# The catalytic role of *Acidithiobacillus ferrooxidans* for metals extraction from mining - metallurgical resource

## Abstract

Mining and metallurgy are the necessary economy branch in the global technological development on which relies a large part of other industries. Mining and ore processing are changing geological conditions, producing a tremendous amounts of waste worldwide, causing negative consequences for the environment and lead to climate change. Negative effect on the environment can be mitigated by treatment of resource from mining and metallurgy with microorganisms. Microorganisms can be used for the leaching of metals from ore, concentrates and waste materials, but also for the bioremediation of acide mine drainage. On that way, it reduces the release of metals into the environment. In this paper is considered the application of Acidithiobacillus ferrooxidans in biological treatment of resource from mining and metallurgy of copper in order to utilization of it for recovery of metals.

**Keywords:** mining, bioleaching, a. ferrooxidans, copper

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## Introduction

Mining and metallurgy have negative consequences for the environment[1–3] and lead to changes in geological[4] and climatic conditions.[5] Mining-metallurgy operations include the extraction, preparing of ores (crushing ore and flotation), processing of concentrates, and the disposal of waste rock and by-products. The main waste in mining and metallurgy are waste rocks, flotation tailings and slag, which often contain significant amounts of valuable metals; sometimes even more than the ore. Because of the increased exposure of surface and quantity of minerals in ores and waste to water and air, the natural dissolution of sulfides is increased in mining regions which leads to formation of acid mine drainage (AMD) rich with metals. The ores, concentrates and waste from copper mining and metallurgy can be subjected to biological treatment in order to utilize this resource for recovery of metals. In addition, the microorganisms can be used for remediation of AMD.

The dissolution of metals from mineral ores by action of microorganisms, and subsequently recovery of metals from solution is known as bioleaching. It is a cost-effective method for recovery of metals from minerals, particularly low-grade ore, and waste from current mining operations; requiring moderate capital investment and low operating costs.[6] There are many publications on development of bioleaching i.e. biohydrometallurgy.[7–13] The advantages of bioleaching of ores and concentrates are compared to conventional metodes, such as pyrometallurgy, reflected in: the potential for processing of low-grade deposits and deposits with significant amounts of arsenic,[14–18] reprocessing waste, lower energy consumption, as well as environmental benefits (e.g., no toxic gases).[19]

In the industrial leaching processes, microorganisms that are found in nature can be used. Dumps of sulphide ores, waste rocks and acid mine water are very complex microbial habitats. Most acidophilic microorganisms can be isolated from these sources.[19–22] The most important role in the development of industrial bacterial leaching is played by the acidophilus bacteria *Acidithiobacillus ferrooxidans*.[12,23,24] In this paper we review the physiology of acidophilic microorganisms, and application areas of bioleaching with *Acidithiobacillus ferrooxidans* in copper mining and metallurgy, as well as the impacts of electrochemistry, type of minerals and addition of catalysts to recovery of metals on the catalytic activity of the bacteria .

## Physiology of acidophilic microorganisms

Many microorganisms can survive extreme physical and geochemical conditions (extreme temperature, pH values, salinity, pressure, drying, radiation, etc). They are extremophiles. Acidophiles are a type of extremophiles which progress in an acid environment with pH < 3,[6,19,25] and many of them cannot grow in neutral pH environments.[19] They grow up in naturally acidic environments (sulfur basins, geysers) and human made acidic environments (coal mining and metal ores). This diverse group of organisms includes archaea, bacteria, fungi, algae, and protozoa.[26]

The *Acidithiobacillus* group of microorganisms is important for dissolution of Cu, Zn, Fe and As, from different ores and waste.[21] They accelerate the dissolution of sulfide minerals, which leads to an increased production acid mine drainage.[3,27] Bacteria possess genes for most of elements known to human society. These genes determine transport systems of nutrient substances, including K, P and Fe, which are necessary to intercellular balance between needs and toxicity, as well as for detoxication or elimination of toxic elements such as Hg, Pb, As, Cr, Cd and Ag.[28]

*Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) is a Gram negative, acidophilic, chemolithoautotrophic bacteria involved in bioleaching and acidic drainage.[29] Its previous name was *Thiobacillus ferrooxidans*, and it was reclassified in 2000.[30] This microorganism plays a key role in the microbial communities involved in bacterial-chemical processes of bioleaching under mesophilic conditions. It uses $Fe^{2+}$, $H_2S$, $S^0$, reduced sulfur inorganic compounds and molecular hydrogen as energy sources.[31] In aerobic conditions, the ferrous iron and/or reduced sulfur compounds present in ores, are oxidized to the ferric iron and sulfuric acid, respectively. Following an initial oxidation of the substrate, the electrons from the ferrous iron and sulfur are included in the respiratory chain and climb over several redox proteins on oxygen. However, the oxidation of ferrous iron and reduced sulfur compounds also must provide electrons for the

reduction of NAD (P), which is later required for many metabolic processes, including $CO_2$ fixation. Oxidation products then chemically attack the metal sulfides, which leads to solubilization of metal ore, and removal of sulfur.[29] The oxidation of sulfur to sulfuric acid produces a strongly acidified environment.[31] When *A. ferrooxidans* grows under anaerobic conditions, it accelerates the dissolution of the ferric iron oxy-hydroxide by oxidation of elemental sulfur, which is a common reduction of iron. This is a key reaction in the "Ferredoks" process for extracting nickel from ore laterite.[32]

Biological copper is generally associated with the three types of ligand: the chains of histidine (His), cysteine (Cys), and methionine (Met), with a few exceptions. Combinations of Met/His or Met/Cys provide organisms with a dynamic, multi-functional domains that may facilitate the transfer of copper at different extracellular, subcellular and tissue specific conditions in terms of pH, redox environment, as well as the presence of other carriers of copper, or target protein.[33] A lot of sulfur is incorporated in proteins, as thiolate or thioether. Sulfur which is bound to metal centres of enzymes give specific properties to Cu enzymes. Sulfur atoms from thiolates, thioethers or rarely disulfide, or inorganic sulfur donors, act as ligands in a variety of copper complexes.[34]

The iron-oxidizing bacteria *A. ferrooxidans* produces a type I copper protein, rusticyanin, which is involved in the oxidation processes of $Fe^{2+}$.[35–37] Rusticyanin is blue colored, as well as the other type I copper protein.[38] It is an acid stable protein, distinct from other types of copper protein I, which are unstable at low pH values.[26] In addition, it characterized by a high redox potential (+680mV),[39,40] and is functional at pH 2 [41]. The copper in this copper protein is bound to one molecule of cysteine and methionine, and two molecules of histidine.[39,42] Cys138 is crucial for bonding of Cu.[42] This protein is the main component in the iron respiratory electron transport chain. It is assumed the next respiratory transport chain of electrons from $Fe^{2+}$ to oxygen:[36,37]

$$Cyc2 \rightarrow rusticyanin \rightarrow Cyc1(c_{552}) \rightarrow cytochrome\ aa_3\ oxidase \tag{1}$$
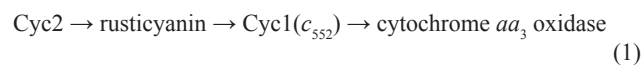
From *A. ferrooxidans,* a Hip iron sulfur protein with a high potential (high-potential iron-sulfur protein, HPIP) was isolated. This is a redox protein with a high redox potential, required to achieve electron transfer reactions at extreme pH values. Structural analysis of the protein showed the presence of two cysteine residues and a high content of proline residues. The high content of proline is essential for stabilizing the folding of proteins at low pH. Unusual is the presence of a disulfide bridge, which fixes the N terminus of the protein.[43] During the contact *A. ferrooxidans* with chalcopyrite, a significant increase occurs in the activity of the enzymes glutathione reductase (GR), superoxide dismutase (SOD) and thioredoxin reductase (TrxR),[44] indicating the formation of reactive oxygen species (ROS).[44,45]

## Chemistry and mechanism of bioleaching

In natural conditions, sulfide minerals chemically oxidize in the presence of oxigen/chemical oxidans and water/wet air (Figure 1). The reactions of the natural chemical oxidation of chalcopyrite and pyrite are shown in Table 1. When the microorganisms are present, they are using $Fe^{+2}$ and $S^0$ for metabolism where upon $Fe^{+3}$ and $SO_4^{-2}$ are formed (Figure 1). Because of the increased concentration of $Fe^{+3}$ and $SO_4^{-2}$ as a result the metabolism of microorganisms, the dissolution of sulfide minerals and the amount of acidic water increase. Thus, the microorganisms play the role of biological catalysts.
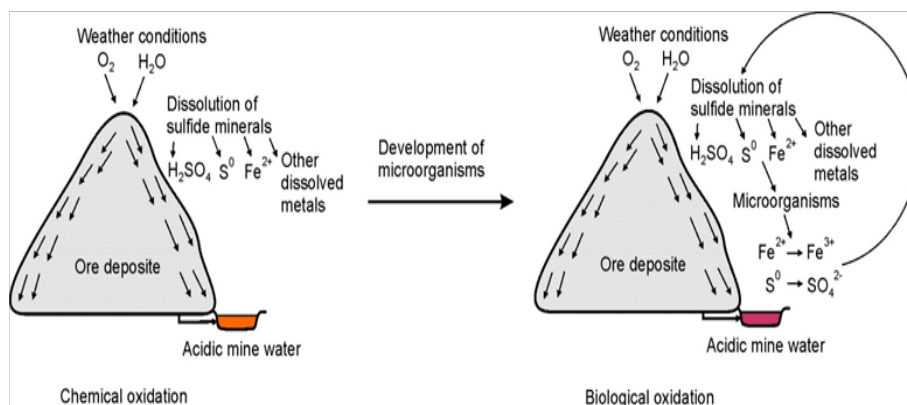


**Figure1** Natural oxidation of sulfide minerals in mining area.

**Table 1** Reactions of chemical oxidation of sulphide minerals

| Sulfide minerals | | | |
|---|---|---|---|
| **Chalcopyrite** | | **Pyrite** | |
| $CuFeS_2 + 2H_2O + 3O_2 \rightarrow Cu^{+2} + Fe^{+2} + 2H_2SO_4$ | (2) | $FeS_2 + 2H_2O + 3O_2 \rightarrow Fe^{+2} + 2H_2SO_4$ | (9) |
| $CuFeS_2 + H_2SO_4 \rightarrow Cu^{+2} + FeSO_4 + 2H^+ + 2S^0$ | (3) | $FeS_2 + H_2SO_4 \rightarrow FeSO_4 + 2H^+ + 2S^0$ | (10) |
| $CuFeS_2 + 4H^+ + O_2 \rightarrow Cu^{+2} + Fe^{+2} + 2H_2O + 2S^0$ | (4) | $FeS_2 + 4H^+ + O_2 \rightarrow Fe^{+2} + 2H_2O + 2S^0$ | (11) |
| $4FeSO_4 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 4H^+$ | (5) | $4FeSO_4 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 4H^+$ | (12) |
| $4Fe^{+2} + O_2 + 4H^+ \rightarrow 4Fe^{+3} + 2H_2O$ | (6) | $4Fe^{+2} + O_2 + 4H^+ \rightarrow 4Fe^{+3} + 2H_2O$ | (13) |
| The resulting $Fe^{+3}$ disolves $CuFeS_2$: | | The resulting $Fe^{+3}$ disolves $FeS_2$: | |
| $CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S^0$ | (7) | $FeS_2 + 2Fe_2(SO_4)_3 \rightarrow 5FeSO_4 + SO_4^{2-} + 2S^0$ | (14) |
| $CuFeS_2 + 4Fe^{+3} \rightarrow Cu^{+2} + 5Fe^{+2} + 2S^0$ | (8) | $FeS_2 + 4Fe^{+3} \rightarrow 5Fe^{+2} + 2S^0$ | (15) |

The resulting primary sulfur product during metal sulfide dissolution, depends on which sulfide mineral is bioleached. The resulting primary sulfur product is subsequently chemically or biologically transformed into elemental sulfur or sulfate.[46] Disulfides $FeS_2$, $MoS_2$ and $WS_2$ are degraded over thiosulfate as the main intermediate. Exclusively iron (III) ions are oxidative solubilizing agents. Thiosulfate degrades to sulfate, with elemental sulfur as a by-product. This explains why only iron (II) ion-oxidizing bacteria are capable of oxidize these metal sulfide.[47]
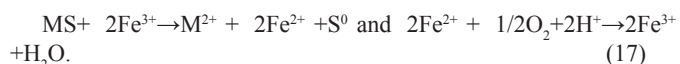
All sulfide minerals, which are soluble in acid to a certain degree, produce elemental sulfur by reacting with the ferric ions, formed from the intermediate polysulfides.[48] Metal sulfides, PbS, ZnS, $CuFeS_2$, $MnS_2$, $As_2S_3$, $As_4S_4$ decompose by the iron (III) ions and proton attack. The main intermediates are polysulfides and elemental sulfur (thiosulfate is only a byproduct in later degradation stages). The dissolution takes place by means of $H_2S^*$- radicals and polysulfides to elemental sulfur. These metal sulfides can be decomposed by any bacteria capable to oxidize the sulfur compounds.[47]

There are two mechanisms that improve the degree of leaching the metals from mineral ores by microorganisms in comparison to the pure physically chemical processes. In the direct action, the microorganism directly oxidized minerals and dissolved metals:[49]

$$MS + H_2SO_4 + 1/2O_2 \rightarrow MSO_4 + S^0 + H_2O \text{ and } S^0 + 11/2O_2 + H_2O \rightarrow H_2SO_4, \quad (16)$$
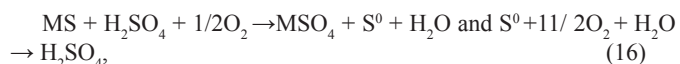
where M is bivalent metal.

In the indirect action of microorganisms, $Fe^{3+}$ is an oxidizing agent for minerals, and the role of organisms is a simple regeneration of $Fe^{3+}$ from $Fe^{2+}$ :[49]

$$MS + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} + S^0 \text{ and } 2Fe^{2+} + 1/2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O. \quad (17)$$

In real microbial leaching, leaching of mineral sulfides is probably a combination of both direct and indirect mechanisms.[50] Fowler & Crudwell,[51] found no evidence that there is a direct mechanism for bacterial leaching.[51] The observations from the scanning slectron microscopy suggested a greater involvement of the indirect oxidation mechanism which utilises the oxidant ferric iron than direct bacterial attachment.[52] Today, some scientists believe that only an indirect mechanism is present.

## Application of acidithiobacillus ferrooxidans in bleaching of metals in mining – metallurgical operations

Industrial bioleaching processes can be: *In situ* bioleaching, bioleaching of dump, bioleaching of heap and bioleaching in reactor (Figure 2). After bioleaching processes, metals are recovery by solvent extraction and electrowinning from the collected pregnant leach solution.

The application *A. ferrooxidans* in bioleaching of metals in mining-metallurgical operations, is possible starting from leaching metals from ores, to leaching of metals from concentrate, till waste (Table 1) Besides that, *A. ferrooxidans* can be used for pretreatment of acid mine drainage,[53,54] as well as for the pretreatment of gold containing ores to the dissolution of sulfides in which the gold is encapsulated[55] and bioassisted phytomining.[56] Because of tolerance to metals[57] and potential absorption of metals,[28] *A. ferrooxidans* can be very effective for bioremediation of heavy metals from polluted environments.[28,58] Biological oxidation of ferrous ions by *A. ferrooxidans* has a positive application and for desulphurization of coal [59,60] and the removal of hydrogen sulfide from gaseous effluents,[61] also.

Bioleaching, as each process involving living creatures, is influenced by the environment, physico-chemical and biological factors, which affect the yield of metal extraction. Once the optimal conditions are maintained, the yield of Cu can be high. The optimal conditions for the growth of microorganisms, such as aeration, humidity, pH, temperature, energy sources and nutrients, as well as the absence of potential inhibitors have to be ensured.[62] Selection of the suitable microorganisms for the leaching is one of the important factors. Various studies have shown that a mixed culture containing the iron and sulphur oxidizing bacteria is more effective than pure culture.[23,63–65] Iron and sulfur-oxidizing cultures are important for efficient degradation of chalcopyrite, probably due to demands for ferric iron as an oxidizing agent; and for the removal of elemental sulfur that could be formed on the mineral surface[64] (Table 2).
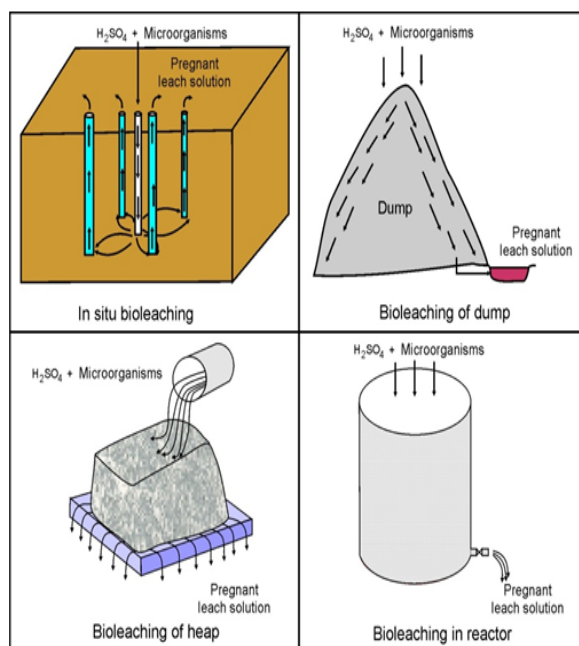


**Figure2** Processes for bioleaching.

**Table 2** Application of *A. ferrooxidans* in bioleaching of metals in mining metallurgical operations

| Type of substrate | Culture | Technological Process | Reference |
|---|---|---|---|
| Waste rocks | mix: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* | collum | 66 |
| Waste rocks | mix: *Acidithiobacillus ferrooxidans*, pure *Acidithiobacillus thiooxidans* mix: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* | collum | 67 |
| Tailing | pure *Acidithiobacillus ferrooxidans* | shake flasks | 68 |
| Tailing | mix: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* | shake flasks | 69 |
| Low grade chalcopyrite ore | pure *Acidithiobacillus ferrooxidans* | percolation columns | 70 |
| Low grade chalcopyrite ore | mixed culture of predominantly of *Acidithiobacillus ferrooxidans* | heap | 71 |
| Low grade chalcopyrite ore | mixed culture of predominantly of *Acidithiobacillus ferrooxidans* | heap | 72 |
| Low grade copper ore | pure *Acidithiobacillus ferrooxidans* | collum | 73 |
| Low grade complex sphalerite | pure *Acidithiobacillus ferrooxidans* | collum | 74 |
| Low grade copper ore | mix: *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* | collum | 75 |
| Pyritic chalcopyrite concentrate | pure *Acidithiobacillus ferrooxidans* mix: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* | shake flasks | 64 |
| Anilite concentrate | pure *Acidithiobacillus ferrooxidans* | shake flasks | 76 |
| Pyrite concentrate Fly lignite ash | pure *Acidithiobacillus ferrooxidans* | shake flasks | 77 |
| Chalcopyrite concentrate | mix: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans, Leptospirillum ferriphilum* | column reactor | 78 |
| Conventer slag | pure *Acidithiobacillus ferrooxidans* | conical flasks | 79 |
| Final slag | mix: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, *Leptospirillum ferrooxidans*, *Sulfobacillus thermotolerans* | stirred tank reactor | 80 |
| Smelter's dust | mix: *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* | stirred tank reactor | 81 |

In addition, the strain which is used should also be taken in to account, because there are variations of the biological characteristics of the bacterium with the same genome.[82] Unadapted mixed cultures of mesophiles are able to tolerate moderate concentrations of different metal ions. By continual growth of the bacteria in an environment that contains an increasing concentration of metals, the bacteria adapts and becomes tolerant to increasing toxic metals and copper.[83,84,85] Treatment of adapted cells *A. ferrooxidans* which are more tolerant of copper than unadapted cells by protein hydrolyzing enzymes leads to a loss of tolerance to copper.[86]

## Application of acidithiobacillus ferrooxidans in leaching of metals from ores

Leaching of metals from ores *in situ* (Figure 2) is classified as solvent mining, and according to some, a preparatory process in mining operations. In this type *in situ* of mining, the effect of microorganisms in these operations and the occurrence of bioleaching are observed. Commercial bioleaching of dumps can be economically justified, since it is considered a cheap technological process. However, the process of bioleaching and metal extraction can be more efficient by constructing specially designed heaps. The planned forming of the heaps creates favorable conditions for optimization of the bioleaching process. By crushing ore and putting it on an impervious surface is more efficient for the distribution of the leaching solution, aeration and collection system.

When the heaps are stacked and irrigated, there is a lag period of the growth and metabolism of the bacteria which contribute to the oxidation of the sulfide. Lag period may be shortened by recirculation of the solution (e.g. raffinate from the solvent extraction) which already contains populations of microorganisms adapted to the conditions of leaching. This ensures that the active bacterial population is distributed throughout the ore. By using the recirculating solution for irrigating the heap with a similar population, the active bacterial population is maintained during the leaching process and it can be used to inoculate the following heap.[6]

Quantity of leached Cu is proportional to the heap height.[70] By increasing the aeration degree, the leaching of Cu is improved.[87] Also, the leaching degree is increased with decreasing dimensions of particles and flow of the leaching agent.[70] Addition of sulphuric acid to maintain pH 2.0, addition of nutrients and a shorter time of leaching agent recycling lead to an increased concentrations of bacteria in the leaching agent and thus improving the efficiency of the leaching.[88]

## Application of acidithiobacillus ferrooxidans in bioleaching of the ore concentrate

Replacement of pyrometallurgy by biohydrometallurgy in the production of metals from centrates is desirable, due to reduced production cost.[11] For commercial bioleaching of the concentrates used projected heaps or reactors. The favorable conditions for bacterial growth can be better controlled in reactors than on heaps.[89] Efficiency of leaching depends on retention time of the leaching solution in the reactor.[78] The supply of oxygen and carbon-dioxide is very important for cells. Carbon-dioxide is needed as a source of carbon, while oxygen is significant as final electron acceptor in the oxidation process. In reactors, these gases are usually obtained by injection of air into fluid.[89] Due to the relatively high costs of supplying the reactor with air, knowing the volume of oxygen consumption of *A. ferrooxidans* during the oxidation of iron in combination with the external parameters, concentration of dissolved metal ions and the pH of the solution, it is possible to estimate the amount of air required for the commercial bacterial leaching process.[82]

## Application of acidithiobacillus ferrooxidans in bioleaching of waste in mining and copper metallurgy

The main waste - waste rocks, flotation tailings and slag- from mining and metallurgy of copper, are often rich in metal content, and sometimes an even richer source of metals than natural ores. Therefore, different possibilities to recover metals from waste[90] have been examined. Bioleaching processes cause significant changes in the mechanical and chemical properties of the waste rocks, and this way they affect stability of dumps and dissolution of metals.

Release of $Cu^{2+}$ from waste rock by action of acid and bacteria is continuous, and erosion of rocks increases with increasing height of dump and leaching time.[67,75] The mineral content of the leached rocks affects the degree of dissolution and precipitation during bioleaching. Porosity of dump and change of size of the rocks are influenced by dissolving, precipitation and clay transport.[66] Grinding of particles under pressure from rocks is another factor that affects the stability of dump.[67,75] From waste rocks,[66,67] flotation tailings[68,69] and slag formed in the pyrometallurgy production of copper,[79, 80, 91] a significant amount of metals can be recovery by bioleaching although they contain arsenic. The results of Bakhtiari et al,[81] indicated that bioleaching is a feasible process for copper recovery from a smelter's dust,[81] also.

## Application of acidithiobacillus ferrooxidans in treatment of acid mine drainage

Flotation tailings and waste rock containing sulfides of heavy metals, which oxidize by weather processes and the action of native sulfur and iron oxidizing bacteria, as well as dissolved metals, contaminate ground and surface water. This leads to the occurrence of acid mine waters - acid mine drainage (AMD), which adversely affect the ecosystem. Therefore, various methods have been developed[92] for the treatment of the acidic, metal-rich mine waters. One of these methods is the biological remediation of acidic mine waters.

*A. ferrooxidans*, which is primarily responsible for the generation of AMD, is used to oxidize ferrous iron in the contaminated flows before the addition of neutralization materials as an alternative for the addition of chemical oxidants or post-neutralization aeration AMD.[53] Oxidation of ferrous iron in to ferric form is possible selectively to precipitate the iron from the solution, because the ferric iron is precipitated at a much lower pH than ferrous iron. Another advantage of this procedure is that after the ferric iron is precipitated (typically at a pH of 2-3), the calcium carbonate can be used for neutralization, which is a much less expensive reagent than calcium hydroxide.[54]

## Electrochemical aspects of the catalytic activity of the bacteria

The understanding of interfacial processes during bacterial leaching and biocorrosion generally is very matters. Good knowledge of electrochemical processes can enables to improve bioleaching[93] and prevention, monitoring and control of biocorrosion.[94] Magnin et al.[95] are created an original process for the production of porous materials by improving the bacterial corrosion of iron in the iron-titanium hot--pressed plates by *A. ferrooxidans*.[95]

In the bioleaching of sulfide, a significant part of the bacteria is usually either temporarily or permanently attached to a solid substrate. These attached bacteria form a biofilm.[96] Extra polymeric substan-

ces (EPS) allow bacteria to attach easily to the metal sulfide,[97] which is useful for bacteria in unfavorable environments.[98] The initial degree of adsorption of *A. ferrooxidans* is improved for the hydrophilic substrate, indicating that the affinity the mineral-to - bacteria is dictated by wetting properties of the substrate.[99,100] The hydrophobic surface repels oxidation ions, reducing the degree of oxidation of the treated pyrite.[101] Electrostatic interactions do not control the attachment of *A. ferrooxidans* to minerals. The number of accessible binding sites is dictated by the chemosensory system of *A. ferrooxidans*, which regulates the chemotaxis response of bacteria to initiate contact of bacteria-to- mineral, and the production of extracellular polymeric substances that mediate the attachment of bacteria.[99] Attachment is facilitated by the extracellular polymeric substances, which render the non-polar surface more polar and allow for water penetration, promoting the attachment of *A. ferrooxidans* cells.[102] The change of surface charge on cells is due to differences in the protein content which is synthesized by the bacteria when exposed to different conditions.[103]

The extracellular polymeric substances of *Acidithiobacillus ferrooxidans* consist mainly of neutral sugars and lipids.[104] Bacterial EPS release $H^+$ and concentrate $Fe^{3+}$. Layers of EPSs with $Fe^{3+}$ are precipitated on the surface of chalcopyrite, and become a barrier for oxygen transport to chalcopyrite; and create an area of high redox potential through the concentration of $Fe^{3+}$ ions.[98] The EPS concentrate ferric ions, probably complexed by glucuronic acid or other residues at the mineral surface.[104] Ions of $Cu^{2+}$ can be more stimulative than $Fe^{3+}$ ions for bacteria production of EPS.[105]

*A. ferrooxidans* can grow by using the $Fe^{3+}/Fe^{2+}$ redox pair either as an electron donor or aceptor, depending on the relative value of the potential of other existing redox pairs in the system. It is interesting to note that in that case, bacteria can increase energy for growth when the electron acceptor and donor are in the periplasmic zone.[46] Strains of *A. ferrooxidans* with a high amount of ferric ions in extra polymeric substances have higher oxidation activity than those with lower ferric ions.[104,106] If the bacterial activity of oxidation of ferric ions is much higher than their consumption by ore, then each further bacterial activity can be harmful for the process of bioleaching.[107] A specific degree of oxidation of ferrous ions is decreased by the increasie of ferric ions: with the increase of ferric ions in *A. ferrooxidans,* the iron-oxidizing enzyme system is competitively inhibited with ferric ions.[108]

The life of microorganisms occurs roughly at -500 mV to +800 mV.[109] The bioleaching conditions typically exhibit a relatively high redox potential around 0.65-0.70 V SHE.[6] Ferric ions and high Eh formed by action of bacterial metabolism inhibit leaching,[107] because at the high solution potential ferric ion readily precipitates as a basic sulfate, like jarosite Eq. (18) in an environment containing monovalent alkali cations and sulfate ions,[6]

$$3Fe^{3+} + 2SO_4^{2-} + 6H_2O + M^+ \rightarrow MFe_3(SO_4)_2(OH)_6 + 6H^+$$

(18)
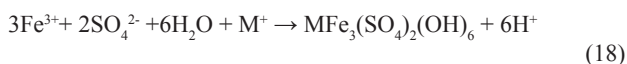
where: $M = K^+$, $Na^+$ or $NH_4^+$.

Precipitation of ferric ions (as jarosite) is responsible for passivation of chalcopyrite.[110,111] Potassium jarosite is the initial product which cover chalcopyrite in the presence *A. ferrooxidans*. The next stage is formation of ammonium jarosite.[112] In addition to the jarosite, the formation of a layer of secondary minerals on mineral surface becomes a diffusion barrier to fluxes of reactants and products. Covellite and elemental sulfur were also detected in passivation layer. Passivation can be reduced by controlling the precipitation of jarosite and by previous adaptation of bacteria.[112] Addition of ferrous ions increases

the leaching degree,[113] and the external addition of high concentrations of ferric ions is unfavorable for the leaching.[114] Accordingly, it is useful to know the significant conditions for precipitation of jarosite and biofilm formation leaching,[114–116] that is to say the limited bacterial activity and control of ferric ions are preferable for effective bioleaching.[107]

## Influence of the type of mineral in leaching process on the catalytic activity of bacteria

The main contribution of *A. ferrooxidans* to the extraction of metals, is its capability to attack sulphide minerals and convert insoluble sulfides of metals like copper, lead, zinc, or nickel to the corresponding soluble sulphates of metals.[117] It is considered that differences in crystal structure of minerals cause different sensibility to oxidation with *A. ferrooxidans*.[118–120] Purified recombinant aporusticianine and intact cells of *A. ferrooxidans* showed an identical pattern of adherence to same minerals. Addition of ferrous ions or organic chelating compounds prevents the binding of any aporusticianine or intact cells to pyrite. Binding of apoprotein to solid pyrite is realized partially by coordination of free ligands of copper with iron atoms on the exposed edge of the crystal lattice of pyrite.[121] When *A. ferrooxidans LR* is exposed to bornite, its metabolic activity changes, where in the detoxication routes generally are activated. This response is not found for chalcopyrite. The exposition of the *A. ferrooxidans* cell to bornite causes a reaction against the oxidation stress, to protect the cell's functions and to maintain homeostasis. Due to the higher solubility of bornite compared to chalcopyrite, the concentration of copper ions realized in the medium is increased, and this probably causes higher stress in bacteria.[119] Mineral sulfides as semiconducting materials show galvanic interactions in the mixture in the electric contacts, which is often the case in ores. In the interaction, a mineral with a higher electrode potential becomes the cathode, while a corroded (oxidized) mineral with a lower electrode potential becomes the anode. An example of galvanic coupling is the acceleration of copper leaching from chalcopyrite in contact with pyrite. Pyrite, which is characterized by higher potential, acts as a cathode:[49]

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

(19)

While chalcopyrite with lower potential, acts as an anode:

$$CuFeS_2 \rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 4e^-$$

(20)
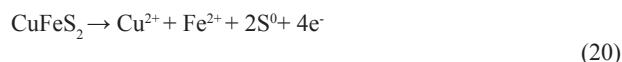
Pyrite may erode sphalerite (ZnS), which has a lower potential. It was found that the mineral with the lowest potential always possesses the highest extent of microbial colonization and dissolution. Thus, the physico-chemical effect (in this case, the galvanic effect) is highly beneficial for bacteria.[49]

Addition of pyrite to chalcopyrite concentrate significantly increases the efficiency of the extraction of copper.[122] When adding activated carbon, *A. ferrooxidans* improves the galvanic interaction between chalcopyrite and carbon, which in turn speeds up the copper dissolution in the bioleaching of chalcopyrite concentrate with *A. ferrooxidans*.[123]

## Influence of silver on the catalytic activity of the bacteria

Silver is an effective catalyst for bioleaching of copper from low-

grade chalcopyrite ore.[124–128] The use of chelating agents (thiosulfate or thiosulfate plus cupric ions) does not influence the extraction yield of copper and iron.[125] Dissolution of copper in the initial phase of bioleaching is increased by addition of a silver chloride solution with respect to the case with silver sulfate.[129] However, a high concentration of chloride (5g/l) represses the catalyst effect of silver.[125] Investigations have shown that use of concentrates or rock with argenit ($Ag_2S$) as catalyst, improves bioleaching of copper from low-grade chalcopyrite ore.[130] Argenit improves the yield of chalcopyrite bioleaching but inhibits biooxidation of pyrite.[131] Metal ions, $Ag^+$ and $Cu^{2+}$, can improve the leaching degree by forming $Ag_2S$, and in smaller quantity CuS, on the surface of realgar.[132] Nutrient composition and pH significantly affect the bioleaching of copper. At pH~3.0, the catalyst effect of Ag vanishes. Bioleaching of low grade copper ore requires a minimum amount of nutrient salts to obtain maximum efficiency. Increasing content of ferric ions has a negative impact on the silver catalyzed bioleaching, but the effect is very positive for silver catalyzed chemical leaching.[124]

Bioleaching of complex sulfide concentrates at low temperature is catalyzed by different ions, such as silver, bismuth and tin.[133,135] The presence of different cations in the leach solution has a different catalytic activity on the dissolution of copper from concentrates: Ag (I)>>Hg(II)>Co(II)>Bi(III)>As(V)>Ru(III). The density of the pulp affects the efficiency of the bioleaching in the presence of silver. The optimal density is 5%.[136] Presence of some cations (e.g. Hg (II), Ag (I), Pb (II) i Cd (II) in concentration of 10 mg / l, inhibits microbe oxidation of Fe (II). On the other hand, presence of 10 mg / l of As (III), Mn (II), Sn (II), Co (II), Cu (II) i Zn (II) and anions (as Cl⁻i NO³⁻), do not affect oxidative activity of bacteria on Fe (II).[137] Silver probably inhibits Fe (II) oxidative capability of bacteria by several mechanisms in which Ag has to compete with Ferro Fe for active sites in the enzyme, and they are also bound to enzym-substrat complex.[138] If the concentrate contains high quantities of silver, it simplifies the design and operation of reactors. These reactors may be used for concentrate processing at relatively low temperatures (30°C), with simple and known acidophilus.[139] Ultraviolet radiation induces mutations in the silver-resistant isolate,and the mutant exhibits a much higher ferrous ion oxidation capacity and tolerance to silver ions.[140]

In the Indirect Bioleaching with Effective Separation (IBES) process, applied to chalcopyrite/sphalerite concentrates after separation of solid / liquid, ferrous iron in the leach solution, can be biologically oxidized with *A. ferrooxidans* to regenerate ferric iron (phase IBES biological processes); silver can be obtained from the residues and is recycled in this way.[141,142]

### Influence addition of amino acids on the catalytic activity of the bacteria

Bacterial attack on the sulphide surface is based on the use of secondary chemical species (H, $Fe^{2+}$, thiol compounds) that lead to the break of chemical bonds on the sulphide surface and thus causes decay.[143] By adding a small amount of cysteine amino acid in acid solution in contact with pyrite, the activity of *A. ferrooxidans* is greatly improved, while at higher concentration of cysteine, a deleterious impact on bacterial activity is observed.[144,145] In the presence of cysteine, pyrite can be oxidized in the absence of bacteria with a degree of leaching comparable to that obtained with bacteria under normal leaching conditions.[143,145,146] Pyrite surface modified by adsorption of cysteine is a simplified model for the simulation of electron transfer in iron sulfur centers.[147] Chemical interactions regulate the entire process of adsorption.[148] Cysteine can act as a bridge or conductor,

to facilitate the transfer of an electronic charge from the pyrite to the final product.[149] The addition of methionine is harmful for bioleaching of pyrite with *A. ferrooxidans* at all concentrations of methionine.[144] Ghosh et al.[150] have found that the addition of aspartate, glutamine, serine or histidine increases the solubility of chalcopyrite in the presence of *A. ferooxidans*, but the efficiency of leaching of aspartate, glutamine and histidine reduces after a few days.[150]

## Conclusions

*Acidithiobacillus ferrooxidans* is a bacteria which exists naturally in mines; it participate in geochemical processes, where it brings about to the oxidation of sulfide ores and stimulates the formation of acid mine waters. But, this same *A. ferrooxidans*, which is primarily responsible for the generation of AMD, can be used for recovery metals by leaching from mining - metallurgical resource and for remediation of acid mine waters. It's possible to isolate strains from acid mine waters with the best characteristics and perform their adaptation to increased metal concentrations. The application *A. ferrooxidans* for leaching of metals is possible starting from ores, over concentrate, till mining-metallurgical waste. The type of mineral affects on the catalytic activity of the *A. ferrooxidans*. The limited bacterial activity and control of ferric ions are preferable for effective bioleaching. Addition of cations or amino acids can increase the degree of bioleaching of metal sulfides. So, the significant yield of copper and other metals from mining - metallurgical resource can be recovery by addition of catalysts (Ag for example or cysteine) in bacterial inoculum and by control of Eh conditions, values of pH, nutrients, oxigen and other biological and physico-chemical factors. Besides the recovery of metals, this is an ecological and cost-effective manner to reduce the release of metals into the environment.

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## Conflict of interest

The author declare that there is no conflict of interests regarding the publication of this paper.

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