Revista Minelor – Mining Revue
ISSN-L 1220-2053 / ISSN 2247-8590
vol. 29, issue 3 / 2023, pp. 50-59

THE STUDY OF THE BIOTECHNOLOGY OF SULFUR MINERALS

Camelia BĂDULESCU[1*]

[1]University of Petrosani, Romania, sorin_mihai_radu@yahoo.com

DOI: 10.2478/minrv-2023-0023

Abstract: *The paper presents the direct and indirect mechanism of action of different types of microorganisms in the process of chemical-bacterial solubilization of sulfuric minerals. The discovery of some bacteria that favor the dissolution of metals, increasing the speed of the process up to 500,000 times, proved to be able to improve the parameters of hydrometallurgy and create new possibilities for its application in the recovery of useful elements from different minerals and waste. The mechanism of the chemical-bacterial mineral solubilization process does not proceed according to a unitary mechanism, but corresponds to different mechanisms depending on the nature of the metal, the nature of the ore, the microorganisms and the physical-mechanical conditions in which the bacterial culture acts.*

Keywords: *chemical-bacterial solubilization process, bacterial oxidation of iron, bacterial oxidation of mineral sulphides*

1. Introduction

The development of biotechnologies in ore processing began in 1954 when Kennecott Copper Corporation began to exploit old copper ore heaps, through leaching, with the help of bacteria.

Between 1955-1970, numerous researches were carried out, which scientifically substantiated the mechanism of the bacterial solubilization process of various sulfuric minerals.

Research on the bacterial leaching process in Romania began in Baia Mare, in 1964, at the Mining Research Institute. In a first stage, *Thiobacillus ferrooxidans* bacteria were discovered and isolated from the mine waters, conducting research on the possibility of using bacterial leaching for different ores from: Baia Sprie, Altîn Tepe, Ilba, Leșul Ursului, Deva , Roșia Poieni and Moldova Nouă [1].

In time, it has been noticed that in the case of complex sulphide deposits, the waters accumulated in some underground voids, including those created by mining, the acidic character of the mine waters appears, which raises special problems regarding the functionality of machines used underground.

It was assumed then, that apart from the acid resulting from the dissolution of pyrite and other mineral sulfides, in mine waters there is an independent factor that contributes to the formation of mineral acids and to the dissolution of some useful mineral substances in them. Analyzing and researching the factors that could favor oxidation processes, it was determined the behaviour of some autotrophic bacteria, existing practically in all mine waters.

The discovery of bacteria that favor the dissolution of metals, being able to increase the speed of the process proved to be able to improve the parameters of hydrometallurgy and create new possibilities for its application in the recovery of useful elements from different minerals. By separating these bacteria and by creating favorable conditions for their development, these microorganisms proved to be very efficient in the processes of metal oxidation and solubilization.

[*] Corresponding author: Camelia Bădulescu, Assoc. Prof. PhD. Eng., University of Petroșani, Petroșani, Romania, contact details (University st. no. 20, Petroșani, Romania cameliabadulescu@upet.ro)

2. Experimental part

The mechanism of the chemical-bacterial solubilization process of metal sulphides

Taking into account the valorization of different minerals through chemical-bacterial solubilization, the processes of decomposition and leaching of minerals, under the action of some bacteria, are of interest. *The process by which the dissolution of mineral substances or impurities takes place with the help of microorganisms or the products of their metabolism, is called the process of biochemical solubilization, or bacterial leaching.*

This process can be applied individually or combined with the chemical one, if the material subjected to solubilization contains oxides and carbonates, which are easily solubilized in an acidic environment, without the need for the catalytic intervention of bacteria. The combined process is called *chemical-bacterial solubilization.*

The main metals currently obtained by chemical-bacterial solubilization methods are: *copper, iron, zinc, nickel, antimony, lead, tin, molybdenum, arsenic, bismuth, vanadium, manganese, gold and silver, rare and dispersed metals, gallium, cadmium, germanium, cobalt, rhenium, selenium, tellurium, titanium, uranium.*

Table 1 presents the main microorganisms used in the solubilization process, the pH and temperature conditions and the area of use [2], [3].

The chemical and biochemical leaching processes of sulfuric ores have been researched and expanded much more than those of non-sulfuric ores, which in many cases did not go beyond the laboratory or pilot phase.

Table 1. Microorganisms used in solubilization process and the applicability domain

Microorganisms	Process	Applicability domain
Bacteria: T*hiobacillus and Leptospirillum*: *Thiobacillus ferrooxidans* *Thiobacillus thiooxidans* *Thiobacillus acidophilus* *Leptospirillum ferrooxidans* *Leptospirillum ferrooxidans* in the mix with *Thiobacillus thiooxidans* and *Thiobacillus acidophilus*	Mineral sulphurs oxidation, of S^0 and Fe^{+2} at pH =1,4 – 3,5 and temperature of 5 - 35˚C	Solubilization of metals from sulphides, complex and concentrated ores, pyrometallurgical waste, coal desulphurisation, in underground or leaching tanks
Acidophilic bacteria such as *Sulfolobus* și *Acidianus*	Mineral sulphurs oxidation, of S^0 and Fe^{+2} at pH =1, – 5 and temperature of 45 - 96˚C	Solubilization of metals from sulphides, complex and concentrated ores, pyrometallurgical waste, coal desulphurisation, in underground or leaching tanks
Organotrophic microorganisms: fungii, bacterii, alge, mucegaiuri	Destruction of sulfuric minerals, aluminosilicates, silicates, manganese reduction and oxidation, gold solubilization and metal biosorption	Extraction of metals from carbonates and silicates, gold leaching, selective extraction of metals from solutions

Generally, the solubilization of sulfuric minerals is obtained by dissociation, according to the solubility of their reaction products [4]:

$$MeS \rightleftharpoons Me^{2+} + S^{-2} \quad (1)$$

where: Me – metall;

Ion S^{2-} can be oxidized to sulfate according to the following electrochemical reactions:

$$S^{2-} + 4H_2O \rightleftharpoons SO_4^{2-} + 8H^+ + 8e^- \quad \text{(anodic reaction)} \quad (2)$$

$$8H^+ + 8e^- + 2O_2 \rightleftharpoons 4H_2O \text{ (catodic reaction)} \quad (3)$$

$$S^{-2} + 2O_2 \rightleftharpoons SO_4^{2-} \quad \text{(general reaction)} \quad (4)$$

The solubilization of the mineral according to equation (1) takes place at a speed proportional to the speed of the reaction, but which can be considerably increased by the intervention of microorganisms.

The solubilization equation is obtained by combining equation (1) with equation (4):

$$MeS + 2O_2 \xrightarrow{\text{microorganisms}} MeSO_4 \quad (5)$$

If it is present the ioron ion, the reaction is:

$$MeS + 2Fe^{3+} \rightleftharpoons Me^{2+} + S^0 + 2Fe^{2+}$$

Microorganisms are very important in the iron oxidation, Fe^{2+} at Fe^{3+} :

$$2Fe^{2+} + 1/2O_2 + 2H^+ \rightleftharpoons 2Fe^{3+} + H_2O \quad (6)$$

2.1. Bacterial oxidation of iron

It is a process that takes place in several stages: in the first stage, the complexation of the bivalent iron takes place, outside the bacterial cell, by certain organic substances produced by the bacteria and released into the environment.

Iron, in the form of an organo-metallic compound, is transported from the external environment to the cytoplasmic membrane [5].

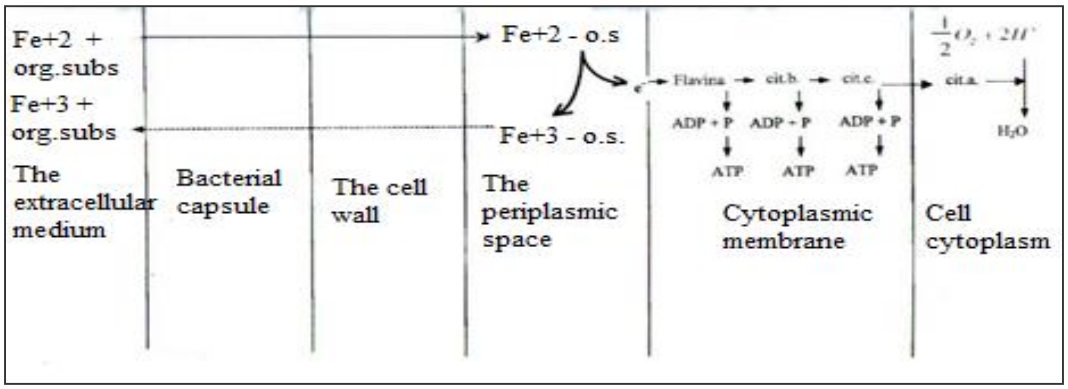

Figure 1. Scheme of the process of bacterial oxidation of bivalent iron

Figure 1 shows the diagram of the process of bacterial oxidation of bivalent iron with the transport of the electron to oxygen and the storage of energy in the adenosonetriphosphate-ATP molecule.

The actual oxidation of iron takes place in the periplasmic space between the cell wall and the cytoplasmic membrane.

The process of bacterial oxidation of iron consists of pulling an electron from the Fe^{2+} ion, taking over this electron by the bacteria, transporting it inside the cell to oxygen - which is the final acceptor and which is reduced, forming water. Several steps are involved in the transport of the electron from the bivalent iron to oxygen, with enzymatic reactions based on cytochromes which are also called electron transporters.

During the path of the electrons, the energy released by iron oxidation is gradually released and is taken over by the bacterial cell and stored in the form of phosphate chemical bonds in the adenosonetriphosphate - ATP molecule, according to the reaction:

$$ADP + P + energie \longleftrightarrow ATP \quad (7)$$

where: ADP – adenozindifosfat;
P – fosfor anorganic;
ATP – adenozintrifosfat.

When the bacterium needs energy, either for metabolic processes of carbon dioxide assimilation or for other energy-consuming actions, the bacterium carries out the reverse reaction of ATP decomposition with the formation of ADP and inorganic phosphate, releasing the energy it needs.

After the oxidation of iron, which took place in the periplasmic space, the trivalent iron formed here is transported through the cell wall and then through the cell capsule to the outside and is eliminated in the external environment.

The transport is also carried out by means of ferro-organic complexes, as well as the transport of bivalent iron inwards, ultimately resulting in solutions with high concentrations of trivalent iron. Iron-oxidizing bacteria are present in waters and springs with a high content of reduced iron. Their role in iron metabolism is uncertain, because Fe^{2+} ions are quickly oxidized in contact with air. Therefore, ferrous iron does not accumulate in sufficient quantities to ensure the growth of bacteria in neutral waters. In acidic mine waters, spontaneous oxidation does not occur, favoring the development of *T.ferrooxidans*, which multiplies and transforms Fe^{2+} into ferric hydroxide. Due to their impermeability to hydrogen, these bacteria can multiply, remaining active even in very acidic environments. They have an important role in the solubilization of some non-ferrous metals [6].

In the solubilization of metals from sulphide ores, *T.ferrooxidans* acts in 2 ways:

- ***The direct way***, through which the bacteria act directly on the sulfide ore, oxidizing the solid sulfides to soluble sulfates. This is done without the participation of the ferrous sulfate produced by bacteria, because the metals are released from the insoluble mineral directly through the oxidative metabolism of the microorganism. The existence of this method of leaching was suspected after the physical binding capacity of bacteria on the surface of metal sulphides was described. In this process, the bacteria attack the mineral components susceptible to oxidation, transferring during the energy metabolism, the electrons from iron or sulfur to the oxygen atoms. So, the process is aerobic and is mediated by transport proteins, with a role in driving the electrons released during the oxidation reactions, from the level of the cell membrane to the oxygen atoms. The existence of this mechanism was demonstrated experimentally by exposing some synthetic metal sulfides, lacking iron, to the action of the bacterium *T.ferrooxidans.* The process takes place with oxygen consumption and aims to solubilize the metal (Me) after the reaction:

$$MeS + 2O_2 \rightarrow MeSO_4 \quad (8)$$

The direct mechanism is suggested by the reduced rate of oxidation of iron-free sulfur minerals such as coveline (CuS), chalcosine (Cu_2S), molybdenite (MoS_2), as well as synthetic sulfides. The addition of Fe^{3+} doubles the extraction rate. This mechanism is difficult to demonstrate on sulfuric minerals that contain iron, such as: pyrite (FeS), arsenopyrite ($FeS_2.FeAs_2$), bornite (Cu_5FeS_4) and chalcopyrite ($CuFeS_2$), due to the release of soluble iron during oxidation and probably the simultaneous appearance of the indirect mechanism.

- ***The indirect way*** is the process by which metals are released from insoluble minerals by means of chemical oxidants produced by microorganisms. The most studied system, considered as a model, is the one related to pyrite, which has the advantage of being frequently encountered and easily oxidizable. Oxidative reactions characteristic of indirect leaching can also occur as purely chemical oxidations. However, the presence and activity of microorganisms greatly accelerates the speed of chemical reactions. In indirect leaching, the bacterium *T.ferrooxidans* produces Fe^{3+} in the form of $Fe_2(SO_4)_3$ by oxidizing soluble ferrous iron. Ferric sulfate is a strong oxidizer, it can dissolve a wide variety of metals, which it transforms into soluble oxidizing ions, in a sulfuric acid solution. Leaching produced under the action of ferric sulfate is called indirect because it takes place in the absence of viable bacteria and oxygen. During this reaction, ferrous iron reappears and is quickly reoxidized by bacteria. In this process, the intervention of the bacterium *T.ferrooxidans* increases the speed of the oxidation reaction over a million times.
  If the direct mechanism occurs after the following global reaction:

$$2\,FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4 \quad (9)$$

in the indirect mechanism, the reactions that take place in the case of the participation of the bacterium T.ferrooxidans and the chemical oxidation of pyrite are the following:

$$2FeS_2 + 7O_2 + 2H_2O \xrightarrow{bacterii} 2FeSO_4 + 2H_2SO_4 \quad (10)$$

$$FeS_2 + Fe_2(SO_4)_3 \xrightarrow{chimic} 3FeSO_4 + 2S^0 \quad (11)$$

$$2FeSO_4 + 1/2\,O_2 + H_2SO_4 \xrightarrow{bacterii} Fe_2(SO_4)_3 + H_2O \quad (12)$$

Comparatively, the role of the two biosolubilization mechanisms is difficult to estimate. As iron is almost always present in natural environments where leaching processes take place, they could occur simultaneously. More precisely, the process could start with direct leaching, which releases the iron, after which the indirect leaching reactions would immediately be triggered.

Research undertaken since 1967 by Duncan and Landesman, point out the two pathways of action occur simultaneously and independently. The authors followed the bacterial oxidation of iron from chalcopyrite and pyrite in the presence of some selective inhibitors.

The results of the mechanisms of action of bacteria in the processes of solubilization of metals from sulphide ores are:

- the formation, through the oxidation of ferrous sulfate, of ferric sulfate, which is an important leaching agent, mainly responsible for the indirect action of oxidizing bacteria;
- the formation of sulfuric acid (through bacterial oxidation of sulfur) which is another metal leaching agent;
- the passage of metals into water in soluble form through the oxidation of insoluble sulfides, a process catalyzed by bacteria.

In the oxidation experiments of iron from pyrite carried out by Arkesteyn (1979), he used a culture of *T.ferrooxidans* grown on Fe^{2+}, FeS_2 and S^O. The processes through which the oxidation of ferrous iron, sulfur and sulphides by the bacteria belonging to the genus Thiobacillus takes place, are carried out by means of the

enzymes produced by these bacteria, which act as catalysts. Cells grown on sulfur have a low oxidation rate of ferrous iron and pyrite. The rate of sulfur oxidation was always relatively low, independent of the previous growth substrate.

2.2. Bacterial oxidation of sulfur

Sulfur-reducing bacteria oxidize elemental sulfur to sulfate or sulfuric acid. Oxidation can be carried out not only on elemental sulfur but also on its compounds: mineral and synthetic sulfides, sulfites, thiosulfates, etc.

The oxidation reaction of elemental sulfur is:

$$S^0 + H_2O + 3/2O_2 \xrightarrow{\text{bacteria}} SO_4^{2-} + 2H^+ + 6e^- \quad (13)$$

Sulfur being an element insoluble in water, it is necessary to make intimate contact between sulfur and bacteria, in this process the phospholipid substances in the bacterial capsule and in the cell wall of thiobacilli play an important role. These substances reduce the surface tension of the liquid and make better contact with the sulfur possible. Sulfur oxidation takes place in the periplasmic space.

The transport of sulfur from the external space to the level of the cytoplasmic membrane is carried out by:

- active transport, being linked by phospholipid molecules;
- through a process of inclusion, similar to phagocytosis (the inclusion of microbes by blood phagocytes);
- transport of sulfur in the form of polysulfides, linked to protein substances that contain sulfur in their molecule
- amino acids with sulfur such as glutathione, according to the reaction:

$$G\text{-}SH + S^0 \longrightarrow G\text{-}S\text{-}SH \quad (14)$$

where: GSH – glutation.

At the level of the oxidation site, sulfur is released and under the action of enzymes that catalyze the different stages of the process, sulfur is oxidized first to sulfite and then to sulfate:

$$GSSH \longrightarrow GSH + S \quad (15)$$

$$S + O_2 + H_2O \longrightarrow SO_3^{2-} + 2H^+ + 4e^- \quad (16)$$

$$SO_3^{2-} + 1/2O_2 \longrightarrow SO_4^{2-} + 2e^- \quad (17)$$

During this process, 6 electrons are released for each elemental sulfur atom oxidized to sulfate. These electrons are energy carriers, being taken over by the bacterial cell and transported by means of the cytochromic electron carrier chain inside the cell, to oxygen, which is the final acceptor. This last phase of the oxidation process - the transport of electrons and the reduction of oxygen - is identical for the bacterial oxidation of iron and sulphur. During the electron carrier chain, the energy that is stored by the bacteria in the macroenergetic bonds of adenosine triphosphate is released in stages.

Figure 2 shows the schematic representation of the processes of bacterial oxidation of sulphur to sulphuric acid, with the transfer of electrons to oxygen and the storage of energy in ATP molecules.

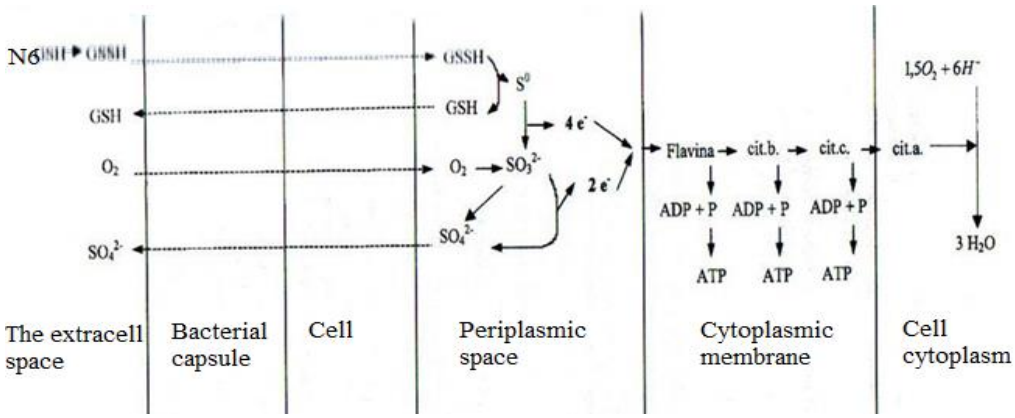

Figure 2. Bacterial oxidation of sulfur

The other sulfur compounds follow the same oxidation path. The sulfite starts the process in step two, the thiosulfate is cleaved into a sulfite molecule and an elemental sulfur atom, according to the reaction [7]:

$$S_2O_3^{2-} \longrightarrow SO_3^{2-} + S^0 \quad (18)$$

Next, sulfite and elemental sulfur enter the oxidation process, each starting from the appropriate stage: sulfite enters the last part of the process and sulfur starts the process from the beginning. The biochemistry of sulfur-oxidizing processes is complex. In a first phase, the oxidation of H_2S and sulfides to elemental sulfur takes place, under the action of enzymes, with the loss of 2 electrons. Through these reactions, in the structure of chemolithotrophic bacteria that use hydrogen sulphide as a substrate, the rapid formation of intracellular elemental sulfur inclusions is observed. Sulfides react with sulfhydryl groups in cells, forming a complex with intracellularly bound sulfur, after which oxidation to sulfite (SO_3^{2-}) occurs. Thiosulfate ($S_2O_3^{2-}$), can be considered as a sulfite sulfide ($SS_2O_3^{2-}$), enters the metabolic pathway after splitting into sulfite and sulfur, under the action of the thiosulfate cleavage enzyme.

Biological significance

Sulfur-oxidizing bacteria are present in natural environments rich in S, H_2S and Fe and with predilection in aquatic sediments (lakes, estuaries, seas, etc.), in canals, hot acidic springs, mine drainage effluents. Their presence is conditioned by the simultaneous existence of reduced compounds of sulfur and oxygen (or nitrates) [8].

Therefore, in aquatic environments they are frequently found on the border between aerobic and anaerobic, their distribution in soil, water and sediments being controlled by complex, physical, chemical and microbial interactions. In general, in nature, the coexistence of H_2S and O_2 is ensured only in systems where they are introduced continuously, because in the presence of oxygen, H_2S is not stable, being oxidized without the intervention of microorganisms. In this case, the speed of the oxidation reaction depends on pH, temperature, the presence of catalysts and/or inhibitors. In this way, the respective natural environments are enriched with a wide range of S compounds (polysulfides, sulfites, sulfates, thiosulfates, etc.), which represent potential substrates for chemolithotrophic oxidation processes.

Sulfur-oxidizing microorganisms tolerate large variations in pH (pH 1.0-9.0) and temperatures up to 60-80^0C. They had and continue to have an important role - along with other microbial activities - in the biogeochemical cycle of sulfur and in the evolution of the biosphere. The currently exploited sulfur deposits are of biogenic origin and were formed by sulfate reduction, followed by an oxidation step, in which sulfur-oxidizing bacteria participated. The proof is the existence of sulfides, in which sulfur accumulates even today.

The oxidation of H_2S and sulfides to sulfate is an important stage in the regeneration of oxidative power in water basins, where sulfur plays a role in anaerobic mineralization. In open waters, seas and oceans, sulfur-oxidizing bacteria oxidize volatile sulfur compounds from constituents released during sediment decomposition.

Sulfur-oxidizing bacteria have an important role in the quantitative regulation of biologically produced H_2S emanations, but at the same time they contribute to environmental pollution with acids and metals released through their action on various residual materials. In the soil, they solubilize sulfur compounds, making them available in the form of sulfates, which can be assimilated by microorganisms, plants, but at the same time favor the production of acidic soils.

Sulfur-oxidizing bacteria produce sulfuric acid (up to 1.5 N) and ferric iron, a process frequently associated with the corrosion of pipes, concrete and other metallic structures, especially submerged ones. This property is exploited in biometallurgy for the leaching of non-ferrous metals from poor deposits and tailings deposits.

Some members of the group of sulfur-oxidizing bacteria can couple the oxidation of inorganic sulfur compounds with other processes:

- *T. ferrooxidans* is at the same time, a sulfur and iron-oxidizing bacterium, capable of oxidizing elemental sulfur, H_2S, $Na_2S_2O_3$, as well as Fe^{2+} to Fe^{3+};
- *T. denitrificans* is a facultatively anaerobic, sulfur-oxidizing and denitrifying bacterium, because in aerobiosis it oxidizes S0, H2S and Na2S2O3, and in anaerobiosis it uses nitrates.

The second group of mesophilic sulfur-oxidizing bacteria is made up of members of the genera: *Achromatium, Macromonas, Thiobacterium, Thiospira, Thiovulum, Beggiatoa, Thiothri and Thioploca*. With the exception of representatives of the genus *Beggiatoa*, which were cultivated on organic media, the other genera could not yet be cultivated and obtained in a pure state.

3. Bacterial oxidation of mineral sulphides

Regarding the mechanism of the chemical-bacterial mineral solubilization process, from the existing data in the specialized literature, it is found that the process does not proceed according to a unitary mechanism, but corresponds to different mechanisms depending on the nature of the metal, the nature of the ore, the microorganisms and the physical-mechanical conditions in which the bacterial culture acts.

Thiobacillus type bacteria cannot break down organic substances that even have a harmful effect on the bacteria's metabolism, therefore, their presence is as undesirable as possible. As a source of carbon for the synthesis of its own substances, *Thiobacillus* uses carbon dioxide (CO_2) as in the case of plants, with the difference that these bacteria use carbon dioxide dissolved in the water in which they live.

$$CO_2 + H_2O \xrightarrow{\text{chemical energy}} (CH_2O) + O_2 \quad (19)$$

However, there are some general features of the chemical-bacterial solubilization processes of metal sulfides, namely the fact that solutions of ferric salts (Fe^{3+}), oxygen (O_2) and sulfuric acid (H_2SO_4) represent the oxidizing chemical factors of heavy metals, their oxides and sulfides . The main stages of the chemical-bacterial leaching mechanism can be presented as follows [9], [10], [11].

- ***The reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+})*** could be considered as a starting reaction that can proceed chemically, without bacterial input, but in the presence of bacteria, the reaction is much accelerated. The ferric ion can come from the dissolution of various minerals in an aqueous environment, but most often it comes from the pyrite that generally accompanies almost all sulphide deposits. The ferric ion can easily accept an electron, reduce and thus oxidize an element willing to give up this electron, according to the reaction:

$$Fe^{3+} \underset{}{\overset{+1\,e^-}{\rightleftharpoons}} Fe^{2+} - 1\,e^- \quad (20)$$

- ***The dissolution of the metal sulfide molecule***, followed by the oxidation of sulfur and the reduction of iron. This stage can proceed through the action of the acidic solution of ferric sulfate existing in the solution and which determines the dissolution of the sulfide ion, with the elimination of the metal ion in the solution and the reduction of iron, according to reaction (21). The entire reaction is also accompanied by the oxidation of sulfur, because of the sulfide molecule, only it can give up electrons. Sulfur is oxidized in several stages, from the S^{2-} form in the sulfide, to the S^{6+} form in the sulfate anion, present in the sulfuric acid.

$$Fe_2(SO_4)_3 + MeS \xrightarrow{\text{chemical}} 2FeSO_4 + MeSO_4 + S^o \quad (21)$$

$$Fe^{3+} \xrightarrow{+1e^-} Fe^{2+} \quad \text{reduction} \quad (22)$$

$$S^{2-} \xrightarrow{-2e^-} S^o \quad \text{oxidation} \quad (23)$$

- ***The formation of sulfuric acid by the further oxidation of the S^o ion to S^{6+} with the help of bacteria as a catalyst.*** The oxidation of S^{2-} from the sulfide under the action of ferric iron proceeds according to the previous stage, only up to elemental sulfur (S^o). This, being a very little active element, can be further oxidized to S^{6+}, only under energetic or catalytic conditions, where the role of catalyst is played by bacteria, under the action of dissolved oxygen in the aqueous medium, according to the reaction:

$$2S^o + 3O_2 + 2H_2O \xrightarrow{\text{biochimical (bacteria)}} 2H_2SO_4 \quad (24)$$

$$S^o \xrightarrow{-6e^-} S^{6+} \quad \text{oxidation} \quad (25)$$

$$O^o \xrightarrow{+2e^-} O^{2-} \quad \text{reduction} \quad (26)$$

- ***Secondary reactions that change the concentrations of Fe^{3+}, Fe^{2+} and the pH of the solution.*** The first and most important reaction is that of the oxidation of ferrous sulfate into ferric sulfate, in the presence of dissolved oxygen, a reaction in which bacteria again play the role of catalyst, so it is a biochemical reaction:

$$2FeSO_4 + 1/2O_2 + H_2SO_4 \xrightarrow{\text{biochimical (bacteria)}} Fe_2(SO_4)_3 + H_2O \quad (27)$$

$$Fe^{2+} \xrightarrow{-1e^-} Fe^{3+} \quad \text{oxidation} \quad (28)$$

$$O^o \xrightarrow{+2e} O^{2-} \quad \text{reduction} \quad (29)$$

In addition to this reaction, it is important to remember that through hydrolysis reactions, part of the iron present in the solution is eliminated by precipitation. This phenomenon is very important, because it leads to the clogging of the pores of the ore and the free spaces between the particles, or it is deposited on the pipes and channels for transporting mine water.

$$8FeSO_4 + 2O_2 + 2H_2O + H_2SO_4 \longrightarrow 3Fe_2(SO_4)_3 + 2Fe(OH)_3 \quad (30)$$

$$Fe_2(SO_4)_3 + 6H_2O \longrightarrow 2Fe(OH)_3 + 3H_2SO_4 \quad (31)$$

The hydrolysis reactions of trivalent iron take place at higher pH values (pH>2.2) and at pH=3.8 the precipitation of trivalent iron is almost total. To avoid these unwanted phenomena, it is mandatory to maintain the pH at values as low as possible, which also correspond to the optimal development of the bacterial culture.

Another secondary chemical reaction that takes place within the complex process of chemical-bacterial leaching is that of extracting the metal ion from the sulfide and releasing it into the solution, through the action of sulfuric acid, which causes the metal ion to pass into a soluble compound, in an aqueous environment . The chemical reaction that takes place is also accompanied by the production of hydrogen sulphide, which is released from the solution:

$$MeS + H_2SO_4 \longrightarrow MeSO_4 + H_2S\uparrow \quad (32)$$

Regarding the speed of the chemical-bacterial process, the oxidation reaction of elemental sulfur under the action of oxygen (reaction 24) and the oxidation of ferrous sulfate under the action of sulfuric acid and oxygen (reaction 27) they are reactions that take place very slowly through purely chemical means. In the presence of bacteria such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, the speed of these reactions increases from 1000 to 500,000 times, according to the opinion of some researchers in this field.

The direct and indirect chemical-bacterial leaching mechanism of mineral sulphides

The chemical reactions that are the basis of the bioleaching process of various minerals can be found in large-scale works that describe the process in its vast complexity. Practically, following the research carried out, the main chemical reactions possible to take place in the process of biological leaching of metalliferous minerals from ores are presented. Most of the time and with a determining role in the process, we find pyrite as the ***predominant mineral,*** in deposits of complex non-ferrous ores. The mechanism of bacterial oxidation of pyrite is particularly important because it "triggers" the process of water formation mine acids, in the presence of water and oxygen, ensuring conditions for the survival and action of bacteria of the *Thiobacillus* type. The biochemical reactions underlying pyrite oxidation are the following:

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad (33)$$

$$2FeS_2 + 7O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2FeSO_4 + 2H_2SO_4 \quad (34)$$

$$2FeSO_4 + 0{,}5O_2 + H_2SO_4 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O \quad (35)$$

$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3FeSO_4 + 2S^o \quad (36)$$

$$2S + 3O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2H_2SO_4 \quad (37)$$

If there is no trivalent iron in the environment, reaction (33) can be considered as the starting reaction of the bioleaching process, because this reaction can take place chemically or in the presence of bacteria, but in this second case, it proceeds at a much higher speed . After initiating the process through this reaction, the bacteria can then activate in two ways:

- The first way is the one in which the bacteria act on the Fe^{2+} ion resulting from reaction (34), oxidizing it to Fe^{3+}, according to reaction (35) which takes place in an acidic environment, with the consumption of sulfuric acid and oxygen, the bacteria using to synthesize substances own, part of the energy that is released. The accumulation of ferric ion in the acidic environment will determine a strong oxidizing potential, trivalent iron becoming an oxidizing factor for pyrite molecules, according to reaction (36), which is a chemical reaction without the participation of bacteria. For this reason, this process is called the indirect mechanism of bacterial oxidation of mineral sulphide, the bacteria having an indirect role here, that of reoxidizing the bivalent iron resulting from the reaction.As can be seen from reaction (36), the chemical process of pyrite oxidation proceeds with the reduction of iron from ferric sulfate to ferrous sulfate and with the oxidation of sulfur in the crystalline network from the S^{2-} form to elemental sulfur S^o.

Thus, the sulfur comes out of the crystalline network of pyrite, releasing at the same time the iron in the ionic form Fe^{2+}. As specified in the presentation of the generalized mechanism, sulfur oxidation stops at the elemental sulfur stage, in the absence of bacteria it cannot be further oxidized to sulfuric acid. For this reason, when there are few bacteria in the system, or when their activity is not intense enough, it is possible to reach a decrease in the content of trivalent iron, an increase in the content of bivalent iron in the solution and the accumulation of elemental sulfur on the surface of the pyrite particles, with the inhibition of the solubilization process.To ensure the continuity of the process, it is necessary for the bacteria to reoxidize the bivalent iron according to reaction (35) and also to oxidize elemental sulfur according to reaction (37), up to sulfuric acid. Both reactions are biochemical, the bacteria ensuring the resumption and intensification of the pyrite solubilization process, contributing to the increase in the concentration of trivalent iron in the solution and to the formation of the acidic environment, which has favorable effects in the kinetics of the bioleaching process;

- The second way is that in which the bacteria act directly on the pyrite, according to reaction (33). For this, the bacteria attach themselves tightly to the surface of the mineral and act oxidatively directly on the S^{2-} and Fe^{2+} ions in the crystalline network. This is the direct bacterial oxidation mechanism of mineral sulphides.

The two mechanisms described above are suggestively presented in figure 3, for the case of bioleaching of the blende, showing the main reactions that take place in the case of the direct and indirect bioleaching mechanism, with the active participation of bacteria as a catalyst of the chemical solubilization process - bacterial of mineral sulphides. Most of the sulphides accompanying pyrite, namely chalcopyrite, blende, galena, arsenopyrite and others, are oxidized by bacteria through the direct and indirect mechanism.Among the sulfides mentioned, galena raises some problems in the bacterial solubilization process, since, although the bacteria oxidize the galena, the lead is not solubilized because it arrives in the form of lead sulfate, insoluble in water, which remains in the form of sludge together with the undissolved material.

It has been demonstrated that the bacteria increase the speed of these reactions by the fact that the ferrous iron contained in the acid mine waters, in concentrations of 200 mg/l, was completely oxidized in a few days by a young *T.ferrooxidans* culture. Comparatively, the chemical oxidation of iron from a sterile solution (without bacteria) with the help of atmospheric oxygen, at the same water acidity, required more than 2 years. Table 2 compares the efficiency of the bacterial leaching process for different mineral sulphides [12].

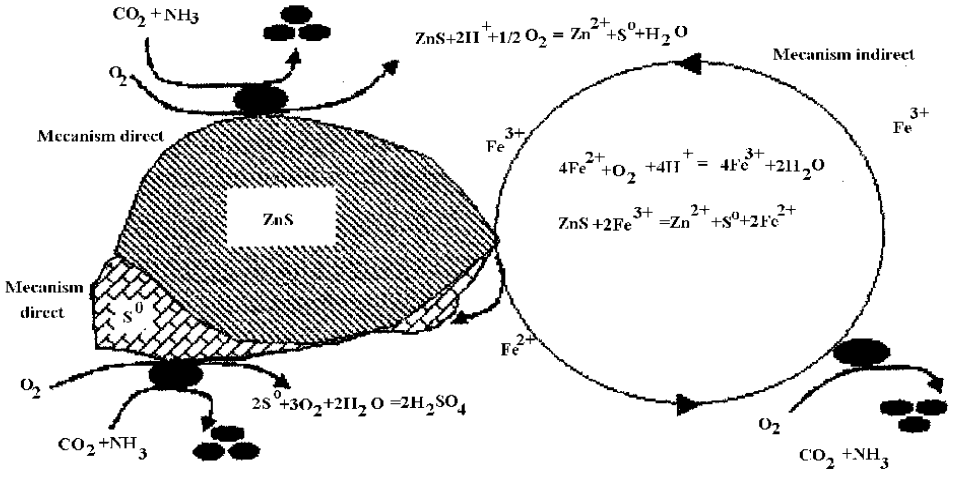

Figure 3. Mechanisms of blende bioleaching process

Table 2. The metall recovery in the leaching processes of different mineral sulphides

Microorganisms	***Metall content (mg/l/h)***					
	Fe from FeS_2	***Cu from $CuFeS_2$***	***Cu from CuS***	***Zn from ZnS***	***Ni from NiS***	***As from AsFeS***
Thiobacillus ferooxidans	59	109	224	444	468	53
Thiobacillus thiooxidans	Nd	Nd	Nd	244	230	Nd
Leptospirillum ferrooxidans	39	41	46	163	144	23
S.thermosulfidooxidans	51	86	155	374	381	39
Thiobacillus sp.	49	81	152	361	365	35
Sulfolobus sp.	52	91	177	420	431	41

4. Conclusions

The discovery of some bacteria that favor the dissolution of metals, being able to increase the speed of the process up to 500,000 times, proved to be able to improve the parameters of hydrometallurgy and create new possibilities for its application in the recovery of useful elements from different minerals;

In the solubilization of metals from sulphide ores, T.ferrooxidans acts in 2 ways:

- the direct way, through which the bacteria act directly on the sulfide ore, oxidizing the solid sulfides to soluble sulfates;
- the indirect way is the process by which metals are released from insoluble minerals by means of chemical oxidants produced by microorganisms;

The main stages of the chemical-bacterial leaching mechanism are:

- Reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+});
- The dissolution of the metal sulfide molecule, followed by the oxidation of sulfur and the reduction of iron;
- Formation of sulfuric acid by further oxidizing the S^o ion to S^{6+} with the help of bacteria as a catalyst;
- Secondary reactions that change the concentrations of Fe^{3+}, Fe^{2+} and the pH of the solution.

Most of the time and with a determining role in the process, we find ***pyrite as the predominant mineral*** in deposits of complex non-ferrous ores, and knowing the mechanism of bacterial oxidation of pyrite is particularly important because it "triggers" the process of water formation mine acids, in the presence of water and oxygen, ensuring conditions for the survival and action of bacteria of the *Thiobacillus* type.

References

[1] **Bădulescu C.,** 2010
Biotehnologii in protectia mediului, Ed Universitas, Petrosani

[2] **Lundgren D.G., Silver M.,** 1980
Ore leaching by bacteria, Rev.Microbiology, p.263

[3] **Brierleyi J.A., Brierleyi C.L.,** 1986
Microbial mining using thermophilic microorganisms, In General, Molecular and Applied Microbiology (Ed. by T.D.Brock), p.279-305

[4] **Zarnea G.,** 1984
Tratat de microbiologie generală, vol.II, Ed.Academiei, Bucureşti

[5] **Lizama H.M., Suzuki I.,** 1989
Synergistic competitive inhibition of ferroua iron oxidation by T.ferrooxidans by increasing concentrations of ferric iron and cells, Applied Environmental Microbiology, 55, p.2588-2591

[6] **Malik A., Dastidar M.G., Roychoudhury P.K.,** 2004
Factors limiting bacterial iron oxidation in biodesulphurization system, International Journal of Mineral Processing, vol.73, No.1

[7] **Yakhontova L.K.,** 1985
The role of sulphide constitution in the process of the bacterial leaching. Biotechnology of metals. Proceedings of International Seminar, Moscow, pg. 216-226

[8] **Fečko P.,** 2007
Biotechnology - technologies of 21century. Institute of Environmental Eng., Mining University of Ostrava, Czech Rep.

[9] **Plkin S.I., Adamov V.V., Karavaiko E.,** 1975
Theory and Practice of Utilizing Microorganisms in Processing Difficult-to-Dres Ores and Concentrates, in 11th International Mineral Processing, Cagliari, pg. 901

[10] **Tătaru V., Ardelean A.,** 1996
Valorificarea minereurilor prin solubilizare şi biotehnologie, Fundaţia Vasile Goldiş, Arad, pg.111-119

[11] **Goktepe F., Pololey F.D.,** 1998
Recovery of Copper from a sulphide Tailings by Bacterial Leaching, Innovations in Mineral and Coal Processing, Proceedings of the 7-th International Mineral Processing Symposium, Istanbul, pg.483-486;

[12] **Tuovinen O.H., Niemela S.I.,** 1971
Tolerance of Thiobacillus ferooxidans to Some Metals, J.Microbiology, nr.37