphingomonas* sp. that was detected by biomolecular methods, and also isolated in pure culture where it was shown to be an obligate acidophile, the first such species of *Sphingomonas* to have this trait. The fact that acid streamer communities can be very different from planktonic communities in the same ecosystem was also shown by a study of long (1.5 m) filamentous biofilms found in the Rio Tinto, Spain. Whereas the dominant microorganisms in the Rio Tinto water column are *Acidithiobacillus, Leptospirillum, Acidiphilium*, and archaea (Thermoplasmatales), the streamer-like growths examined were composed of gamma- and alphaproteobacteria; Gram-positive bacteria and betaproteobacteria were also detected in smaller numbers. As with the Cae Coch streamers, *Sphingomonas*- and *Ralstonia*-like bacteria were identified in clone libraries constructed from Rio Tinto streamers.

A more complex streamer/mat community in another mine site in southwest Spain (Cantareras) has been described recently (**Figure 7**). Both solar and chemical (mostly ferrous iron) energy drives primary production in an adit drainage channel at the site, and consequently acidophilic microalgae and chemoautotrophic iron-oxidizing Bacteria both thrive. The streamer growths that fill the ~100-m-long drain channel show distinct stratification (**Figure 7**). The surface layer is green due to the presence of *Zygnema, Chlamydomonas*, and other phototrophic eukaryotes that both aerate the anoxic mine water and provide organic carbon, which supports the growth of heterotrophic acidophiles. The lower layers are (in sequence) cream-brown, turquoise, and gray-black in color, and are almost exclusively bacterial. In contrast to most other acid streamer communities that have been described, heterotrophic (mostly iron-reducing) acidophiles dominate subsurface layers at Cantareras. This is particularly the case with the bottom mat layer, which is composed almost exclusively of *Acidobacterium*-like bacteria and novel strains of sulfate reducers. Complex biogeochemical cycling of iron (acting as both electron donor and electron acceptor) and sulfur in the Cantareras streamer community helps to sustain the highly diverse population of acidophiles that occurs there (**Figure 8**).

Figure 7 Acidophilic microbial communities in an abandoned copper mine (Cantareras, Spain): (a) acid mine drainage (AMD) channels draining the mine adit, showing deposition of copper salts on the adit walls; (b) stratified acid streamer and mat growths in the main drain channel; (c) scanning electron micrograph of the surface streamer layer, showing filaments of microalgae (*Zygnema*) and aggregates of bacteria (the bar scale represents 20 μm); (d) scanning electron micrograph of lower zone streamers, showing rod-shaped bacteria embedded in dehydrated exopolymeric material (the bar scale represents 5 μm).

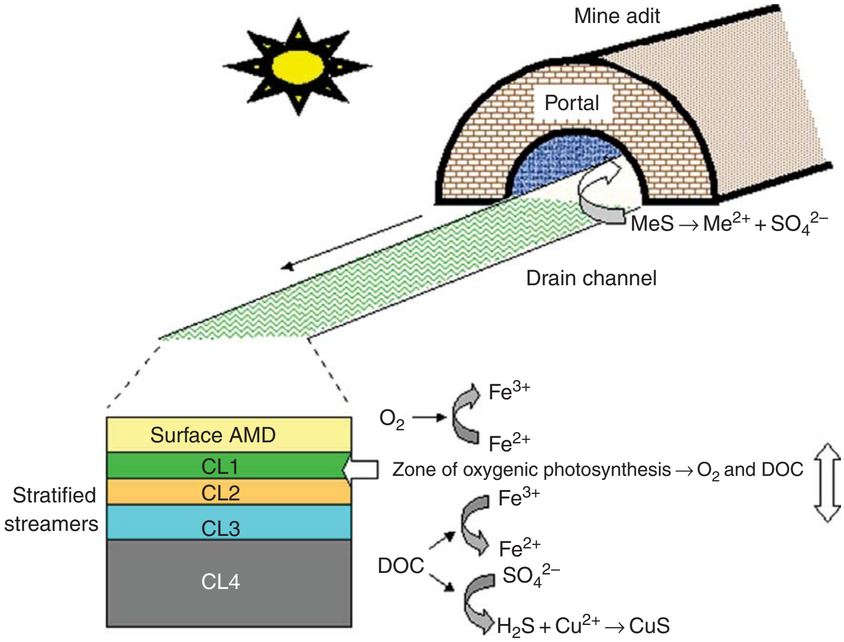

Figure 8 Proposed model of the biogeochemical cycling of iron and sulfur at the abandoned Cantareras mine. Dissolution of sulfide minerals in the exposed mine workings gives rise to a highly acidic, metal- and sulfate-rich effluent. The anoxic water draining the mine is oxygenated by photosynthetic acidophilic algae in the surface (CL1) layer of the acid streamer growths that develop immediately outside of the adit, which facilitates oxidation of ferrous iron in the surface AMD (catalyzed primarily by *Acidithiobacillus ferrooxidans*). Dissolved organic carbon (DOC) originating from photosynthetic and chemosynthetic primary producers serves as substrates for the (dominantly) heterotrophic bacteria in the deeper zone (CL2–4) streamer layers. Ferric iron is used as terminal electron acceptor in streamer layers CL2 and CL3, while in the thick CL4 layer sulfate is also used, resulting in the deposition of copper sulfide (CuS). The gradual buildup of ferric iron concentrations as the AMD flows through the channel results in the elimination of the microalgae, thereby removing the major primary production system that supports the streamer microbial community. Reproduced from Rowe OF, Sánchez-España J, Hallberg KB, and Johnson DB (2007) Microbial communities and geochemical dynamics in an extremely acidic, metal-rich stream at an abandoned sulfide mine (Huelva, Spain) underpinned by two functional primary production systems. *Environmental Microbiology* 9: 1761–1771, with permission from the publishers.

Acidophilic snotite-like biofilms have also been found in at least one other, very different, location. The Frasassi complex, located in central Italy, is a large and actively developing sulfidic cave, hosted in limestone rock. Large concentrations ($\sim 0.3\,\mathrm{m\,mol\,l^{-1}}$) of hydrogen sulfide have been found in groundwater in deep sections of the cave system, and this gas has been shown to support the growth of sulfur-oxidizing acidophiles that grow in biofilms on the cave roof. Although the pH of the cave stream water is 7.0–7.3, droplets of liquid at the tips of the snotites have pH values of between 0 and 1, as a result of microbiological oxidation of sulfide, forming sulfuric acid. A bacterium related to *Halothiobacillus* was isolated from a snotite sample from Frasassi, together with clones related to *Acidithiobacillus* and *Sulfobacillus*. A later study showed that most of the clones obtained (65%) from a snotite sample from Frasassi were related to the mesophilic sulfur-oxidizing acidophile *At. thiooxidans*. The second most phylotype identified (31% of clones) was most closely related to *Am. ferrooxidans*, which is interesting as this is a moderately thermophilic acidophile that can oxidize ferrous iron but not reduced sulfur.

Outlook and Applications

Knowledge of the phylogenetic and physiological diversities of acidophilic microorganisms has expanded greatly in the past 25 years. Data from biomolecular studies of extremely acidic sites, however, suggest that a large number of acidophilic prokaryotes still await isolation and characterization. There is a great deal of interest in acidophiles, not only from the standpoint of understanding how these microorganisms can thrive in conditions that are hostile to most life-forms, but also due to their importance in environmental pollution (mine spoils and mine drainage waters) and in biotechnology (their central role in biomining and in removal of metals from contaminated soils). Significant research effort is currently concerned with finding acidophiles that can also tolerate other environmental extremes, such as temperature (above that of currently known thermoacidophiles) and salinity. There will doubtless be new opportunities to exploit existing and novel acidophilic microorganisms in future biotechnologies that will harness their unique abilities to thrive in conditions that are moderately or extremely acidic and mediate transformations of inorganic as well as organic chemicals.

See also: Autotrophic CO_2 Metabolism; Biofilms, Microbial; Extremophiles: Hot Environments; Heavy Metals, Bacterial Resistance; Iron Metabolism; Metal Extraction and Biomining; Sulfur Cycle

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