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Research Paper

Bioleaching, Viable Alternative For The Treatment Of Some Metallic Sulphides In Peru.

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ABSTRACT: *The benefit of minerals through bioleaching is a viable alternative to reduce the environmental problems faced by the metal extraction industry and to reduce production costs and also to address economic changes by that the extractive companies go through.*

The isolation and adaptation of the strains of Aciditiobacillus Ferrooxidans was made by modifying the 9k medium of Silverman and Lundgren and the following conditions: 22.4 gr/L of iron sulfate, pH in the range of 1.6 to 2.4, bacterial concentration at 10% v/v , average temperature 22 ° C, Orbital agitation 160 rpm., from rock and mine effluents are characterized by their acidity and dissolved metal content, they are usually natural habitat of bacteria of the genus Acidithiobacillus Ferrooxidans and Thiooxidans, Bacteria widely studied for their importance in the biooxidation of minerals and able to obtain energy from the oxidative catalysis of compounds of sulfur, iron and metal sulphides. The final identification was performed by the polymerase chain reaction to a 98% probability. The adaptation was carried out in different means containing varied quantities of iron and sulphide minerals with the presence of oxides from high mineralization quarries (presence of copper, lead, zinc, sulfur, silica, gold, silver and others).

The copper solubility, achieved versus Fe concentration (II) was determined that with 6 gr/L of $FeSO_4.7\ H_2O$, 72.64% copper is obtained at the maximum growth point of the bacterium, 4.75 $x10^7$ cell/ml. in a period of 24 days. During the second stage with the absence of $FeSO_4.7\ H_2O$, it is possible to extract 85.6% of copper at the maximum growth point of the bacterium, 4.41 $x10^7$ cell/ml. Within a period of 25 days.

KEYWORDS *- bioleaching, metal sulphides, acid drainage, acidithiobacillus Ferroxidans.*

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I. INTRODUCTION

The accelerated development of the biological sciences and the knowledge of applied biotechnology mineral processing through the use of native microbial strains in the recovery of metals contribute to the whole of studies in the area of Hidrometallurgy, which is being developed in several national and international universities, to implement clean and economical technology of application in the mining community, providing solutions not only to the problems of environmental impact, but also to the economic character, and enabling the recovery of various elements from sulphide minerals. This technology has been gaining a great boom in recent decades, due to the improvement in the kinetics and efficiency of the mechanisms involved in the processes of metal extraction.

The mine Acid drainages (ADM) characterized by extreme acidity (pH 2.5 – 4.0) and high concentrations of heavy metals, contain native populations of oxidizing bacteria, responsible for the progressive oxidation of sulphides. Within the microorganisms found in the acidic environments and within the family of the **prokaryotes** has been identified the bacteria of the genus of Thiobacillus, Leptospirillum and Acidophilium (Pachon D., 1998) able to contribute to the degradation of sulphides Metal. The **Thiobacillus genus** bacteria have great greed for metallic sulphide compounds, obtaining their energy source from the oxidation of reduced sulphides and ferrous salts and in a medium with moderate temperature (mesophiles) and acidity in the range from 0.5 to 4. The bacteria of **Leptospirillum genus**, are developed oxidizing to ferrous iron in ferric compounds like pyrite (Sands W., et al 1979). This genus of bacteria considered as strict aerobics are developed

in the range 1.5 to 4.0 pH, is widely distributed in deposits of altered minerals and drainages that are generated through them (Sand, W. et al 1992). The bacteria of the **Acidophilium genus**, are cells of form of straight bacilli, Gram-negative, mesophilic, chemoorganotrophs obliged that are inhibited from certain concentrations of organic matter (Hann, M. et al, 1993).

In the genus Acidithiobacillus have studied the Acidithiobacillus Thiooxidans, Acidithiobacillus Caldus and Acidithiobacillus Ferrooxidans, characterized by its favorable evolution at temperatures between 28 and 34 °C, use CO_2 as a source of carbon and obtain sulfur oxidation energy, iron and metal sulphides.

The high acid content in mine drainage has caused the whole world to look for new forms of treatment, reduction of metals in polluted sites and also represent an economically feasible alternative. Thus, in biological and microbiological activities are being explored which include bioleaching, biosorption, bioaccumulation and in general, processes of oxidation and biological reduction.

II. EXPERIMENTAL DEVELOPMENT

Biotechnology a viable alternative for the processing of various materials and derivatives, one of its areas of development is Biohidrometallurgy, which refers to the oxidation of elemental sulphur, ferrous iron and sulphide minerals (Cu, Pb, Zn, Fe, Ni). The biooxidation in the treatment of gold ores is aimed to the oxidation of the sulfide matrix present, remaining the gold in native form. This mechanism is already used in the pre-treatment of minerals and gold concentrates, replacing the calcination and oxidation at pressure prior to leaching in the conventional way. However, it continues with the traditional application of biomining, initiated in the decade of 1960 with the construction and irrigation of landfills for copper recovery in the Kennecott Bingham Canyon Copper mine, Utah, USA (Brierley 2001). Since the decade of 1980, there has been a large expansion in the number of leaching operations for copper recovery from low-grade minerals, and several operations started in Chile. (Chandra Sekhar Gahan, 2012). In Peru, bacterial bioleaching accompanied by chemical leaching is being successfully applied in Toquepala (Southern Peru), where the leaching liquors of the Cuajone and Toquepala dumps are treated at the solvent extraction plant and Electrodeposition to produce more than 500.000 tons per year of copper.

The experimental design followed by this research was:

1. Sampling from natural drainages and old mining works, with high presence of dissolved metals and marked acidity.
2. Isolation and generation of the microbial consortium of acidophilic organisms by progressive concentration in liquid and solid media. Sowing of colonies Acidithiobacillus Ferroxidans in substrate nutrients.
3. Adaptation to mineral medium and analysis of the bacterial growth cycle by oxidation rate and manual counting of bacterial population against the variation of pH and concentration of iron sulphate.
4. Bioleaching and biooxidation tests on various minerals containing metallic sulfides and the presence of gold in considerable quantities. 5. Mineralogical characterization of mineral before bioleaching and tailings of bioleaching and biooxidation processes.

III. ANALYSIS OF RESULTS

3.1 Extraction of samples

The effluents were taken from two sources, being them from acid effluents from natural watercourses, known as Acid rock drainages and others from mining works known as acid mine drainages; Sent by the Environmental Directorate of the Buenaventura Mines Company (see table 3.1 and Fig 3.1).

Table 3.1. Acid waters of the Huancavelica region

SAMPLE	pH	T(°C)
River Hualchocolpa Tributary	4.60	12.2
PT Palcas Admission	4.45	9.9
U. antapite - NV 3285	3.77	14.4
PT Antapite – NV 3240	3.38	15.6

Figure 3.1. A Drainage of acidic rock water and drainage of acidic mine water (ARD).

3.2 Determination of the bacterial growth cycle.

The growth cycle of the strains was identified during cultivation done to bacteria Thiobacillus Ferrooxidans isolated from acid effluents. Growth was determined by direct counting using the Petroff-Hauser camera in a phase contrast microscope (Leica ®) at 1000 magnifications. The strain reaches the stationary phase of growth after five days. At seven days the precipitation is presented Hydrosulfides and the fall of the potential. Fig 3.2 and Table 3.2 represents the evolution of the bacterial population that takes about 7 days.

Table 3.2 Oxidation tests for the determination of the growth cycle.

Condition	pH	$FeSO_4$ (g/L)	Inoculum (ml)	Oxidation Time (hours)
Before	1.61	33.3	10	80 – 96
Modified	1.84	44.4	10	48 - 60

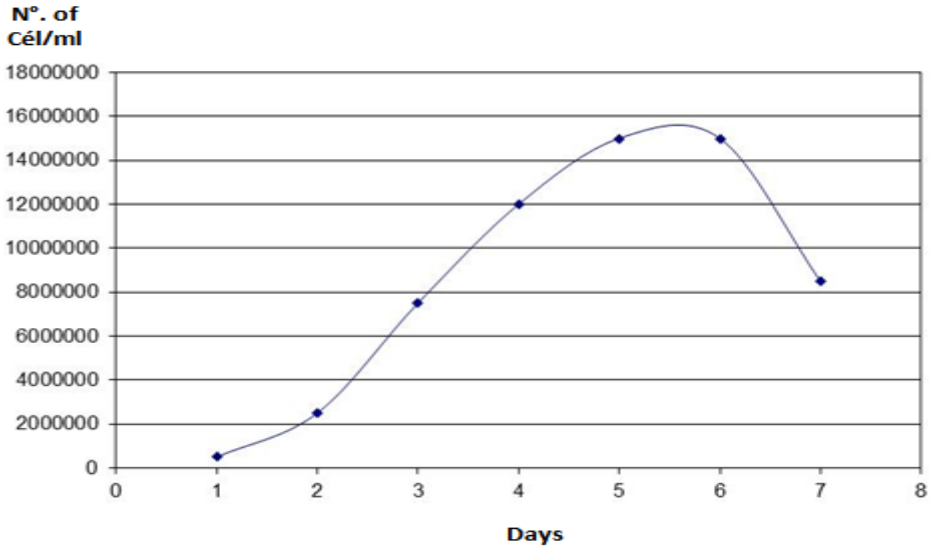

Figure 3.2. Growth kinetics of T. Ferrooxidans isolated from acid drainage, using a substrate containing 22.4% ferrous sulphate. It shows that the strain reaches the stationary phase of growth around the six days.

3.3 Adaptation of the bacterium in the presence of metallic sulphides

Identified the modified medium 9K in its iron content and at a favorable pH, its adaptation was sought in the presence of minerals containing various iron sulphides with an abundance of chalcopyrite (See *Table 3.3*), whose mineralogical distribution is presented in *table 3.4.1* The process of adaptation presents certain difficulties due to the presence of compounds inhibitors to bacterial development (Semenza, G. et al., 2000), being them the sulfides of arsenic and silver. The tests consisted in the adaptation of the bacterial strain to different media with different amounts of ferrous sulphate and sulfite mineral pyrític (Akcil A. et al., 2007). A% by weight of the pyrite was considered and the amount of iron sulphate was varied, as iron sulphate% decreases, the amount of iron increases in the sample. The evolution of the adaptation phase is determined by the variation

of the potential (Juan O. et al, 2012), determining that a marked bacterial growth occurs above 580 mV. In this case, it is possible to keep constant for several days as a sign of an oxidation of Fe^{+2} to Fe^{+3} giving a bacterial growth and then the inhibition and death due to the saturation of the medium and the depletion of nutrients in the medium.

Table 3.3. Distribution of mineral species in the adaptation stages.

Mineral Species	Content, %
Chalcopyrite	33.66
Pyrrhotite	31.00
Sphalerite	4.43
Arsenopyrite	0.65
Goetite	1.21
Pirite	1.28
Covelite	0.12
Galena	0.36
bargains	27.29

3.3.1 First adaptation test

For this purpose, 4 samples of 100% pulverized ore were considered at -400M, medium 9k containing 33.3 g/Lt of iron sulfate.

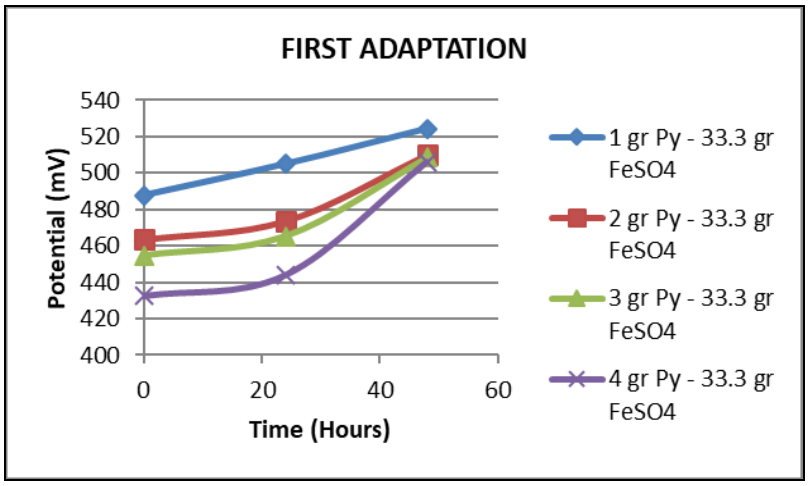

Figure 3.3.1. Changes in oxidation potential as a function of the amount of pyrite.

Figure 3.3.1. shows a considerable increase in the potential of the sample containing 1 gram of mineral, because it presents less damage to the bacteria due to the friction between the mineral particles and the bacteria, determining that the greater the amount of mineral, the greater the inhibition of the bacteria by friction, by the amount of arsenic.

3.3.2 Second adaptation test

From a particle size fraction, we use sieves Numbers. 200, 325 and 400 of the Tylor series, respectively. 5 grams of ore were taken for each trial in a medium containing 11.1 g/L of iron sulphate.

In *Fig 3.3.2.* it can be seen that the sulphide ore pulverized to -400 Mesh presents a favorable response due to the concentration of sulphides and the reduced friction on the bacteria.

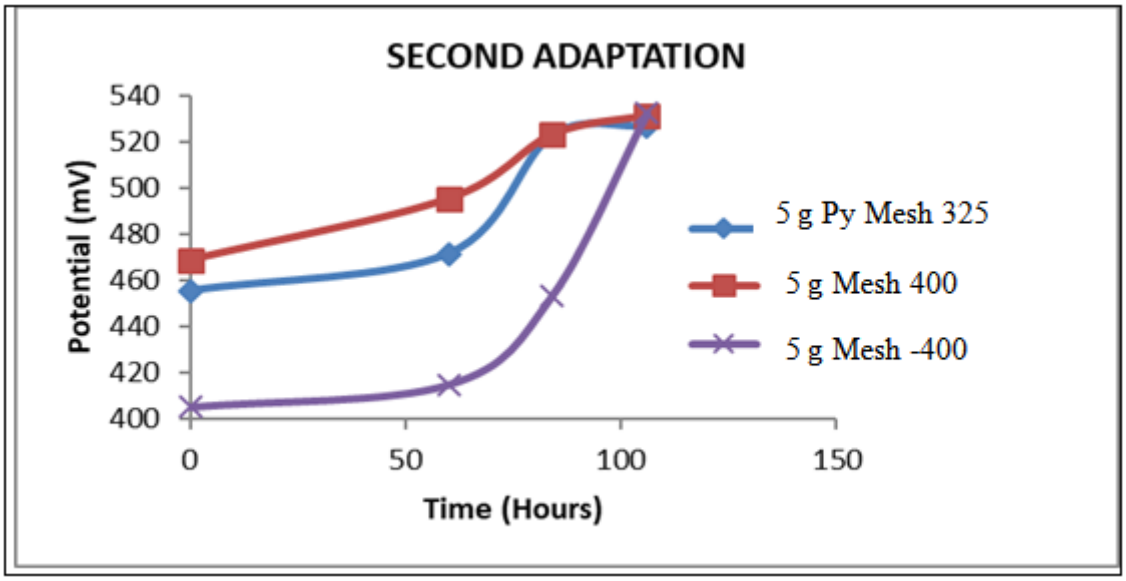

Figure 3.3.2. Changes in oxidation potential as a function of particle size

3.4 Bioleaching test

The bioleaching tests have been carried out in 2 sequential stages using modified 9K solution as a medium at different concentrations of $FeSO_4.7H_2O$ in each stage. The agitation has been constant at 150 rpm and at average environmental temperature conditions of 19.5 °C, in the stages have been started with a pH 1.8, evaluating the ORP variables, the biological density count (# Cell / ml) through a microscope biological and the determination of Copper (Cu) and Iron (Fe) by Atomic Absorption. At the end of each stage the solubility of Arsenic, Iron and Sulfur was analyzed by means of ICP analysis.

3.4.1. First Bioleaching Test

For each mineral, solutions were prepared with the 9K medium with the concentrations of $(NH_4)\ 2SO_4$ (3.00 g / l), KCl (0.100 g / l), K_2HPO_4 (0.500 g / l), $MgSO_4.7H2O$, (0.500 g) / l), Ca (NO_3) 2 (0.01 g / l), modifying the concentrations of $FeSO_4.7H_2O$ from 0.00 to 15.00 g / l in a range of 3.00g / (See *Fig 3.4.1*). In each 500 ml Erlenmeyer has been added 3 grams of mineral (1% W / V), 30 ml of Bacterial Inoculum (10% V / V) and completing with the solution of 9K to 300 ml for each Erlenmeyer at the concentrations of $FeSO_4.7H_2O$, the pH was regulated to 1.8 with concentrated H_2SO_4 solution and subsequently the bioleaching began.

Figure 3.4.1. Beginning of the first stage of bioleaching, at different $FeSO_4.7H_2O$ concentrations.

3.4.1.1 Biological growth.

Fig 3.4.1.1. shows the growth of the biological density at different concentrations of $FeSO_4.7H_2O$, the biological density of the inoculum has been 7.05x107 Cell/ml observing the beginning of the exponential growth 10 days after the beginning of the stage, in days 21 to 24, the biological density at the different concentrations of $FeSO_4.7H_2O$, tend to remain constant, indicating the final stage of biological growth. The maximum biological density reached up to 24 days was $4.75x10^7$Cell/ml at 6 g/L $FeSO_4.7H_2O$. Ranging up to 67% of the inoculum.

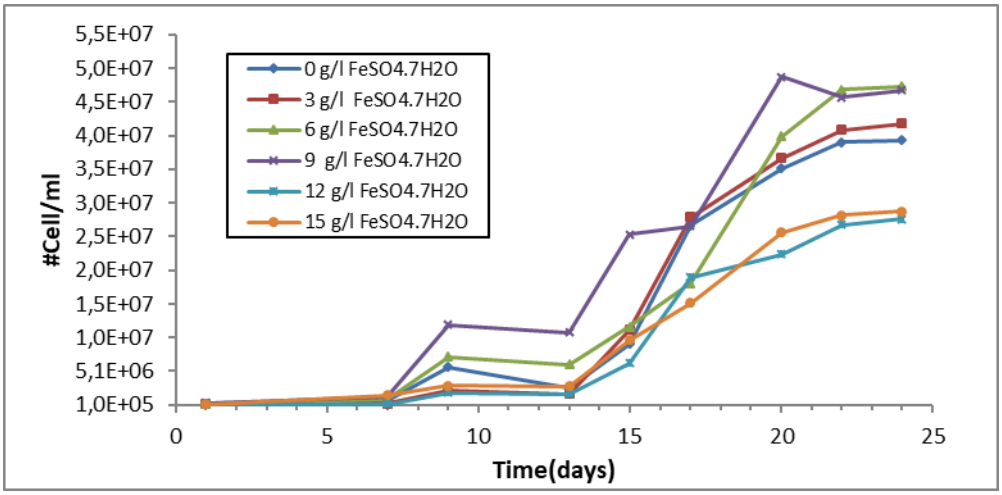

Figure 3.4.1.1. Biological growth during the first stage.

3.4.1.2 Copper Recovery (Cu).

From figure 3.4.1.2, it has been observed that the maximum recovery obtained for this first stage is 72.64% at 6g/l $FeSO_4.7H_2O$ and the minimum recovery is 30.96% at 15 g/L $FeSO_4.7H_2O$.

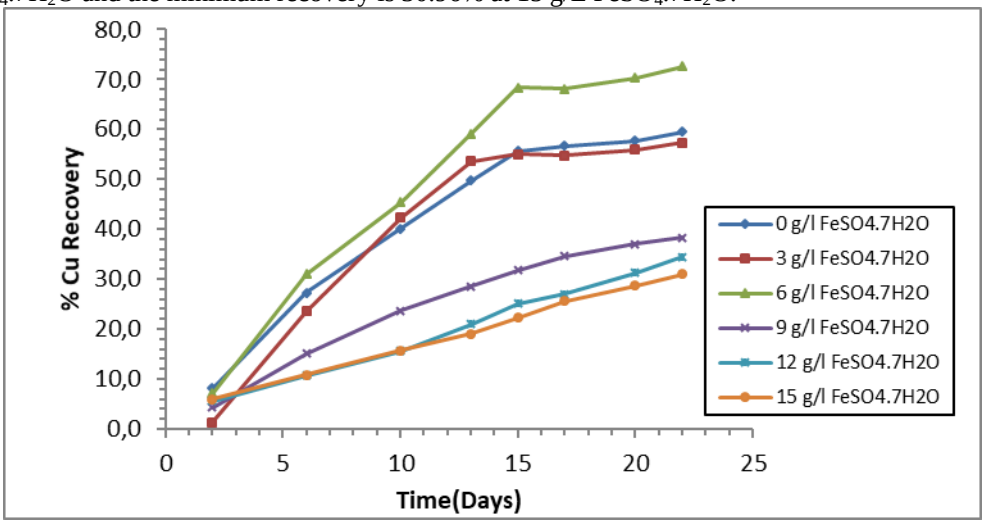

Figure 3.4.1.2. Percentage of copper recovery during the first stage.

After 18 days of bioleaching, it was observed that the percentage of copper recovery does not show a significant increase over the first 17 days. Consider for this behavior the biological growth that involves the recovery percentage because the biological density starts the constant stage, as well as the solubilization of iron product of the oxidation of the sulphur species of the Mineral.

3.4.2. Second Bioleaching Test

For each mineral, solutions have been prepared with the medium 9K with concentrations of $(NH_4)2SO_4$, (3.00 g/L), KCl (0100 g/L), K_2HPO_4 (0500 g/L), $MgSO_4.7\ H_2O$, (0.500 g/L), Ca $(NO_3)_2$ (0.01 g/L), modifying the concentrations of $FeSO_4.7\ H_2O$ at 0.00 g/L, 3.00 g/L, 9.00 g/L and 15.00 g/L. The difference from the first stage is only for opting for a higher range of comparison.

At this stage the pulp density has doubled (2% W/V), by which the weight of each mineral is 6 grams for each Erlenmeyer, the constant agitation of 150 rpm.

The volume of innocuousness has been extracted from the solution of the first stage, considering that there has been a biological adaptation to the mineral, the innocuous volume has been of 30 ml. 10% (V/V) compared to the total volume.

3.4.2.1. Effect on biological growth

The biological density of the inoculum has been of 4.73 $x10^7$ cell/ml, after 3 days of initiation The bioleaching begins the exponential growth, compared to the first stage, has been managed to reduce the time in

5 days, since the onset of exponential growth was given to the Ten days. For this stage it is observed that at 0 g/L $FeSO_4.7\ H_2O$ A maximum concentration of 4.41 $x10^7$ cell/ml with respect to the other concentrations is achieved, concluding the biological adaptation in the absence of $FeSO_4.7\ H_2O$. See *Fig 3.4.2.1.*

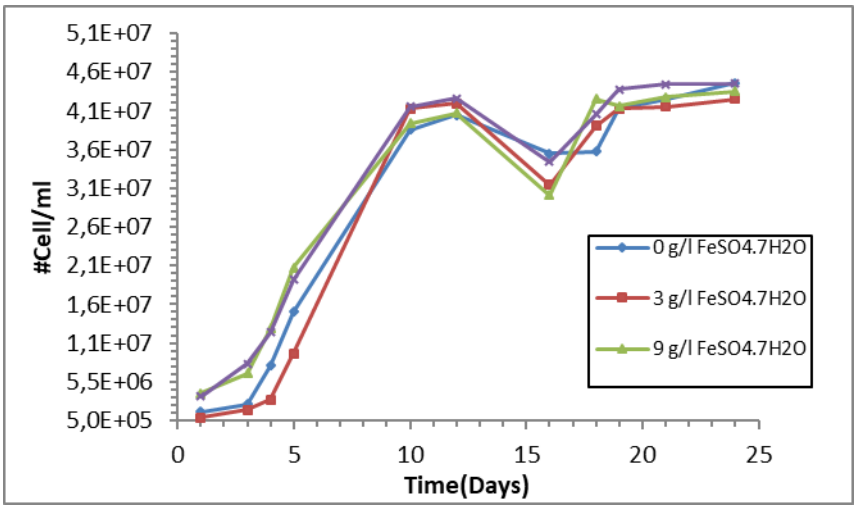

Figure 3.4.2.1. Biological growth during the second stage.

3.4.2.2 Copper Recovery (Cu).

In Fig 3.4.2.2. It is appreciated, that for the concentrations of $FeSO_4.7\ H_2O$ They tend a linear increase during the bioleaching stage, reaching a maximum recovery of 85.6% to 0 g/L $FeSO_4.7\ H_2O$, a 43.98% higher compared to the first stage that was able to recover only 59.45% copper to 0 g/L $FeSO_4.7\ H_2O$.

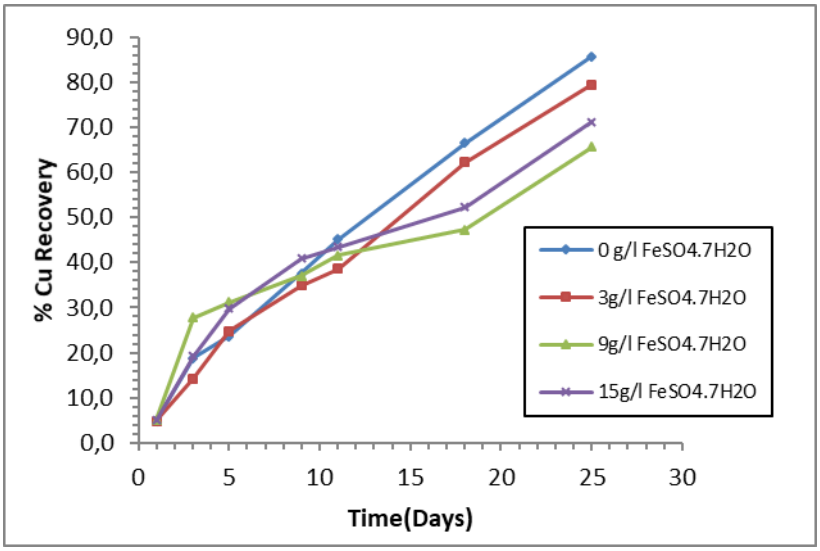

Figure 3.4.2.2. Percentage of copper recovery during the second stage.

3.4.3 Mineralogic characterization

The samples corresponding to this project have been studied using the light microscope polarized by the reflection method, for which previously the polished sections have been prepared and on the basis of this have been determined their respective members Mineralogical, that is to say, its characterization was made, the results obtained are specified below.

3.4.3.1 Analysis of the head mineral

The mineralogical characterization shows the presence and variety of compounds found by direct optic microscopy. See table 3.4.3.1. The metallogenic origin of the sample allows us to identify the way in which the main components of interest are found:

AURIFEROUS MINERALS: In this sample gold has been found in pyrite of a size of three microns. Likewise, gold was also found in the contact between covellite and bargains of a size of 5 microns.

ARGENTINE MINERALS: No silver minerals have been observed.
MINERALS OF FERRIFERS: Ferrous minerals include hematite, pyrite, arsenopyrite, pyrrhotite and goethite.
CUPRIFERO MINERALS: Copper mineral, chalcopyrite, covelite, chalcosite and tenantite.
OTHER MINERALS. In this group are the gangs (within these are the silicates and carbonates*).*

Table 3.4.3.1. Mineral compounds the mineral under investigation.

MINERALS	FORMULA	SHORTHAND.	Volumen, %	% Liberation
Pyrite	FeS_2	py	27.80	91.83
chalcopyrite	$CuFeS_2$	cp	0.10	0,00
Sphalerite	ZnS	ef	0.30	83.33
Arsenopyrite	$AsFeS_2$	apy	1.46	100.00
Pyrrhotite	$Fe_{1-x}S$	po	14.55	93.70
Goetite	FeO.OH	gt	9.95	90.49
Hematite	Fe_2O_3	hm	1.22	100.00
Marcasite	FeS_2	mc	1.22	100.00
Gold	Au	Au	0.01	0.00
Tennantite	$Cu_3AsS_{3,25}$	tn	0.02	0.00
Rutile	TiO_2	rt	0.63	38.50
Bargains		GGs	42.29	94.90

3.4.4. Degrees of release

The degrees of release presented by the minerals that have intervened in the modal analysis of the sample, indicate to us the different difficulties that they have encountered during the milling stage.

3.4.4.1. Interpretation of Degrees of release

According to the two tables shown above the interpretation of the degrees of release is as follows:
Pyrite occupies 27.80% of the total volume of the sample, of this volume 91.83% is free, while the remaining

8.17% is still interlaced.
The chalcopyrite occupies 0.10% of the total volume of the sample, of this volume the 100.00 is still interlaced.
Sphalerite occupies 0.30% of the total volume of the sample, of this volume 83.33% is free, while the remaining

6.67% is still interlaced,
The goethite occupies 9.95% of the total volume of the sample, of this volume 90.49% is free, while the remaining 9.51% is still interlaced.
The pyrrhotite occupies 14.55% of the total volume of the sample, of this volume 93.70% is free, while the remaining 6.30% is still interlaced.
The arsenopyrite occupies 1.46% of the total volume of the sample, of this volume the 100.00 is free.
Rutile occupies 0.63% of the total volume of the sample, of this volume 38.50% is free, while the remaining

61.50% is still interlaced.
The bargains occupy 42.29% of the total volume of the sample, of this volume 94.90% is free, while the remaining 5.10% is still interlaced.

3.4.5 Micrographic views

Below in Fig 3.4.5. the micrographs taken during the study are shown to be a reliable sample of the observed.

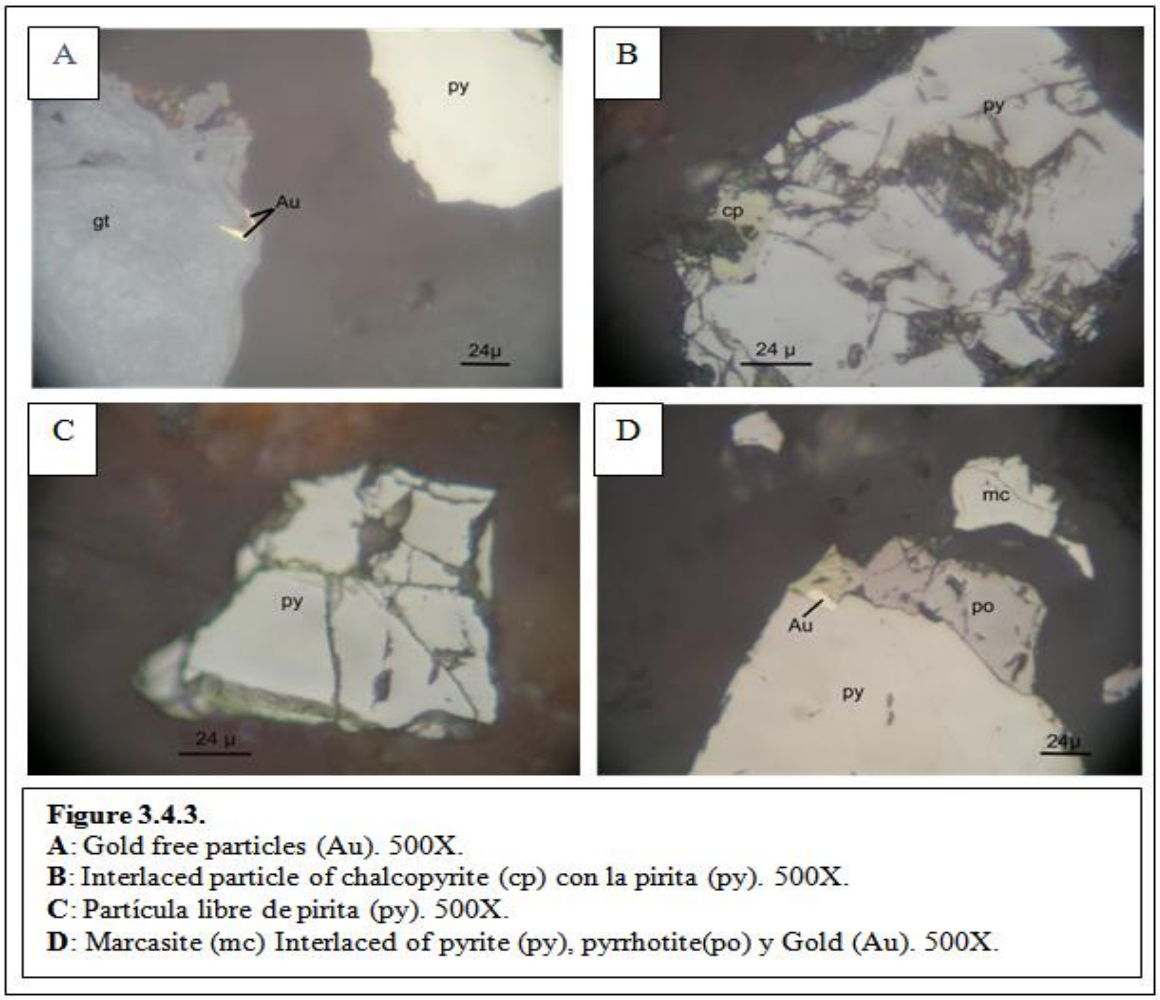

Figure 3.4.3.
A: Gold free particles (Au). 500X.
B: Interlaced particle of chalcopyrite (cp) con la pirita (py). 500X.
C: Partícula libre de pirita (py). 500X.
D: Marcasite (mc) Interlaced of pyrite (py), pyrrhotite(po) y Gold (Au). 500X.

3.4.6 Mineralogy of the bioleached mineral.

The objective of this test is to show the physical change of the minerals by the effect of bioleaching, as well as the release of gold encapsulated in pyrite, chalcopyrite and arsenopyrite minerals. The mineralogy is from the tailings of the ore (see Table 3.4.4.) after being subjected to bioleaching. The sections shown are the treatment given to the samples to perform the microscopic study.

Table 3.4.6.1 Mineralogy to the tailing of the first stage of bioleaching at 06 g/l $FeSO_4.7H_2O$.

MINERALS	FORMULA	Shorthand.	Volumen, (%)	% Liberation
Pyrite	FeS_2	py	24.16	97.63
chalcopyrite	$CuFeS_2$	cp	0.40	99.50
Sphalerite	ZnS	ef	0.40	100.00
Arsenopyrite	FeAsS	apy	0.20	100.00
Pyrrhotite	Fe0.8-1 S	po	1.00	100.00
Goetite	FeO(OH)	gt	17.98	96.78
Hematite	Fe_2O_3	hm	0.00	0.00
Marcasite	$FeO.Fe_2O_3$	mt	0.50	80.00
Gold	Au	Au	0.00	0.00
Ilmenite	Fe_2TiO_3	Il	0.10	0.00
Rutile	TiO_2	rt	0.20	100.00
Bargains		GGs	56.06	99.89

Pyrite occupies 24.16% of the total volume of the sample, of this volume 97.63% is free, while the remaining 0.37% remains interlaced, the reason why it has not been fully released is due to the different geometric types of interlacing in which they are immersed.

Chalcopyrite occupies 0.40% of the total volume of the sample, of this volume 99.50% is free, while the remaining 0.50% remains interlaced, the reason why it has not been fully released is due to the different geometric types of interlacing in which it is immersed.

Arsenopyrite occupies 0.20% of the total sample volume, of which 100.00% is free. The gold is in the order of traces, which is why the volume has been considered 0.00% compared to other mineralogical species.

3.4.6.1. Mineral tailings micrographs.

The figures shown (*Fig 3.4.4.1.)* show cracks and changes in structure that facilitate the release of many species of minerals and gold particles.

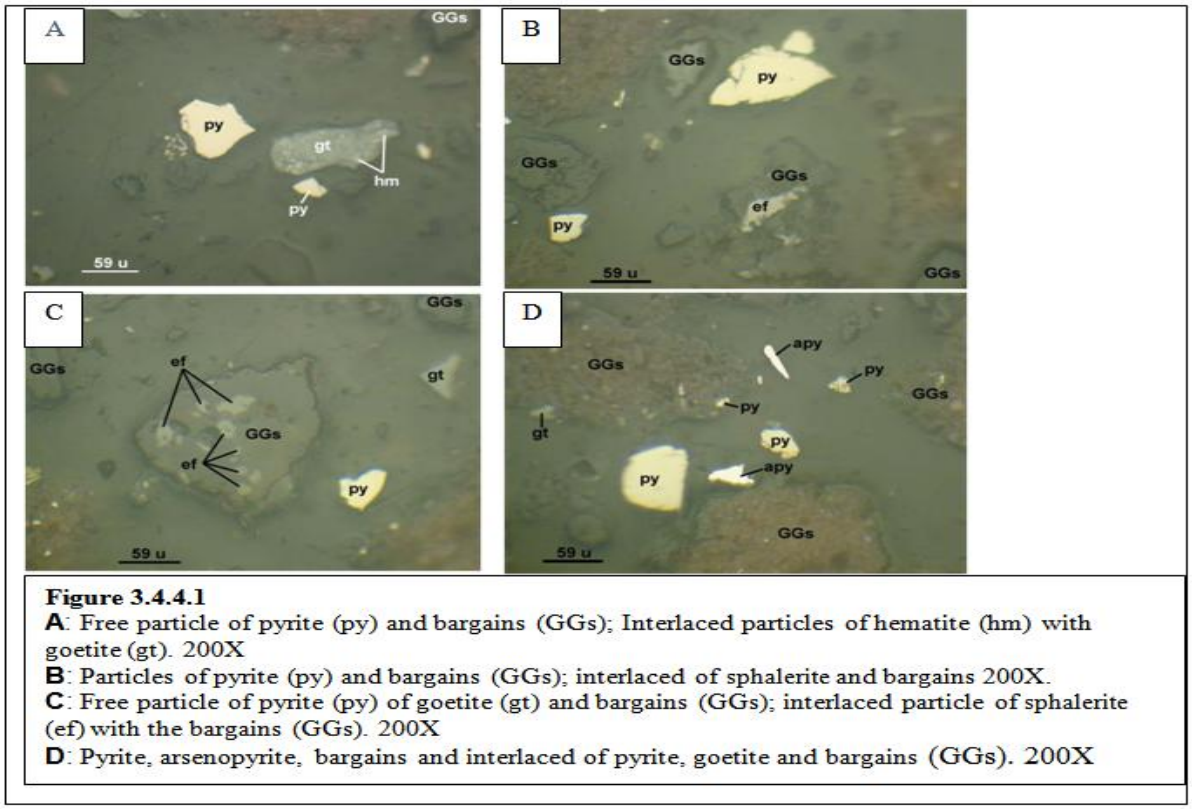

Figure 3.4.4.1
A: Free particle of pyrite (py) and bargains (GGs); Interlaced particles of hematite (hm) with goetite (gt). 200X
B: Particles of pyrite (py) and bargains (GGs); interlaced of sphalerite and bargains 200X.
C: Free particle of pyrite (py) of goetite (gt) and bargains (GGs); interlaced particle of sphalerite (ef) with the bargains (GGs). 200X
D: Pyrite, arsenopyrite, bargains and interlaced of pyrite, goetite and bargains (GGs). 200X

4 CONCLUSIONS

1. The acid drainage of the studied mining work (Huancavelica), presents acidity in a range of 3.0 to 4.5 pH, with a significant amount of metals in solution and a diversity of microorganisms among which is the bacterium Acidithiobacillus Ferroxidans.
2. The formulation used as a modified 9K culture medium was satisfactory to achieve the isolation and growth of the bacterial strain. Its use as a means of isolation is limited to the type of microorganisms accompanying and its ability to adapt to new conditions which also increases with the number of reseeding.
3. Optimal biological growth is achieved in successive stages of adaptation in nutrient substrates and by the gradual increase of pulp density, directly related to the oxidation of metal sulphides.
4. During the evaluation of the influence of the concentration of Fe (II) was determined that with 06 gr / L of $FeSO_4.7\ H_2O$ is achieved to extract 72.64% copper at the point of maximum growth of the bacterium, 4.75 x107cell/ml. During the second stage with the absence of $FeSO_4.7\ H_2O$, it is possible to extract 85.6% of copper at the maximum growth point of the bacterium, 4.41 $x10^7$ cell/ml.
5. The high bacterial growth achieved in substrates with a certain amount of iron does not guarantee the achievement of an optimal catalytic process during the confrontation with sulphide minerals, less the best copper recovery.
6. The biooxidant effect as an indirect mechanism of leaching has been revealed by the identification of gold particles in the micrographs of the treated material.
7. The presence of bargains interferes in the stages of adaptation and bioleaching of minerals, manifesting in the identification of population growth and oxidation of substances, as in the dissolution of copper and

arsenic.

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