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Biodiversity and interactions of acidophiles: Key to understanding and optimizing microbial processing of ores and concentrates

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Abstract: Mining companies have become increasingly aware of the potential of microbiological approaches for recovering base and precious metals from low-grade ores, and for remediating acidic, metal-rich wastewaters that drain from both operating and abandoned mine sites. Biological systems offer a number of environmental and (sometimes) economical advantages over conventional approaches, such as pyrometallurgy, though their application is not appropriate in every situation. Mineral processing using micro-organisms has been exploited for extracting gold, copper, uranium and cobalt, and current developments are targeting other base metals. Recently, there has been a great increase in our knowledge and understanding of both the diversity of the microbiology of biomining environments, and of how the microorganisms interact with each other. The results from laboratory experiments which have simulated both stirred tank and heap bioreactor systems have shown that microbial consortia are more robust than pure cultures of mineral-oxidizing acidophiles, and also tend to be more effective at bioleaching and bio-oxidizing ores and concentrates. The paper presented a concise review of the nature and interactions of microbial consortia that are involved in the oxidation of sulfide minerals, and how these might be adapted to meet future challenges in biomining operations.

Key words: acidophiles; biomining; consortia

1 Biodiversity of acidophilic microorganisms and nature of their interactions

A number of review articles have been published in recent years, which have described the physiologies and phylogenies of acidophilic microorganisms[1−4]. The definition of an “extreme acidophile” which has gained general acceptance is that it is a micro-organism that has a pH optimum for growth of pH 3 or less, while “moderate acidophiles” are those with pH optima of between pH 3 and pH 5. “Acid-tolerant” microorganisms, on the other hand, have pH optima of above pH 5, but are still active in low pH environments.

The diversity of both extreme and moderate acidophiles is now known to be far more diverse that was recognized even a couple of decades ago (Table 1). As with other extremophiles, acidophiles tend to be specialized life-forms, and many are unable to grow in neutral pH environments. The majority of these are prokaryotes, and they comprise a large number of phylogenetically-diverse species of bacteria and archaea. However, there are a significant number of both single-celled and multicellular eukaryotes that can grow in highly acidic ponds and streams, including species of algae, fungi, protozoa and rotifers.

As in more “normal” environments, microorganisms that live in extremely acidic environments, including biomining operations, tend to interact with each other[5]. In some cases the net effect of this interaction is negative for at least one of the partners involved, though in many cases one or both partners benefit from the interaction. An example of this, which is particularly important in the context of commercial bioprocessing of minerals, is the oxidative dissolution of pyrite by mixed cultures of *Leptospirillum ferriphilum* and *Acidithiobacillus caldus*. *L. ferriphilum* is an iron-oxidizer that is unable to oxidize sulfur, while *At. caldus* has the opposite abilities. Pyrite is an acid-insoluble sulfide mineral and is oxidized by ferric iron produced by ferrous iron-oxidizing *L. ferriphilum* in an acid-consuming reaction. The reduced inorganic sulfur compounds(RISCs) produced as a result of ferric iron attack on pyrite are oxidized to sulfuric acid by *At. caldus*, thereby generating the extremely low pH conditions under which *L.ferriphilum* thrives and mineral

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Table 1 Examples of phylogenetic diversity of acidophilic microorganisms

Bacteria domain	
Acidobacteria	*Acidobacterium capsulatum*
Actinobacteria	*Acidimicrobium ferrooxidans*
Firmicutes	*Sulfobacillus* spp.
Nitrospira	*Leptospirillum* spp.
Alpha-proteobacteria	*Acidiphilium* spp.
Beta-proteobacteria	*Thiomonas* spp.
Gamma-proteobacteria	*Acidithiobacillus* spp.
Archaea domain	
Euryarchaeota	*Ferroplasma* spp. *Thermoplasma* spp.
Crenarchaeota	*Sulfolobus* spp. *Acidianus* spp. *Metallosphaera* spp.
Eukaryote domain	
Algae	*Euglena* spp. *Chlamydomonas acidophila* *Cyanidium caldarium*
Fungi	*Acontium velatum* *Scytalidium acidophilum*
Protozoa	*Eutreptia* sp. *Urotricha* sp. *Vahlkampfia* sp.
Rotifera	*Elosa woralii*

dissolution is accelerated. Although *L. ferriphilum* (unlike *At. caldus*) is able to catalyze the oxidative dissolution of pyrite when grown in pure culture, acid-production by the sulfur-oxidizer contributes to the greater efficiency of the mixed culture. Interestingly, the greater amount of energy available from oxidizing RISCs compared to ferrous iron results in numbers of *At. caldus* often being much greater than those of the "primary oxidizer", *L. ferriphilum*, in commercial systems. Another important way in which biomining microorganisms interact is by carbon transfer, where the association is mutualistic. Many of the key iron/sulfur-oxidizing bacteria involved in biomining are autotrophs. These fix carbon dioxide and convert it into biomass carbon. Some of this is lost during active metabolism, and a great deal more when cells die and lyse. Where microbial populations are large (as in stirred tanks) this dissolved organic carbon can accumulate to concentrations at which growth of some of the more sensitive autotrophs (notably *Leptospirillum* spp.) is inhibited. Acidophiles that metabolize organic carbon (mixotrophs and heterotrophs) appear to be ubiquitous in biomining systems (where they may also contribute to net oxidation of iron and/or sulfur) and they are though to play an important role in detoxifying leach liquors and maintaining robust bioleaching microbial communities.

2 Biomining: biotechnology based on oxidative dissolution of sulfidic minerals by prokaryotic microorganisms

Microorganisms have significant impact on the extraction and recovery of metals from ores and wastes long before their roles were recognised. Construction of "precipitation ponds" at the Rio Tinto mine (southern Spain) and the Parys mine (Anglesey, north Wales) to recover copper from leached rocks by cementation is documented during the 18th−19th centuries. It was not until the middle of the 20th century that the first bacteria that accelerate the dissolution of metal-containing sulfide minerals at these (and other) sites were discovered. Realisation that the abilities of these microorganisms to oxidize minerals could be harnessed in more precisely engineered operations led to the emergence of a biotechnology, generically referred to as "biomining"[6]. The advantages of bioprocessing of ores and concentrates over more conventional approaches such as pyrometallurgy include the potential for processing low-grade deposits and those that contain significant amounts of arsenic, for re-processing earlier metal-containing wastes, the production of less chemically-active tailings, lower energy inputs and other environmental benefits (zero production of noxious gases etc.).

Bioprocessing of sulfide minerals can be divided into bioleaching, which results in the solubilisation of target metals (e.g. copper from chalcopyrite and covellite) and biooxidation, whereby microbial dissolution of pyrite and arsenopyrite associated with fine-grain gold allows extraction of the precious metal by cyanidation. Besides these two metals, biomining has been harnessed to extract uranium and cobalt, and other metals, including nickel and zinc, will be bioleached from complex polymetallic ores in a heap leaching operation that is currently expanding into full-scale production, in Talvivaara, Finland.

2.1 Mineral bioprocessing: engineering options

These may be divided conveniently into irrigation-based principles (dump- and heap-leaching, and in situ leaching) and stirred tank processes[7]. The earliest engineering technology used ("dump leaching") was very basic, and involved gathering low-grade (otherwise waste) copper-containing ore of large rock/boulder size into vast mounds or dumps and irrigating these with dilute sulfuric acid to encourage the growth and activities of mineral-oxidizing acidophiles, primarily iron-oxidizing mesophiles. Copper was precipitated from the metal-rich streams draining from the dumps using by displacement with scrap iron ("copper cementation").

Later developments on the engineering and hydrometallurgical aspects of biomining have involved the use of thin layer heaps of refractory sulfidic ores (mostly copper, but also gold-bearing material) stacked onto water-proof membranes, and solubilized copper recovered using solvent extraction coupled with electrowinning(SX/EW). In situ bioleaching was developed to scavenge for uranium and copper in otherwise worked out mines. This involves fracturing underground workings using explosives, percolating with acidic leach liquors containing metal-mobilizing bacteria, pumping the pregnant liquor to the surface and extraction of solubilized metals. Since the 1980's, aerated stirred tanks have been used to process sulfidic ore concentrates. These tanks, which may be extremely large (over 1 000 m^3), allow for greater control (e.g. of temperature; sulfide mineral oxidation being an exothermic process) of biooxidation of mineral ores. To date, stirred tank bioreactors used for mineral processing have tended to operate between 40 ℃ and 50 ℃ (i.e. where moderate thermophiles and thermo-tolerant acidophiles would tend to be of greatest significance), though a thermophilic stirred tank, operating at about 80 ℃, has been used successfully to extract copper from chalcopyrite, a mineral that is notoriously difficult to bioleach at low temperatures.

2.2 Microorganisms involved in dissolution of sulfide minerals and extraction of metals

Biomining processes provide a highly specialized growth environment and, irrespective of whether tank or heap processes are used, the microorganisms that catalyze biomining processes are required to grow in an essentially inorganic, aerobic, low pH environment. The most important microorganisms are therefore autotrophic and, although the exact nature of the energy sources may vary from mineral to mineral, they grow by oxidizing reduced forms of sulfur or ferrous iron (or both). The pH within tanks and heaps might also vary, but is highly acidic and typically within the range of pH 1.5−2.0. The characteristics of biomining micro-organisms have been reviewed in detail elsewhere[1] but the extreme conditions in stirred tanks and heaps mean that the number of microorganisms that are likely to play a major role in biomining processes is limited. However, it is important to note that in all pilot-scale and full-scale biomining operations that have been examined, microbial consortia (mixed cultures) rather than axenic (single) cultures have been found.

2.3 Stirred tanks

The environment in a stirred tank mineral-oxidizing bioreactor is highly homogenous as it is operated at a set pH and temperature and controlled aeration. Some operations use single tanks, while others use a series of in-line tanks[7]. Conditions, such as concentrations of soluble metals and metalloids, and often also pH, vary from tank to tank in a continuous flow system as mineral oxidation becomes increasingly extensive, and this can have a significant impact on diversity and numbers of indigenous microbial species[8]. Stirred tanks operate as continuous flow (non-sterile) systems, and the objective is to degrade the in-coming minerals as quickly as possible. The homogeneity within an individual tank in terms of pH, temperature, aeration, dissolved solids results in a limited ecological niche that tends often to be dominated by 2−4 species of acidophiles, although smaller numbers of other microorganisms may be present (Table 1). For example, MIKKELSON et al[9] found that the microbial populations in thermophilic (78 ℃) stirred tanks leaching chalcopyrite were entirely archaeal (as would be predicted from the known thermo-tolerance of acidophilic prokaryotes) and comprised relatively few species of the order Sulfolobales (Table 2).

Table 2 Acidophilic prokaryotes identified in stirred tank mineral bioleaching and biooxidation operations[11]

Mineral concentrate	t/℃	Prokaryotes identified
Zinc/lead pyrite	35−40	*Leptospirillum ferroxidans*[a] *Acidithiobacillus thiooxidans*[b] *Acidiphillum cryptum*[c] *Acidithiobacillus ferrooxidans*[c]
Pyrite/arsenpyrite (gold) Biox® culture	40	*L. ferrooxidans*[a] *At. thiooxidans*[b] *At. Ferrooxidans*
Cobaltiferous pyrite	35	*L. ferrooxidans* *At. thiooxidans* *Sulfobacillus thermosulfidooxidans*
Polymetallic (copper, zinc and iron sulfides)	45	*Leptospirillum ferriphilum* *Acidithiobacillus caldus* *Sulfobacillus* sp. *Ferroplasma acidophilum*
Pyrite, arsenical pyrite and chalcopyrite	45	*At. Caldus* *Sb. Thermosulfidoooxidans* '*Sulfobacillis montserratensis*'
Chalcopyrite	78	(*Sulfolobus shibitae*[d,e]) (*Sulfurisphaera ohwakuensis*[d,e]) *Stygiolobus azoricus*[d] *Metallosphaera* sp.[d] *Acidianus infernus*[d]

a: *L. ferrooxidans* was almost certainly L. ferriphilum as identification methods at the time did not permit the two species to be distinguished from each other; b: *At. thiooxidans* was almost certainly *At. caldus* for the same reason as footnote; c: These two species were found in batch tanks but not in continuous flow tnaks; d: Nearest affiliated cultivated archaea to recovered clones; e: Clones probably represent new species within the order *Sulfolobales.*

2.4 Heap leaching operations

The engineering design of heaps used to leach ores is of critical importance in determining their efficiencies, and greater effort has traditionally gone into this aspect than into their microbiology. Heaps are constructed to pre-determined dimensions using graded ores, irrigated from above with acidic liquors and aerated from below (to provide carbon dioxide required by autotrophic mineral-oxidizing microorganisms, as well as the oxygen to promote iron- and sulfur-oxidation). However, even the most carefully engineered heap reactors are inevitably heterogeneous (both spatially and temporally), in terms of irrigation efficiency, temperature, pH, the presence of anaerobic pockets, redox potential, dissolved solutes, available nutrients, etc. This lack of homogeneity results in a large number of micro-environments compared with the relatively homogenous environment provided by a stirred tank. The variability would be expected to support a much greater diversity of mineral-oxidizing and other microorganisms that colonize different zones and microsites within them, than is the case with stirred tanks. For example, temperatures will be determined by climatic conditions (particularly in the outer layers of a heap), exothermic chemical reactions and heat transfer (conduction, convection, and radiation at the heap surface). The oxidation of sulfide minerals is an exothermic reaction, though heat generation varies between minerals, and is related to their reactivities (e.g. heaps containing appreciable quantities of pyrrhotite ($Fe_{1-x}S$, where x=0−0.2) can become very hot soon after construction). Mineral-oxidizing and other acidophilic prokaryotes often have widely different temperature optima and ranges, and may be conveniently grouped into mesophiles (20−40 ℃; predominantly bacteria) moderate thermophiles (40−60 ℃; bacteria and archaea) and (extreme) thermophiles (60−80 ℃; predominantly archaea). In a heap reactor that experiences fluctuations in temperature, these different groups would be predicted to become more or less dominant, as temperatures increase or decline, assuming that they are present in the first place. Some prokaryotes, notably Sulfobacillus spp. and other Firmicutes, are better adapted to survive adverse conditions, such as excessively high or low temperatures, or water stress (zones and microsites within heaps may experience periodic drying, in contrast to stirred tanks) due to their ability to survive as endospores. It may therefore be predicted that, unlike stirred tanks which are dominated by a relatively small number of different species of prokaryotes, heap reactors contain a much greater biodiversity, and that the dominant species will vary spatially and temporally during the lifetime of a heap. Again in contrast to stirred tanks, heap bioreactors tend to select for acidophiles that attach to the mineral phase, and selection for rapid cell growth is less important.

There have been relatively few studies on the microbiology of heap bioreactors, and most of these have analyzed the liquid phases (pregnant leach solutions (PLS), raffinates etc.) rather than the ore itself. Most studies have been on chalcocite (Cu_2S) heaps, as this copper mineral is particularly amenable to bioleaching. Microbiological data from the limited analyses of heap populations that have been carried out show that a considerable diversity of acidophiles may be present in these reactors.

3 Results from laboratory simulations

Laboratory-scale simulations of both stirred tank and heap bioreactors allow detailed examination and experimentation to be carried out on mineral-oxidizing microbial consortia. The most appropriate simulation of stirred tanks in the laboratory are bioreactors, where factors such as pH and temperature can have the same level of control as full- and pilot-scale stirred tanks, and can also be operated (like stirred tanks) as continuous feed systems, and as single or multiple in-line reactors. The main difference between laboratory and other systems is, of course, in scale; laboratory reactors generally operate with working volumes of 1−2 L, whereas stirred tanks used for mineral processing can be in excess of 1 000 m^3. Shake flask cultures can also be useful where multi-factorial experimental design is required, though pH control and on-line monitoring are usually not possible in this case. In contrast, laboratory simulation of heap leaching usually involves the use of column bioreactors, which may operate as flooded and aerated systems, percolated systems using recycled leachate, or air-lift systems[10]. The capacities of column bioreactors may vary from tens of grams to over a kilogram of mineral ore.

Two contrasting approaches have been suggested and used to determine optimized microbial bioleaching consortia[11]. In the “bottom up” approach, the reference point (or base line) is the rate of mineral oxidation/metal solubilisation by a pure culture of one or more chemolithotrophic iron-oxidizing acidophile (such as a *Leptospirillum* sp., or *At. ferrooxidans*). Mixed cultures containing additional acidophiles with complimentary metabolic abilities, such as being able to oxidize sulfur or to grow heterotrophically, are then compared. The aim is to identify a microbial consortium that not only is highly efficient (in terms of rates) at catalyzing the oxidative dissolution of target minerals, but which is also stable and robust. Information from pilot- and full-scale operations, and detailed knowledge of the physiologies of individual microbial species (e.g. tolerance to metals

that are likely to be present at elevated concentrations when processing a specific mineral concentrate) are important for the "logical design" of consortia using the "bottom up" approach. Using this approach, OKIBE and JOHNSON[12] demonstrated that mixed cultures of moderate thermophiles that contained L. ferriphilum (an autotrophic iron-oxidizer), *At. caldus* (usually classed as an autotrophic sulfur-oxidizer) and *Acidithiobacillus ferrooxidans* (a mixotrophic iron-oxidizer) were far more effective at accelerating pyrite oxidation that were pure cultures of each of these acidophiles or mixed cultures containing any permutations of two of the three species. They also noted that pyrite oxidation by *L. ferriphilum* was suppressed when grown in co-culture with the iron-oxidizing heterotrophic archaeon *Ferroplasma acidiphilum* (strain MT17), but that the inclusion of *At. caldus* to this mixed culture resulted in a highly efficient bioleaching consortium.

The "top down" approach utilizes an inoculum that contains a wide variety of different species and strains of acidophiles, on the basis that the prokaryotes that are most fit for purpose (e.g. for bioleaching a particular mineral concentrate) will establish while those that cannot compete are eliminated. Bacteria and archaea that vary both in the physiologies (e.g. energy and carbon sources; pH and temperature optima; metal and solute tolerance) are recommended for inclusion in the initial inoculum. Using this approach (and over 20 different species of acidophilic prokaryotes) JOHNSON et al[13] showed that different microbial consortia established on different types of mineral concentrates and that, in some cases, these were very different in composition to those previously reported in stirred tank systems (which have so far been mainly used to process pyrite and arsenopyrite-rich concentrates[6]. One unexpected finding was that *Am. ferrooxidans*, a moderate thermophile that has not been detected in full-scale systems, was a member of many of the microbial consortia that established in laboratory cultures. Interestingly, CLEAVER et al[14] isolated a novel *Acidimicrobium* species from a bioreactor operated at 49 ℃ in continuous feed mode (with a nickel concentrate), where it appeared to be the dominant iron-oxidizer present. The apparent absence of A*cidimicrobium* spp. in current commercial-scale stirred tanks might be because it has not been introduced (in the initial inoculum or the feed mineral) or that it better fitted to leaching base metal concentrates and ores than to oxidizing gold concentrates.

There have been a number of reports describing the microbiology of laboratory-scale column bioreactors, designed to mimic mineral heaps[15]. In a study of the microbial dissolution of a polymetallic black schist ore using a "top down" approach[16], it was shown that very different microbial consortia established in shake flasks with fine-grain ore than in column bioreactors containing coarser-grades of ore. The former were dominated by *L. ferriphilum*, with smaller numbers of *At. caldus*, *Am. ferrooxidans* and *Leptospirllum ferrooxidans*, whereas the column bioreactors displayed a far greater biodiversity of acidophiles, and populations showed profound temporal changes, that appeared to be related to changes in the chemistry of the leach liquors. The iron/sulfur-oxidizing bacterium *At. ferrooxidans* dominated the early phase of the ore leaching cycle, but this was replaced after several weeks by a related species (*Acidithiobacillus ferrivorans*). *L. ferriphilum* was detected in significant numbers in the mid-phase of the 40 weeks experiment, but *L. ferrooxidans* was only observed in relatively small numbers. Heterotrophic and mixotrophic acidophiles emerged in greater numbers as ore leaching progressed. In contrast to the results obtained with shake flasks, *At. caldus* was only detected in relatively low number on a few occasions, as *Am. ferrooxidans* not at all. Because of practical difficulties, the ore used in columns (3 kg in each case) was not sterilized. Bacteria that has not been included in the mixed culture used to inoculate the columns were detected in biomolecular analysis of leach liquors and also isolated on selective media. These were identified as mostly novel *Alicyclobacillus* spp. (spore-forming *FIrmicutes*) and, in common with some known species of *Alicyclobacillus*, were able to oxidize ferrous iron. Interestingly, these "indigenous" bacteria were dominant microorganisms detected during the early-mid phase of the leaching cycle, particularly in the case of the coarser grade ore. The results from column bioreactor simulations have suggested: 1) even when temperatures are constant, bioleaching consortia in heaps will be far more diverse than in stirred tank systems; 2) populations in heaps change with time, and these changes may be related to leachate chemistry; 3) inoculating heap bioreactors with a diverse range of mineral-oxidizing and other acidophiles is beneficial; 4) indigenous micro-organisms can have an important role in mobilising metals from sulfide ores, and 5) that particle size has an impact on the composition of the bioleaching consortium.

4 Future challenges and opportunities

The advantages that biological processing have over conventional (pyrometallurgy) and competing technologies (e.g. pressure leaching) have led many people to speculate that biomining will increase significantly both in terms of the range of mineral ores and concentrates processed and the scale of metal produced, in the 21st century. There are requirements,

however, to increase the efficiencies of biomining operations, both in terms of rates (bioprocessing is far slower than conventional and other innovative technologies) and in metal recovery. One of the most challenging mineral to bio-oxidize is chalcopyrite ($CuFeS_2$), the most abundant copper sulfide mineral in the lithosphere. It is known that copper extraction, which is typically about 20%−30%, can be greatly improved by bioleaching at elevated 70−80 ℃) temperatures. This was demonstrated by a pilot plant set up jointly by BHP Billiton and Codelco in Chuquicamata (Chile) that successfully processed 200 t of copper concentrate per day[17]. While using even higher temperatures might be deemed as desirable, the upper temperature range for known bioleaching microorganisms (certain species of archaea) is about 80 ℃. New species of hyper-thermophilic mineral oxidizing archaea could have great biotechnological potential, though other factors of a more physicochemical nature (e.g. the far lower solubility of oxygen and carbon dioxide, both of which are required for aerobic autotrophic archaea) are equally challenging. An alternative to high temperatures for effective leaching of chalcopyrite has been suggested to operate tanks at relatively low redox potentials[18]. While this can be achieved by careful monitoring of dissolved oxygen concentrations, it is known that different species of iron-oxidizing bacteria develop and operate at different redox potentials, even under conditions of oxygen saturation[19−20]. The possibility therefore exists that specifically designed microbial consortia could be used to maintain stirred tank systems at pre-determined redox potentials and thereby improve copper extraction from chalcopyrite.

A major challenge faced by biomining companies, particularly those operating heap bioreactors in arid and semi-arid zones, is the quality of water used for irrigating the heaps. Brackish and saline waters are problematic because biomining bacteria and archaea are, in general, highly sensitive to elevated concentrations of anions, with the exception of sulfate. The need to identify and characterize species (and consortia) of salt-tolerant mineral-oxidizing acidophiles has been recognized as a research priority.

A pertinent question is, even if "ideal" consortia are devised and shown to be highly effective at leaching a particular ore or concentrate under laboratory conditions, would it be possible to maintain such a consortium in a full-scale industrial situation, given the fact that both stirred tanks and heap bioreactors operate as open, non-sterile systems? This might be more feasible in tanks, where pH, temperature etc. can be used to maintain conditions that are non-conducive (or less conducive) for the growth of a microorganism that has been identified as undesirable for bioleaching a particular material, but which is likely to be present on the feed material. For example, the high redox-potential generating bacterium *L. ferriphilum* (which is frequently the dominant iron-oxidizer in stirred tanks operated at 37−45 ℃, but which may be considered less desirable when relatively low redox potentials are required) can be suppressed by maintaining temperatures above 50 ℃. In the case of heap bioreactors, however, the concept of controlling bioleaching microbial consortia would appear untenable. Here it is more important to ensure sufficient biodiversity of mineral-oxidizing and other associated prokaryotes (e.g. carbon-degraders) in the liquors used to inoculate and to irrigate the heaps, which may necessitate modifying inoculation ponds or using a variety of ponds maintained at different pH and/or temperature. Where brackish and saline waters have to be used, then the priority would be to facilitate the growth of salt-tolerant acidophiles in the ponds.

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