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RESEARCH PAPER

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Introduction about Thiobacillus ferrooxidans

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ABSTRACT

Thiobacillus ferrooxidans is recognized as being responsible for the oxidation of iron and inorganic sulfur compounds in areas such a mine tailings and coal deposits where these compounds are abundant Acidithiobacillus is a genus of the Acidithiobacillia in the "Proteobacteria". Like all "Proteobacteria", Acidithiobacillus spp. are Gram-negative. Some members of this genus were classified as Thiobacillus spp., before they were reclassified in 2000. Acidithiobacillus ferrooxidans (basonym Thiobacillus ferrooxidans) can be isolated from iron-sulfur minerals such as pyrite deposits, oxidising iron and sulfur as energy sources to support autotrophic growth and producing ferric iron and sulfuric acid.Acidithiobacillus thiooxidans (basonym Thiobacillus thiooxidans, Thiobacillus concretivorus) oxidises sulfur and produces sulfuric acid; first isolated from the soil, it has also been observed causing biogenic sulfide corrosion of concrete sewer pipes by altering hydrogen sulfide in sewage gas into sulfuric acid. Thiobacillus ferrooxidans *is a gram negative, obligately autotrophic and aerobic Proteobacteria. These bacteria are motile, and possess polar flagella. T.* ferrooxidans *is an acidophile, living in environments with an optimal pH range of 1.5 to 2.5.* T. ferrooxidans *is also thermophilic, preferring temperatures of 45 to 50 degrees Celsius.*

The high temperature tolerance of the bacteria may be due in part to its high GC content of 55 to 65 mole percent. T. ferrooxidans derives energy from oxidation of ferrous iron to ferric iron, and reduced-sulfur compounds to sulfuric acid. Fine sulfur deposits may accumulate in the cell wall of the bacteria.
Key words: **Thiobacillus, Bacteria and Iron.**

INTRODUCTION

The mining industry is a major force in the world economy, occupying a primary position at the start of the resource supply chain, supporting 14.4 % of the world's total economy, while using less than 1 % of the global surface area. Production patterns are driven by consumption, which continues to rise in middle- to high-income countries, and is reaching unprecedented levels in low-income countries, whose appetite for the world's minerals reflects their rapid development. However, extraction and processing are associated with a number of sustainable development challenges, including economic, environmental and social issues. For example, poor waste management practice, one of the most conspicuous features of the global mineral industry, can result in severe and long-term environmental and social consequences. Furthermore, it can also impose costs on mining companies by eroding share value, increasing the risks of temporary or permanent shut down, exposure to compensation, fines and litigation costs, lost future opportunities and increased remediation and monitoring. A way to alleviate the negative consequences of mining is through the application of microbial processes, referred generically as “biomining”. They do not require the high amounts of energy used during roasting or smelting and do not produce sulfur dioxide or other environmentally harmful gaseous emissions. Furthermore, mine tailings and wastes produced from physicochemical processes when exposed to rain and air may be biologically leached, producing unwanted acid and metal pollution. Tailings and wastes from biomining operations are less chemically active, and the biological activity they can support is reduced by at least the extent to which they have already been bioleached. From an economical point of view, biomining has a clear advantage in the extraction and recovery of precious and base metals from low-grade ores, where many metals are not economically recoverable by non-biological methods ores of copper, nickel, cobalt, zinc and uranium). At least 20 % of the copper produced worldwide today comes from bioleaching (Kelly and Wood, 2000).

Bacterial leaching versus abiotic leaching

Simple laboratory experiments can show that chemical reactions catalyzed by bacteria are the essential processes which lead to decay of sulfidic heavy metal minerals and some other minerals and those abiotic reactions play a negligible role. If sulfidic ores are percolated with simple water or diluted salt solutions under aeration in laboratory percolators in parallel sets, one set not sterilized or inoculated with natural acid mine effluent, another set under sterile conditions, it can be seen that disintegration of ore and leaching of metals proceeds in the not sterilized or inoculated percolators very much quicker than in the sterilized ones, the ratio being about 104 or higher (Selman and Joffe 1922).

In such percolator experiments it is observed that almost all the bacteria adhere to the pieces of ore and especially to the surfaces of the sulfidic minerals. Only a small amount of bacteria is floating free in the medium. So the bacteria are in close contact to the almost insoluble substrate which they oxidize to yield energy. This seems to be necessary because we can assume, that solubilization of the minerals by some direct mechanisms requires direct contact.

The rate of dissolution of the metal minerals is essentially limited by the accessible surface of the minerals and can be enhanced by grinding the minerals or the pieces of ore resp. to smaller grains. If the sulfidic minerals are not freely exposed, but are embedded in rock, as is normally the case with heavy metal ores, the rate of leaching is limited above all by the diffusion rates of solutes through fissures. Oxygen, ferric ions and hydrogen ions have to diffuse from the outside of the piece of ore, to the metal minerals inside and, conversely, metal, sulfate and hydrogen (Sand and Bock, 1987).

There are two major microbial mediated processes in biomining. The first is bioleaching, which is a strategy for metal recovery, whose underlying mechanism is the oxidation of metallic and/or sulfuric compounds by either enzymatic or mediated chemical oxidation caused by the catabolism of microorganisms. Depending on the mineral, chemical attack is by a combination of ferric iron and acid (protons), whereas the role of the microorganisms is to generate the ferric iron and acid. The second process is called biooxidation.

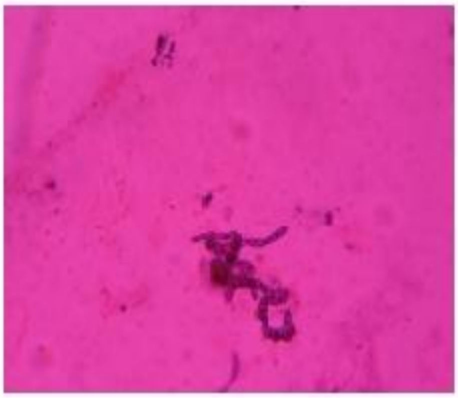
This strategy applies mainly to the recovery of gold from difficult-to-treat arsenopyrites ores and concentrates. The aim is to use biooxidation to decompose the mineral matrix and expose entrapped gold These processes are mediated by a consortium of Gram-negative bacteria (*Acidithiobacillus, Leptospirillum, Sulfobacillus, Acidimicrobium*) and archaeal genus (*Ferroplasma, Sulfolobulus and Metallosphaers*). There are many factors that affect the microbial composition of ores, such as, the type of mineral to be treated, temperature, and type of reactor used. Industrial applications use both mixed populations as well as isolated cultures (Gadd, 2004).

Bioleaching processes are extensively used in copper extraction. Typically, these processes can be summarized in three steps. First, copper ores are pulverized and placed in heaps. Second, In order to promote the microbial consortia metabolism for iron and sulfur compound oxidation, the heaps are sprinkled with sulfuric acid. In this step, the microbial consortium oxidizes Fe (II) to Fe (III). And thirdly, the Fe (III) generated from the microbial metabolism is used to oxidize Cu (I) to the more soluble form Cu (II). One of the most important, and by far the best characterized members of the bioleaching microbial consortia is *Acidithiobacillus ferrooxidans* formerly known as *Thiobacillus ferrooxidans,* it is a gram-negative, highly acidophilic, chemolithoautotrophic γ-proteobacterium. For bioleaching, this organism is particularly important, since it drives Fe (II) oxidation thus allowing the copper solubilization for further recovery by physic-chemical means.

Beyond its biomining capabilities, *A. ferrooxidans* offers exceptional opportunities to study life under extreme conditions. It typically grows at an external pH of 2 or lower using the oxidation of ferrous ions (Fe_2) by oxygen (O_2), producing ferric ions (Fe_3) and water (H_2O), while fixing carbon dioxide (CO_2) from the environment. It can also obtain energy by the oxidation of Reduced Inorganic EPS aids the process by mediating the bacterial adhesion to the sulfide mineral surface, and by concentrating ferric ion in the mineral-microorganism interface by complexation with uronic acids or the EPS residues, allowing the oxidative attack on the sulfur to take place. Due to the lack of well-developed systems for genetic manipulations, the study and exploration of the molecular biology and physiology of *A. ferrooxidans* has proven to be deficient. In terms of the behavior of the complete system, different aspects of metabolism, such as, iron oxidation, CO_2 uptake and fixation, and the anaerobic metabolism of sulfur-coupled iron reduction remain little described. Furthermore, this organism has often proved to be the source of some confusion, because it requires understanding of the consequences of both growing at very acidic external pH and of using a relatively weak reductant (ferrous iron) as the sole source of electrons for respiration Several aspects regarding its energetic metabolism remain weakly described in quantitative terms, such as, how it balances the use of iron as both a micronutrient and as a required energy source and how proton-driven membrane transport and energy processes function in face of a proton motive force across the inner membrane that is several orders of magnitude higher, and how the large pH gradient is maintained across the cytoplasmic membrane of *A. ferrooxidans*.

Table 1. Growth of Acidithiobacillus thiooxidans (Glu + and Thio +) on 1% carbon sources as an energy source at 440 nm optical density after 24 hours.

Carbon source	Strains	Growth at 440 nm optical density					Maximum growth at
		24	48	72	96	120	
Glucose	Glu^{+}	0.001316	0.004997	0.003512	0.002841	0.004193	48h
	$Thio^{+}$	0	0.0034235	0.0072867	0.037241	0.19134	After 120h
Sucrose	Glu^{+}	0	0.001704	0	0.002196	0.23639	Up to 120h
	$Thio^{+}$	0.001713	0.14264	0.18097	0.28433	0.26996	After 72h
Fructose	Glu^{+}	0	0	0	0.000254	0	Up to 72h
	$Thio^{+}$	0.000	0.0008698	0.0014500	0.00316	0.12966	After 72h
Raffinose	Glu^{+}	0.000734	0.000375	0.10697	0.11545	0.12358	After 72h
	$Thio^{+}$	0.14922	0.00687	0.002738	0.004638	0.008871	After 120h
D-sorbitol	Glu^{+}	0.21647	0.02142	0.5661	0.15344	0.12259	Up to 120h
	$Thio^{+}$	0.23211	0.26231	0.2558	0.20217	0.1629	After 48h
Galactose	Glu^{+}	0.19135	0.17611	0.35102	0.38479	0.64757	After 24h
	$Thio^{+}$	0.007190	0.001846	0.11681	0.009810	0.007098	After 96h
Lactose	Glu^{+}	0.0070912	0.0024145	0.005698	0.003151	0.008390	
	$Thio^{+}$	0.18805	0.006727	0.005663	0.005480	0.000	After 24h
Maltose	Glu^{+}	0.0074301	0.0063735	0.085993	0.69364	3.33500	After 120h
	$Thio^{+}$	0.18928	0.0084773	0.18231	0.21245	0.19117	After 72h
Rahammanose	Glu^{+}	0.22787	0.22433	0.33156	0.57322	1.2788	After 48h
	$Thio^{+}$	0.11206	0.0074445	0.0094829	0.009584	0.18174	Different
Mannose	Glu^{+}	0.12652	0.11039	0.74809	0.14488	1.55176	After 48h
	$Thio^{+}$	0.13461	0.0086341	0.14731	0.30430	0.15634	



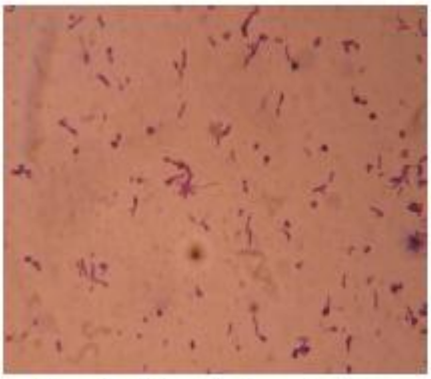


Figure 3. Glu+ bacteria after gram's staining and Thio+ bacteria after gram's staining Genus Acidithiobacillus.

Acidithiobacillus are acidophilic obligate autotrophs (Acidithiobacillus caldus can also grow mixotrophically) that use elementary sulfur, tetrathionate and ferrous iron as electron donors. They assimilate carbon from carbon dioxide using the transaldolase variant of the Calvin-Benson-Bassham cycle. The genus comprises motile, rod-shaped cells that can be isolated from low pH environments including low pH microenvironments on otherwise neutral mineral grains.

Bioleaching

Acidithiobacillus ferrooxidans has emerged as an economically significant bacterium in the field of biohydrometallurgy, in the leaching of sulfide ores since its discovery in 1950 by Colmer, Temple and Hinkle. The discovery of A. ferrooxidans led to the development of "biohydrometallurgy", which deals with all aspects of microbial mediated extraction of metals from minerals or solid wastes and acid mine drainage.[4] A. ferrooxidans has been proven as a potent leaching organism, for dissolution of metals from low-grade sulfide ores. Recently, the attention has been focused upon the treatment of mineral concentrates, as well as complex sulfide ores using batch or continuous-flow reactors.

Acidithiobacillus ferrooxidans is commonly found in acid mine drainage and mine tailings. The oxidation of ferrous iron and reduced sulfur oxyanions, metal sulfides and elementary sulfur results in the production of ferric sulfate in sulfuric acid, this in turn causes the solubilization of metals and other compounds. As a result, A. ferrooxidans may be of interest for bioremediation processes.(Acidithiobacillales entry in LPSN Euzéby, 1997).

Table 2. Characteristics of Thiobacillus ferrooxidans.

Characteristics of Thiobacillus ferrooxidans.(1)

Condition	Characteristic
Optimum growth pH	1.3-4.5
Temperature range	10-37^{0}C
Optimum temperature	30-35^{0}C
Motility	0 to several polar or peritrichous flagella
Mol% G+C	56-59
Gram staining	Gram-negative
Spore formation	none
Shape	rod, 0.5-1 micrometers
Trophy	obligate chemolithoautotroph*
Energy pathway	oxidation of Fe^{2+} and reduced sulfur**
Oxygen requirements	obligate aerobe*
Electron acceptor	oxygen*
Nitrogen source	Ammonium salts, nitrate, fix dinitrogen
Oxygen requirements	obligate aerobe*

Morphology

Acidithiobacillus spp. occurs as single cells or occasionally in pairs or chains, depending on growth conditions. Highly motile species have been described, as well as nonmotile ones.

Motile strains have a single flagellum with the exception of A. albertensis, which has a tuft of polar flagellae and a glycocalyx. Nitrogen fixation also is an important ecological function carried out by some species in this genus, as is growth using molecular hydrogen as a source of energy - neither property are found in every species. Ferric iron can be used by some species as a terminal electron acceptor.

Phylogeny

Main article: Proteobacteria § taxonomy

This genus the other genus in the order Acidithiobacillales (i.e. Thermithiobacillus (Williams and Kelly, 2013) were formerly members of the Gammaproteobacteria, with considerable debate regarding their position and that they could also fall within the Betaproteobacteria, but the situation was resolved by whole-genome alignment studies and both genera have been reclassified to the new class Acidithiobacillia (Kuenen J. Gijs, et al. 1992).

In table 2 T. ferrooxidans is generally assumed to be obligately aerobic, but under anaerobic conditions, T. ferrooxidans can be grown on elemental sulfur using ferric iron as an electron acceptor. These results indicate that T. ferrooxidans can be considered a facultative anaerobe playing an important role in the iron and sulfur cycles in acidic environments. The ability of T. ferrooxidans to grow in oxygen deficient environments may have important implications in bioleaching processes where anaerobic conditions may often exist.
**T. ferrooxidans may also obtain energy from oxidizing Cu+ and Se2- and from the oxidation of tetrathionate, molecular hydrogen, formic acid, antimony compounds, uranium compounds, and molybdenum compounds.

CONCLUSION

Acidithiobacillus ferrooxidans is a gram-negative chemolithoautotrophic γ-proteobacterium. It typically grows at an external pH of 2 using the oxidation of ferrous ions by oxygen, producing ferric ions and water, while fixing carbon dioxide from the environment. A. ferrooxidans is of great interest for biomining and environmental applications, as it can process mineral ores and alleviate the negative environmental consequences derived from the mining processes. In this study, the first genome-scale metabolic reconstruction of A. ferrooxidans ATCC 23270 was generated (iMC507). A total of 587 metabolic and transport/exchange reactions, 507 genes and 573 metabolites organized in over 42 subsystems were incorporated into the model. Based on a new genetic algorithm approach, that integrates flux balance analysis, chemiosmotic theory, and physiological data, the proton translocation stoichiometry for a number of enzymes and maintenance parameters under aerobic chemolithoautotrophic conditions using three different electron donors were estimated. Furthermore, a detailed electron transfer and carbon flux distributions during chemolithoautotrophic growth using ferrous ion, tetrathionate and thiosulfate were determined and reported. Finally, 134 growth-coupled designs were calculated that enables Extracellular Polysaccharide production. iMC507 serves as a knowledgebase for summarizing and categorizing the information currently available for A. ferrooxidans and enables the understanding and engineering of Acidithiobacillus and similar species from a comprehensive model-driven perspective for biomining applications.
Genomic evidence indicates that *A. ferrooxidans* relies on diverse standard iron uptake mechanisms to obtain both Fe (II) and Fe (III) (93). The type strain has candidate genes (AFE2523-AFE2525) potentially encoding the Feo ABC Fe (II) inner-membrane transport

system and an NRAMP dual Mn (II)/Fe(II) MntH-like transporter (AFE0105). Previously reported gene context analysis indicated that *feo A, feo B*, and *feo C* form part of an iron-regulated operon, along with an ORF (AFE2522) encoding a putative porin (designated *feoP*) that could facilitate entrance of Fe (II) into the periplasm.

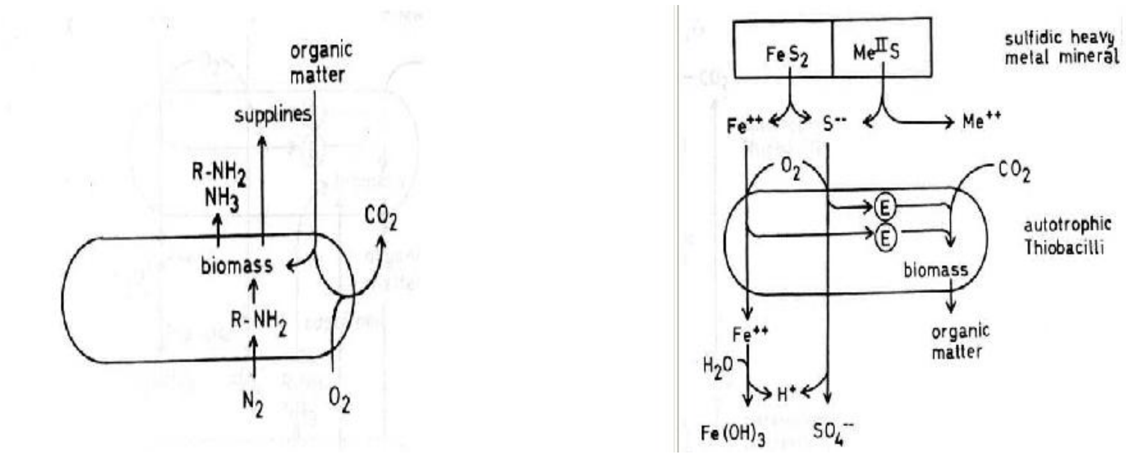


Fig 2. Growth of heterotrophic bacteria at the expense of organic matter, able to reduce molecular nitrogen and bacterial leaching of heavy metals from sulfidicore minerals.

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