A child-centric microbiology education framework

Life in Acid

Daddy: Why is that stream the colour of blood, and what are those things waving around in the flowing water?

D. Barrie Johnson

School of Natural Sciences, Bangor University & Life and Health Sciences, Coventry University, UK

Life in Acid

Storyline

Extremophiles are a group of mostly microorganisms that are able to live in environments that are hostile or toxic to most life forms, including ourselves. Among these are microorganisms that thrive in solutions of sulfuric acid which often contain very elevated concentrations of iron, other metals and sometimes extremely noxious elements such as arsenic. Acidophiles are widely distributed in the "tree of life" and how they have learned to adapt to live and grow at low pH (while at the same time losing the ability to compete in more "normal" environments) has fascinated scientists since their discovery. Because of their unusual abilities, communities of acidophiles can thrive both on the land surface and in deep dark caverns underground, and can form large slimy growths, especially in flowing waters. Biotechnologies have developed that harness the abilities of acidophiles, mostly to extract and recover metals from mineral ores and metallic wastes.

The Microbiology and Societal Context

The microbiology: extremophiles; acidophiles; pH and acidity; metal oxidation; metal tolerance; biomining; bioremediation; *Sustainability issues:* clean water; sustainable industry; sustainable production; marine conservation; land conservation.

Life in Acid: the Microbiology

1. What is acidity? Acidity and alkalinity is usually measured and referred to as "pH", with 7 being neutral, values above 7 being alkaline and those below 7 being acidic. Neutrality is when the concentration of positively charged hydrogen H^+ (protons) is the same as (is balanced by) the concentration of negatively charged hydroxyl (OH^-) ions When there is an imbalance in these, the pH is either lower or higher than pH 7. Degrees of acidity (and alkalinity) can vary greatly, which is disguised to an extent by just referring to pH numbers, as pH is a logarithmic measurement. This means that a microorganism living in a pH 1 environment is challenged with acidity levels that are 1,000-times greater than one living in a pH 4 environment, and 1,000,000 greater than one living in a pH "neutral" site.

2. ***Acidophiles in Context.*** We all encounter acid on a regular basis. The fruit juice we drink at breakfast-time, the wines and beers enjoyed by adults, and foods such as jam, honey and cheese are all acidic. Although the largest habitable environments on our planet (the seas and oceans) are mildly alkaline, many terrestrial areas, such as peatlands, soils and volcanic areas are acidic. In many situations, such as mineral soils, which rarely record pH values of less than 4.2, the natural ability of environments to counter acid production – buffering: the tendency of a system to counteract an increase in acidity or alkalinity - prevents them falling to too low a pH value.

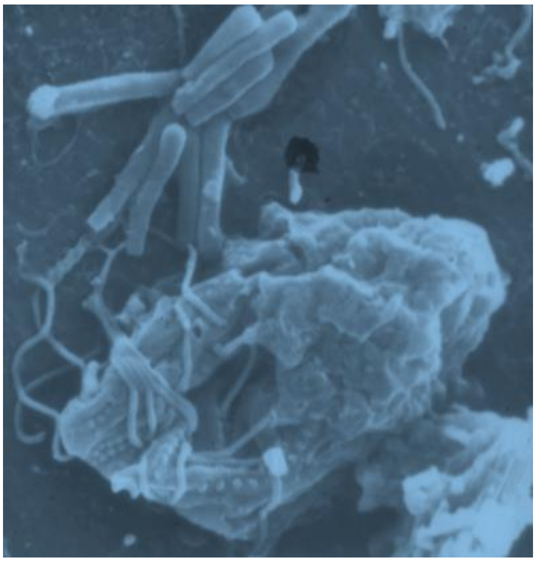
However, in some situations the potential for generating large amounts of acidity is overwhelming, and these environments can have measured pH values of 2 or less and, in some really extreme cases, even negative pH values. In these situations, the low pH is due to the presence of mineral acids, rather than the organic acids found in foods, fruit juices and peatlands, and by far the most important of these is sulfuric acid, which is the sort found in car batteries. For this reason, extremely acidic environments are mostly associated with the presence of relatively large accumulations of sulfur – for example in volcanic and geothermal areas such as

Yellowstone National Park – or of minerals such as pyrite ("fools gold") that are also sulfur-rich. Only two other ingredients are required to generate strong acid from these - oxygen and water, and some specialized microbial life-forms have learned the trick of getting energy from converting sulfur into sulfuric acid and, not only surviving but thriving is the strongly acidic liquids they make.

The Norris geyser area in Yellowstone; a hot acidic environment in a geothermal zone

When there are significant amounts of iron (the most abundant element within planet Earth) also present as, for example, in pyrite and similar minerals, the water bodies in the vicinity can become highly coloured, ranging from light orange to blood red. These streams, ponds and lakes are commonly found in an around metal and coal mine sites and are the most commonly-encountered extremely acidic environments in many European countries and others that have long histories of mining. While these water bodies are incredibly hostile to most life forms, specialized microorganisms, extreme acidophiles, have learned to thrive within them and to take advantage of the sometime unique opportunities they provide.

3. ***Living in extremely acidic environments.*** Most, though not all, life-forms that can thrive in sulfuric acid solutions are unicellular. They include representatives from all three main branches of the "tree of life" (archaea, bacteria and eukarya), and evidence suggests that this ability has been acquired on numerous independent occasions in evolutionary history. The axiom that as an environmental physico-chemical parameter becomes increasingly extreme, the indigenous biodiversity shrinks, holds true with acidic pH niches. A further complexity is that some very acidic sites can also be very warm or hot (e.g. in geothermal and volcanic areas) which further limits biodiversity. Nevertheless, some microorganisms have been shown to be alive and active in very acidic (pH 0 or less) and hot (up to about 80°C) sites in different parts of the world, and it is quite possible, if not probable, that the known current limits of life at low pH will be extended as research in this area continues to expand in the future.



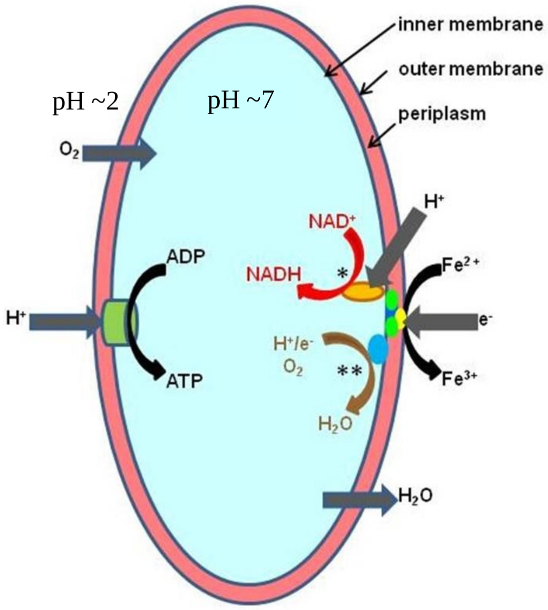
Microorganisms in a low pH environment. The larger object is a ciliate protozoan (a eukaryote) which is grazing (feeding) on the rod-shaped bacteria

4. ***How do microorganisms cope with living in strong acid?*** If you look inside an acidophilic microorganism, you will find that the pH of the vast majority of its interior is close to neutrality. That is: the pH of the inside of the cell is higher than that of the outside. This implies that acidophiles have developed a way of excluding or controlling the influx of positively charged protons (H^+, though strictly speaking these are hydronium ions, H_3O^+) and that their surface structures that are directly exposed to the outside acidic liquors are very resilient and resistant to being degraded by acid. A large group of bacteria (known as Gram-negatives) that are found in low pH environments have a thin space (known as the periplasm) between their outer walls and the membranes that encase their cytoplasm. Intricate measurements have shown that the pH within the periplasm is essentially the same as the outer solutions, and this gives the opportunity for these bacteria to house proteins that are stable and perform highly useful functions at low pH, for example the oxidation of iron, as described below. It is the cell membranes that provide the essential barrier to hydronium ions, and how these differ from membranes of more “normal” bacteria has been an important main focus of study.

5. ***Pros and cons of living at low pH.*** Most living organisms, including ourselves, generate energy by creating a pH gradient across their cell membranes (mitochondrial membranes in eukaryotic cells) and then allowing controlled entry of hydronium ions through specific channels which drives the formation of ATP (the “energy currency” of known life forms). The large pH (proton) gradient (very low pH outside, near neutral pH inside cells) implies that microorganisms that live at low pH have a ready-made energy source, and it is the case that most, if not all, known acidophiles generate ATP using this proton gradient. The obvious problem is that by allowing protons/hydronium ions to enter cells with no concurrent means of neutralizing them or pumping them back out, their cytoplasm would rapidly acidify causing them to die. There is no such thing as a free lunch, even for acidophiles!

6. ***How they pay for the lunch: iron oxidation.*** Microorganisms that grow at low pH have, of course, got around this problem, many of them using a form of a metal that is often much more abundant and bio-available than in neutral pH environments - iron. While iron is the most

abundant metal in the lithosphere, its distinct chemistry means that it has restricted solubility and hence bioavailability in many environments, especially those with pH values around or exceeding neutrality. In contrast, at very low pH both non-metallic forms of iron (known as iron (II) and iron (III) readily dissolve in water and, in contrast to the case in aerated neutral pH environments, iron (II) is chemically stable. A defining characteristic of many acidophilic microorganisms is that they can catalyse the oxidation of iron (II) to iron (III). This releases an electron, which is transported into their cells where it combines with protons (allowed in to generate ATP) and oxygen to form water. A simple reaction, but one which works very effectively at low pH.



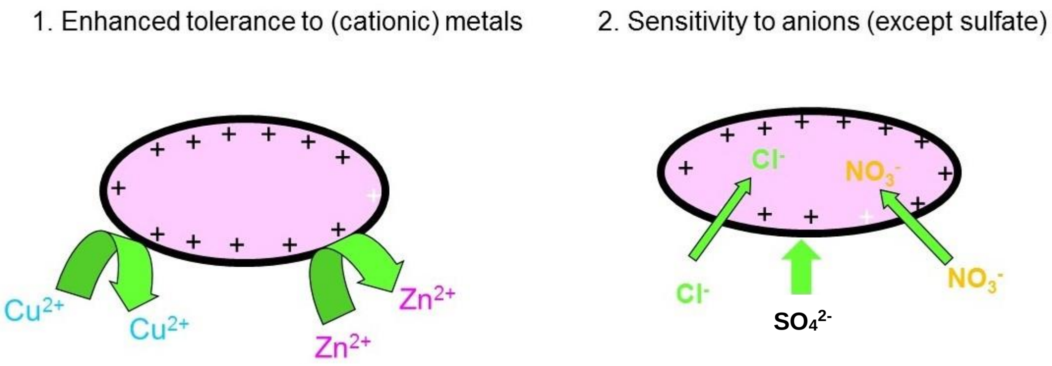
Schematic of an acidophilic bacterium using iron as an "electron donor" to neutralize the positively charged protons channelled in to generate ATP

7. ***The next problem: avoiding becoming rusty.*** One potential problem is that if they were to oxidize iron inside their cells, the iron (III) formed would instantly precipitate as a rust-like deposit, thereby clogging and killing the acidophiles very quickly. For this reason, iron oxidation is mediated in low pH regions, such as the periplasm, where the iron (III) formed remains soluble and diffuses away from the cells, while the electron released is carried inside by transporting proteins. As can be seen from the schematic: H^+ and O_2 in, water out: a very active iron-oxidizing acidophilic bacterium can produce a lot of water inside itself – equivalent to a human body generating and needing to excrete about 1,000 litres every day!

8. ***Acidophiles can use a variety of substrates for energy generation***. The ability to live by exploiting a non-carbon-based "energy source" (strictly speaking, an electron donor) is particularly widespread among acidophiles. Besides iron, sulfur and a variety of sulfur salts are widely used, reflecting the typical abundance of this element in both volcanic and mining environments. Other metals that, like iron, have multiple possible forms – valencies - when they dissolve, can also be used in the same way, or indirectly, with iron acting as an electron shuttle. Some acidophiles can also grow on hydrogen gas. Forms of some metals, such as iron and manganese, can also be used by many acidophiles as replacements for oxygen, allowing them to live and grow in the absence of air, though they may still need carbon dioxide, just like green plants.

9. ***Another advantage of acidophily: resistance to high concentrations of metals.*** One other important consequence of living in strong mineral acid is seemingly more subtle, but has major environmental and technological implications. All microorganisms are differently charged either side of their membranes, and in the vast majority of cases the charge difference is negative. In contrast, bacteria growing in very low pH liquors have net positive trans-membrane charge. This gives them an innate tolerance to positively charged metals in their environment, which is of great advantage since, due to the greatly enhanced solubility of metals such as copper and zinc at low pH, acidophiles encounter metal concentrations that can be orders of magnitude greater than at neutral pH. Now, although all living systems need metals – e.g. we need iron for the haemoglobin in our red blood cells that carry oxygen through our bodies and distribute it to organs like our brains which need a lot when we are thinking – they are also toxic at high concentrations.

10. ***Another problem: intolerance of negatively-charged ions.*** The flipside of acidophile tolerance of high metal concentrations is that they tend to be far less tolerant of negatively-charged ions, including chloride; salt-tolerant acidophiles are relatively rare. The membrane potential charge is, however, a movable feast and becomes less positive and eventually negative as the external pH increases. While this increases their tolerance of chloride etc., it reduces their ability to tolerate copper and other positively charged metal ions.



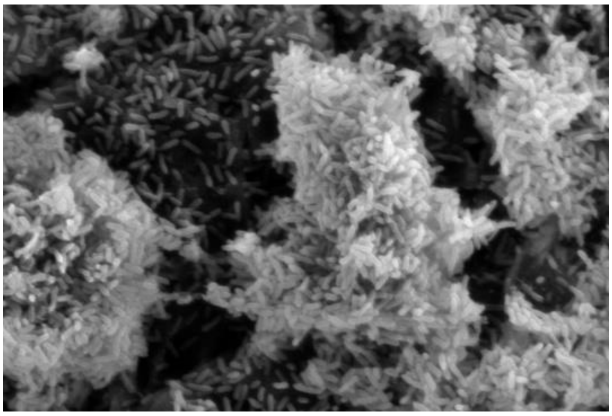
How having a positive charge helps acidophiles tolerate many heavy metals but makes them more sensitive to chloride, nitrate (and other negatively charged ions)

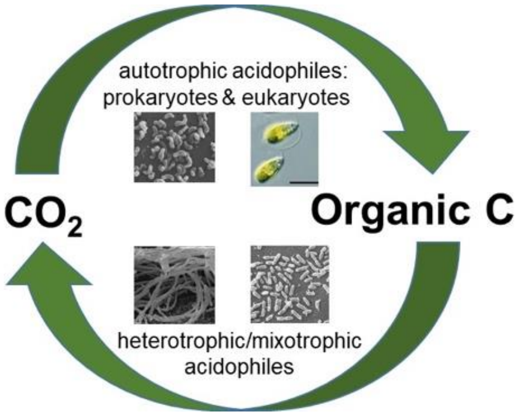
11. ***Macroscopic communities that live at low pH.*** Most life on planet Earth is ultimately sustained by photosynthesis, where most of the energy trapped is used to convert carbon dioxide to organic carbon which underpins the global food web. This is also the case with some low pH sites, though the potential for using energy from inorganic materials including some minerals as well as sulfur to fix carbon, means that large-scale acidophilic communities can also develop deep underground, in caves and abandoned mines. The most dramatic of these are growths of acid streamers, stalactites and slimes that have been found in many different parts of the world. Smaller scale acidophile communities can be found as biofilms on mineral surfaces, where they have direct contact with the minerals they are "feeding" on.

12. ***Interactions between microorganisms that live at low pH.*** It is now known that the range of microbial interactions that are ongoing in low pH sites is similar to those in more "normal" environments. Cross feeding with regard to carbon is particularly critical, since carbon dioxide (CO_2) is very poorly soluble at low pH, making it far less abundant and difficult to access in acidic than in neutral pH waters. The carbon fixers (primary producers: algae, and the bacteria

and archaea that use inorganic forms of energy) leak some of the organic carbon they make when they are alive, and a lot more when they die, which sustains heterotrophic acidophiles. Like ourselves, the latter group converts organic carbon back to carbon dioxide, and some recent work has shown that a proportion of this is fixed again by the primary producers. This is helped by the fact that carbon fixers and degraders often live together in close proximity in biofilms. This recycling of a basic yet precious resource illustrates how intimate and important microbial associations are in low pH environments.

Microbial stalactites and slimes growing deep underground in a mine,
abandoned for over 10 years, in north Wales





Left: a biofilm of acidophilic bacteria growing on a mineral surface;
right, how bacteria cross-feed carbon in these biofilms

Rapid and efficient cycling of metals – most notably iron – also helps sustain life and diversity in these sites. This has been studied in depth in the Rio Tinto, a long (~100 km) river found in the south-west of Spain that remains very acidic blood red in colour from its inland source to the Atlantic Ocean, due to the large concentrations of soluble iron it contains.

The Rio Tinto, a 100 km-long acidic, iron-rich river in the south-west of Spain

13. ***Established and emerging technologies that use acidophiles: biomining.*** The classical extraction of metals from metal-bearing rocks can be highly polluting and energy intensive, and thus not sustainable. Not long after the first discovery of a bacterium that could live by catalysing the oxidation of iron in dilute sulfuric acid, it was realised that this ability could potentially be used to degrade a class of minerals (sulfide minerals) and thereby extract valuable metals that are either part of the mineral (such as copper and zinc) or associated with them (such as gold). The first "biomining" operations started in the USA in the 1960s and has since developed into an important biotechnology for obtaining metals from ores and mineral wastes produced from historic mining operations that still contain appreciable amounts of valuable metals.

These are very large-scale operations, for example the largest tanks used for any "bio" process are currently used to extract gold, using acidophiles. Current developments include using acidophiles to obtain other metals (nickel and cobalt) from a totally different class of mineral ores (oxidised iron ores) and to recover base and precious metals from electronic wastes.

Biomining technologies are often advocated as being more "green" than conventional mining, one reason being that the main bacteria involved fix CO_2 and work at relatively low temperatures, whereas conventional roasting of ores is highly demanding of energy and releases large amounts of CO_2 into the atmosphere. Acidophiles in a single large tank used to biomine gold have been estimated to fix a similar amount of CO_2 each day as 1,000 fully-grown oak trees.

Left: biomining copper in by heap leaching; right: biooxidation of gold ore in stirred tanks

14. ***Established and emerging technologies that use acidophiles: acid mine drainage and its mitigation.*** Other acidophilic bacteria have been used to selectively recover metals from acidic streams associated with abandoned metal mines, thereby helping both to clean up the environment and recycle metals.

Many scientists think that there is still a lot of potential for developing new technologies that harness the unique physiologies and abilities of these amazing microorganisms.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management)*. Classic mining can involve the production of waste heaps that contain reactive minerals and large amounts of metals. Over time, acidophiles produce acidic streams containing toxic metals that pollute both surface and groundwaters, including drinking water supplies: the problem of acid mine drainage. Biomining can help avoid the production of such waste heaps and hence can contribute significantly to SDG 6. Moreover, technologies now exist for the safe extraction of metals from historical polluting acid mine drainage, also contributing to SDG 6.
- **Goal 12. Ensure sustainable consumption and production patterns** *(achieve sustainable management and efficient use of natural resources)*. Classical mining produces mineral wastes that often still contain significant amounts of valuable metals. Biomining can be used to extract these metals and produce a more secure, inert secondary waste, which be able to be used for a totally different purpose, such as in construction. Biomining can therefore be less wasteful; less wastage of natural resources and contribute to sustainable consumption and production protocols.
- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development** *(reduce pollution of marine systems by toxic chemicals)*. Acid mine drainage from coastal regions can pollute marine systems, so prevention of new acid mine drainage and remediation of historical drainage flows will reduce coastal pollution.
- **Goal 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.** Surface mining, the creation of mineral waste stockpiles and the acid mine

drainage that seeps from them, are all industrial activities and the consequences of such activities that degrade terrestrial ecosystems and reduce biodiversity. Biomining and bioremediation using acidophiles can help eliminate future degradation and reduce existing problems.

Pupil Participation

1. ***Exercises***
    a. Which parts of your own body might be acidic? What kind of environment would the acidophiles have there and what might they be doing?
    b. Is there a situation where a part of your body which is normally not acidic becomes acidic? Do you notice it and, if so, how do you change it?
    c. What foods and drinks are acidic? Is it good to eat/drink a lot of them?
    d. If you do eat/drink a lot of them, what can you do to compensate?
    e. How do you think buffering works?

The Evidence Base, Further Reading and Teaching Aids

Johnson, D.B. and Aguilera, A. (2019) Extremophiles and Acidic Environments. In: Schmidt, Thomas M. (ed.) Encyclopedia of Microbiology, 4th Edition. vol. 2, pp. 206-227. UK: Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.90687-3

Johnson, D. B. (2018) The evolution, current status and future prospects for using biotechnologies in the mineral extraction and metal recovery sectors. *Minerals* 8, 343; doi:10.3390/min8080343

Johnson, D. B. (2018) The evolution, current status and future prospects for using biotechnologies in the mineral extraction and metal recovery sectors. *Minerals* 8, 343; doi:10.3390/min8080343

Johnson, D.B. and Schippers, A. (2017) Recent advances in acidophile microbiology: fundamentals and applications. *Frontiers in Microbiology* e-book. doi: 10.3389/978-2-88945-163-0

Quatrini, R. and Johnson, D.B. (eds.) (2016): Acidophiles: Life in Extremely Acidic Caistor Academic Press, Haverhill, UK, 310pp. https://doi.org/10.21775/9781910190333.

Johnson, D.B. and Quatrini, R (2016) Acidophile microbiology in space and time. In: Acidophiles: Life in Extremely Acidic Environments (Quatrini, R. and Johnson, D.B., eds) Caistor Academic Press, Haverhill, UK, pp.3-16

Glossary

ATP – adenosine triphosphate, often considered as the “energy currency” used by all known life-forms
Archaea - a distinct group (domain) of single-celled microorganisms that lack nuclei and other organelles, and often found in extreme environments
Autotroph – a (micro)organism that uses inorganic carbon, such as CO_2, for its growth
Bacteria – a distinct group (domain) of single-celled microorganisms that lack nuclei and other organelles

Biofilm - a group of microorganisms encased in a layer of self-made slime, often attached to a surface

Biomining – a technology that uses acidophilic microorganisms to obtain base and precious metals

Eukarya – a distinct group of single-celled and multicellular life forms, which includes plants and animals

Extreme acidophiles – (micro)organisms that grow optimally at pH 3 or less

Heterotroph - a (micro)organism that uses organic carbon, for its growth

Mixotroph - a (micro)organism that uses either organic or inorganic carbon for its growth

pH – a logarithmic measure of acidity or alkalinity