Revista Latinoamericana de Microbiología (1999) 41:273-278
Asociación Latinoamericana de Microbiología

Gold Recovery from Arsenopyrite Ores by using an Arsenic-Resistant *Thiobacillus ferrooxidans* Strain.

ERASMO ORRANTIA-BORUNDA,[1,5] SIGIFREDO ARÉVALO,[2] CARLOS CERVANTES,[3] LUIS J. GALÁN WONG,[1] HIRAM MEDRANO-ROLDÁN,[4] AND BENITO PEREYRA-ALFÉREZ[1]*

Departamento de Microbiología e Inmunología. Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo León.[1] *Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua.*[2] *Instituto de Investigaciones Químico Biológicas, Universidad Michoacana.*[3] *Instituto Tecnológico de Durango.*[4] *Present address CIMAV, Chihuahua, Chih., México*[5]

*Autor para la correspondencia: A. Postal 2990. San Nicolás de los Garza, N. L. CP. 66450. México.
Phone: (52 8) 376-2813. Fax: (528) 376-2813; 376-4537. E-mail: bpereyra@ccr.dsi.uanl.mx

ABSTRACT. Twenty-nine strains of *Thiobacillus ferrooxidans* from acid mine drainage were isolated . Based on their natural resistance to arsenic, we choose one which was resistant to 50 mg/l of arsenic and it was called T1. This strain was subject to selective pressure through successive cultures using since 100 to 2000 mg/l of arsenic. We select one strain resistant to 1800 mg/l, named T18. Both strains were evaluated in gold recovery from ores containing high level of pyrite and arsenopyrite. These mineral concentrates were subject to analytical analysis and percentage distribution of arsenic, iron and gold. Our results showed that these elements were mainly associated, to particle size less than 38 mm containing 2.1% As; 28.4% Fe and 9.8 g/ton Au. The metabolic activity, measured as an increase in arsenic and ferric iron in solution, was almost five times higher in the arsenic-resistant strain than native one almost five times. Bioleaching results showed that gold recovery was 6 and 10-folds higher with T1 and T18 than with the non-bioleached mineral, respectively. These results show that the gold recovery from refractory minerals can be increased by using arsenic-resistant strains.
Key Words: *Thiobacillus ferroxidans*, bioleaching.

RESUMEN. A partir de agua de minas logramos aislar 29 cepas de Thiobacillus ferrooxidans y en base a la resistencia natural a arsénico, seleccionamos una llamada T1, la cual fue resistente a 50 mg/l de arsénico. La cepa T1 fue sometida a presión selectiva a través de cultivos sucesivos usando arsénico desde 100 a 2000 mg/l, logrando seleccionar una resistente a 1800 mg/l, denominada T18. Ambas cepas fueron evaluadas para recuperar oro a partir de minerales con alto contenido de pirita y arsenopirita. Estos concentrados de mineral fueron analizados para conocer el tamaño de partícula y la distribución de arsénico, fierro y oro. Nuestro resultados mostraron que estos elementos estaban asociados, principalmente, a partículas menores de 38 mm, las cuales contenían 21% de As, 28.4% de Fe y 9.8 g/tonelada de Au. La actividad metabólica, medida como un incremento de arsénico y fierro en solución, fue casi 5 veces mayor en la T18 que en T1. Los resultados de la biolixiviación mostraron que la recuperación de oro fue 6 y 10 veces mayor con la T1 y T18 que con el mineral no lixiviado. Estos resultados muestran que la recuperación de oro a partir de minerales refractarios, puede ser incrementado usando cepas resistentes a arsénico.
Key Words: *Thiobacillus ferroxidans*, bioleaching.

INTRODUCCION

Gold usually occurs in nature in elemental form, alloyed with silver or in association with other minerals. Gold mainly occurs in three distinct categories of ore types: free-milling ores, base-metal ores and refractory ores. Refractory precious ores, in which gold and silver are finely disseminated in sulfide minerals, such as pyrite (FeS_2) and arsenopyrite (FeAsS) are becoming of greater interest and importance to the mining industry, due to a depletion of simple, free-milling ores and to a significant increase in the price of gold.[7,8]

Gold recovery from refractory ores requires that the these be subject to a pretreatment to liberate the gold. The pretreatment is usually an oxidation step and can be performed by several methods, such as roasted to remove arsenic and to break down the gold-bearing sulfides to render the ores amenable to cyanide process.[2,10] However, these traditional methods are often no longer satisfactory, due to economical and environmental considerations. Recently, many efforts have been addressed to develop alternative methods for refractory ore treatment. The greatest interest in the biological treatment of gold ores relay on the oxidation of sulfide refractory ores to liberate the precious metals in agitated

reactor systems; however, the extreme conditions of pH and soluble concentration of metals such as silver, mercury, cadmium, and mainly arsenic (due to the presence of arsenopyrite) can be a limiting step for the biological breakdown of these ores.[2,10,11]

Several types of microorganisms are known to be able to derive energy from the utilization of sulfide minerals, metabolic active under extreme pH conditions and soluble heavy metal concentrations.[12,18] Among this ones *Thiobacillus ferrooxidans,* a chemolitotrophic and acidophilic bacteria, has been the most used microorganism for mineral oxidation and leaching applications.[7,15] This bacterium is frequently active wherever oxygenated waters contact exposed sulfide mineral deposits, such as at mine sites and tailings pilets; this reaction breaks down the sulfide minerals, releases metals in solution and, usually, increases the acidity of the solution. *T. ferrooxidans* may be used therefore to break down gold bearing sulfides, such as pyrite and arsenopyrite liberating the precious metal.[1,4]

Several mines located at North of México have serious problems, because the gold is associated with pyrite and arsenopyrite. Under these conditions the arsenic content and other heavy metals inhibit the bioleaching process.[2,13,14] In this paper we report the isolation of an arsenic-resistant strain of *T. ferrooxidans* which increased the gold recovery from pyrite-arsenopyrite ores.

MATERIAL AND METHODS

Isolation and characterization of *T. ferrooxidans* strains. *T. ferrooxidans* strains were isolated from an acid drainage samples from a domestic mining located at the Northeast of Mexico. Samples, 10 ml, were inoculated in Erlenmeyer flasks containing 90 ml of 9K basal salts liquid-medium plus $FeSO_4$ (9KF medium) and incubated 8 days at 30°C with constant agitation 175 rpm.[17] Samples of 1 ml were withdrawn and plated on 9K pH 2.6-3.0 modified medium and incubated 7-8 days at 30°C.[16] Typical *T. ferrooxidans* colonies were isolated and identified as mentioned elsewhere.[5] Isolated colonies were plated on 9K medium supplemented with 50, 100, 200 and 300 mg/litre of sodium arsenite (m-$NaAsO_2$) Those colonies growing at higher arsenic concentrations were inoculated in on a sucesive serial 250 ml Erlenmeyer flasks containing 50 ml 9KF medium supplemented with increased sodium arsenite amounts (200 to 2,000 mg/litre). The flasks were inoculated with 5×10^8 cells/ml of actively growing cells and incubated as mentioned before. One ml was taken and viability observed on Petri dishes containing same arsenic concentration. The arsenic resistance was evaluated by the maximum arsenic concentration at which bacteria growth was observed.

Resistance to heavy metal. Growth inhibition tests were performed for each strain by measuring the heavy metal resistance at 30°C in 9KF, containing different amounts of the follow salts: copper sulfate, cadmium chloride and sodium molybdate, 50-200 mg/liter; mercury chloride, 10-15 mg/liter and silver nitrate, 1-5 mg/liter.

Mineral Sampling. The refractory concentrate used in this study was obtained from a domestic mining site located at Northeast from Mexico. A sample of 50 kg of ore with a size particle about 10 mesh (1.70 mm size particle), was dried and homogenized in a Sepor Precision Sampler and pulverized in a Disc/Plate Pulverizer (Bico-Braun); the mineral was passed through different mesh screens to a final mesh of -400 (50%). The presence of pyrite and arsenopyrite and other components in the concentrate was determined by megascopic and mineragraphic analysis, X-Ray diffraction, and chemical analysis. The total analysis of the sample agreed closely with the formula for pyrite (FeS_2) and arsenopyrite (AsFeS) with regard to the ratio of iron to sulfur and arsenic, as well as compounds like quartz, lime and other sulfides.

Leaching of pyrite-arsenopyrite concentrate by *T. ferrooxidans*. The pyrite-arsenopyrite concentrates (-400 mesh at 50%, 10 % weight/volume pulp density) were leached in 1,000 ml conical flasks with 250 ml of water at 30°C, and 175 rpm. Actively growing cells of T1 and T18 strains (25 ml, 108 cells/ml) were added separately to flasks containing pyrite-arsenopyrite concentrate. The cell numbers in the mixture were determined by using a Petroff-Hausser bacteria counting chamber, and a phase contrast microscope. Uninoculated flasks served as sterile controls. After a brief equilibration period (2 h), an initial sample of 5 ml was taken, centrifuged and analyzed for total soluble iron, ferric iron, ferrous iron, total arsenic and pH, as described below. The time course was continued with periodic sampling each 24 h for 21 days.

Determination of total arsenic and iron oxidation. In order to determine arsenic and iron levels, periodic sampling from leached and non-leached liquors was carried out; 3 ml were dissolved in a final concentration of 3N HCl, and analyzed by atomic absorption in a Perkin-Elmer spectrophotometer Mod. 3110. For the Fe^{+2} analysis, 1 ml of liquor was dissolved in 5 ml of diluted H_2SO_4 1:1, and titrated with 5 mM $KMnO_4$ as previously mentioned.[6,10,13] The Fe^{+3} was determined adding 1 ml of liquor to 1 mL of 6N HCl and 1 ml of 50% sodium-acetate, titrating with 0.01 N ascorbic acid, 0.01 N EDTA disodium salt, using Bindschedler's green leuco base as indicator. pH determinations were made by routine procedures.

Cyanide process. After the bioleaching process, each ore was filtered in order to remove the arsenic and iron present in the bioleaching liquor, washed and dried at 60°C. Samples of 10 g were placed in 150 ml of a sodium hydroxide solution pH=10.5, and subjected to a leaching by using a modified method from Habashi,[10] adding 0.5 % NaCN, during 16 h at 175 rpm; the same procedure was done with the non-bioleached mineral that served as a control. Solubilized gold was determined by atomic absorption spectrophotometer. To confirm the gold recovery, the metal remaining in each residue, was determined using fire assay.

Revista Latinoamericana de Microbiología (1999) 41:273-278
Asociación Latinoamericana de Microbiología

RESULTS AND DISCUSSION

Isolation and characterization of a native and an arsenic-resistant *T. ferrooxidans* strains. Twenty nine independent strains of *T. ferrooxidans* derived from three different mining sites were isolated. Notwithstanding, the *T. ferrooxidans* strains were isolated from acid drainage of mineral deposits containing arsenopyrite, it was interesting to find out that 4 strains were unable to growth at 50 mg/liter of arsenic. However, we did not find any native microorganism able to growth at concentrations higher than 200 mg/liter of arsenic (Table 1). The isolate growing at 200 mg/liter was named T1, and was subjected to arsenic-selective pressure and a strain named T18, which was able to grow even at 1,800 mg/liter was derived. The doubling times for T1 and T18 strains growing on 9KF, were 12 and 11 h respectively.

The ability of *T. ferrooxidans* to growth on media containing heavy metals is an inherent characteristic of this bacteria. In this sense, both strains showed a very similar growth inhibition by salts of Cu, Cd, and Mo at 200 mg/liter and by silver at 5 mg/liter. However, the mercury inhibited to T1 but not to T18 strain at 15 mg/liter of mercuric chloride. This fact is very important because main *T. ferrooxidans* strain are inhibited by this metal even at less than 0.5 mg/ml.[15] At the present we do not know the reason why the arsenic-selective pressure modified the mercury-resistance of T18 strain. The resistance to several heavy metals play a very important role, because sometimes one of these elements can abolish the activity of *T. ferrooxidans* even more than arsenic.[2,7]

Chemical analysis of the refractory ore. In order to determine the distribution of gold, arsenic, iron and other heavy metals across the refractory mineral, the concentrate was subject to a mesh screening. Figure 1 depicts that gold and arsenic are associated in an almost homogeneous distribution, whereas iron had a different distribution. The close association between gold and arsenic even at less than 38 μm (-400 mesh), allow us to conclude that gold is mainly present at the level of arsenopyrite crystals.

The microanalysis showed that 75% of gold was within arsenopyrite and the remaining 30% is in a free form (data not shown). These results are agree with those reported by Lawrence (1990) and Bosecker (1997) who reported ores containing higher pyrite contents, where the gold was disseminated within the mineral but at fine particle size. This event supports the usefulness of an improved bacteria with high resistance to arsenic in a bioleaching process of this refractory ore, because there is a relationship between the mineral oxidation and the amount of gold released.[9]

Bioleaching process. Because of the major operating costs of a biooxidation system are directly related to the amount of sulfur oxidized, the benefits of minimizing the quantity of sulfide oxidation are obvious. The partial selectivity in the oxidation of pyrite-arsenopyrite minerals, that gives to the biological treatment an advantage over the pre-

Table 1. Arsenic effect on the growth of *T. ferrooxidans* isolates[a]

Arsenic (mg/l)	Isolates	Growing (%)
50	25	86.2
100	7	24.1
200	1	3.4
300	0	0

[a] Concentration at which the growth could be observed

treatment alternatives.

T1 and T18 were tested for their ability for leaching the pyrite-arsenopyrite ore, followed by a cyanide process. The bioleaching process was carried out and the following parameters were measured in the leaching liquor: total iron, oxidation of ferrous iron, increase of ferric iron, solubilization of total arsenic content, and increase of pH. Initial pH in all flasks was 2.3, and in the inoculated flasks decreased to 1.5, due to sulfuric acid, generated as a by-product of bacterial metabolism.[7,10]

Since the main difference between T1 and T18 is the resistance to arsenic, and that the mineral had a high content of arsenic and the gold was associate with it (Table 2 and Fig. 1); this fact support our proposal that an improved arsenic-resistant strain could be helpful in the bioleaching process of this kind of refractory ores.

Results of the kinetics of the fig. 1 show that, there is a clear solubilization of total iron exclusively in the leached minerals, and a decrease of ferrous iron, concomitantly with an increase of ferric iron. However, T18 had a higher metabolic activity, increasing the solubility of total iron and the oxidation of ferrous iron, by 3 and 500-fold in relation to T1 and non-treated mineral, respectively.

These data suggest that even though both strains were

Table 2. Resistance of *T. ferrooxidans* strains to several heavy metals.[a]

Heavy Metal mg/liter	Mineral real content	Mineral in Biolixiviation	T1	T18
As	4238.4	424.46	200	1,800
Cu	50.00	5.13	200	200
Cd	30.00	3.34	200	200
Mo	40.0	4.03	200	200
Ag	7.50	0.82	<5	<5
Hg	9.00	0.97	<15	15

[a] Concentration at which the growth could be observed

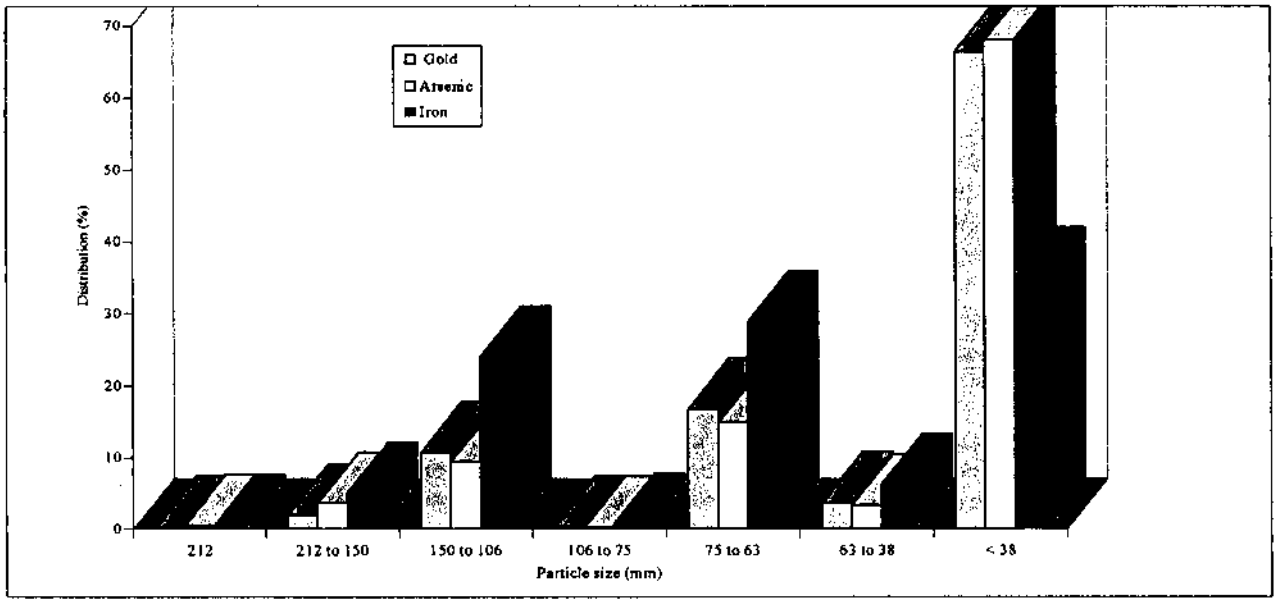


Fig. 1. Distribution of gold arsenic and iron in the mineral related with the particle size

able to oxidize iron, a toxic component of the concentrate may be responsible for the inhibition of metabolic activity of T1 strain. Since the main difference between T1 and T18 is the resistance to arsenic, and that the mineral had a high content of arsenic (Table 1 and Fig. 1), this supports our proposal that an improved arsenic-resistant strain could be helpful in the bioleaching process of this kind of refractory ores.

The results of Fig. 2 indicate that strains T1 and T18 are able to leach arsenic and interestingly, after a period of equilibration (about 12 days) there is a short period in the process, where the amount of solubilization of arsenic was raised in both strains, with no considerable changes over a long period of time. However, T18 strain is able to leaching about 1.6-fold more arsenic than T1. This suggests that T18 strain has a higher arsenic solubilization ability than T1, and that after a distinct limit of arsenic solubilization is reached, metabolic activity of both strains is stopped. One prediction is that the use of a *T. ferrooxidans* strain with an even higher arsenic resistance than T18, will have a better arsenic-solubilization ability but, essentially, with a similar behavior.

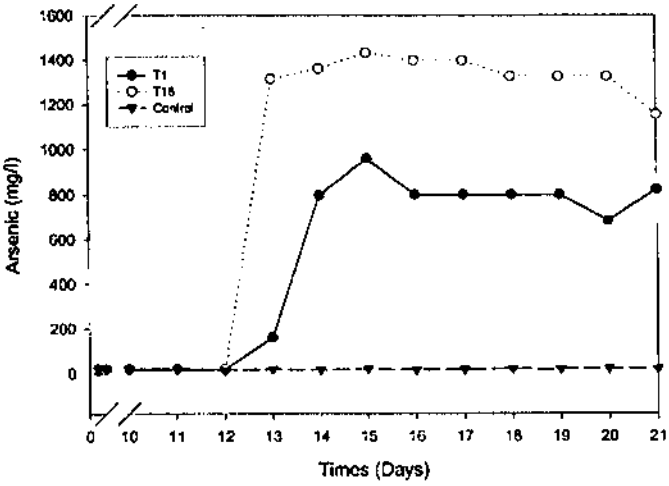
Cyanide process. The native and the arsenic-resistant *T. ferrooxidans* strains were used for the bioleaching process of a pyrite-arsenopyrite mineral, containing 9.8 g of gold per ton. The results showe that a wild type strain is able to produce 6-fold more gold recovery that the non bioleached mineral; moreover, using the arsenic-resistant strain a gold yield as high as 10-fold. This increase in gold recovery is economically important because in this mineral, a direct cyanide process is able to reach only 7.5 % recovery of total gold; this yield was enhanced to 45% with the native bacterium and to 75 % with the arsenic-resistant strain (Table 3). In this study we have used a pyrite-arsenopyrite mineral (in a ratio of 12:1, respectively), with 9.8 g/t of gold. The mesh analysis indicates that the gold is non-homogenous distributed, being present mainly in the arsenopyrite crystals. Although the biological iron oxidation is faster for arsenopyrite

Table 3. Gold recovery from ores containing high arsenopyrite content.

Strain	Gold Recovery (%)	Gold[1] (mg/liter)	Gold[2] (G/ton)	Yield
Control	7.5	0.74	9.3	1
T1	46	4.5	5.0	6
T18	76	7.35	2.5	10

[1] Gold in cyanide solution; [2] Remaining in residues

Revista Latinoamericana de Microbiología (1999) 41:273-278
Asociación Latinoamericana de Microbiología



Arsenic solubilization from ore by *T. ferrooxidans* strains T1 and T18.

than pyrite, the concomitantly solubilization of arsenic in this process, may be a disadvantageous effect, due to its potential inhibitory effect over the growth of the bacteria. In a bioleaching process, we have found that a native arsenic-tolerance *T. ferrooxidans* strain -isolated from the same source of the mineral-, is able to produce a 6-fold more gold yield that the non-treated mineral. Moreover, an arsenic selective pressure over the native strain, produce a series of improved strains, reaching as far as 1800 mg/liter of arsenic resistance. The use of one of the former strains increased the gold recovery almost twice than the native strain, producing a 75% of gold recovery, compared with a 7.5% of recovery with the non-bioleached mineral. We propose that the improved bacteria leaches better this kind of refractory mineral, due to the particular gold distribution within the mineral lattice.

According with the arsenic solubilization and gold recovery results, we estimate that in this particular mineral, the gold is mainly distributed across the arsenopyrite crystals, and that the use of an improved arsenic-resistant strain in the bioleaching process, can be helpful in an industrial process due to its partial selectivity oxidation over arsenopyrite. It is notorious that -whit both strains-, there is a sharp period of time in the bioleaching process where the ferrous iron-oxidation and the arsenic solubilization is abruptly increased, reaching its higher level; these levels do not change considerably, even at longer times of processing. If we considered that the gold recovery is proportionally related with the arsenic solubilization, this data can be very helpful to choose the best time to stop the bioleaching process, and to proceed with the cyanidation. These data must be corroborated at level of a fermentation process and/or a pilot plant, in order to know if is a technical and economical feasible, before to proceed to the gold recovery of this mineral at an industrial level.

AKNOWLEDGMENTS

We thanks to M. Monroy for microanalysis assays. E. Orrantia-Borunda was a recipient of a fellowship from CONACYT (85709). This research was supported in part by a Grant from Sivilla 9501100.

REFERENCES

1. Baldi, F., T. Clark, S. S. Polack and G. J. Olson. 1992. Leaching of pyrites of various reactivities by *Thiobacillus ferrooxidans*. Appl. Environ. Microbiol. 58:1853-1856.
2. Bosecker, K. 1997. Bioleaching: metal solubilization by microorganisms. FEMS Microbiol. Rev. 20:591-604.
3. Gehrket, T., J. Telegdi, D. Thierry and W. Sand. 1998. Importance of extracellular polymeric substances from *T. ferrooxidans* for bioleaching. Appl. Environ. Microbiol. 64:2743-2747.
4. Habashi, F. 1967. Kinetics and mechanism of gold and silver dissolution in cyanide solution. Department of Metallurgy. Montana College of Mineral Science and Technology. Bulletin 59.
5. Kelly, D. P., and A. P. Harrison. 1989. Genus Thiobacillus, Beijerinck 1904b, 597A, In: J.T. Staley, M.P. Bryant, N. Pfennig, and J.G. Holt (ed.), Bergey's Manual of systematic bacteriology, vol. 3 The Williams & Wilkins Co., Baltimore.
6. Krebs, W., C. Brombacher, P. Bosshard, R. Bachofen and H. Bramdl. 1997. Microbial recovery of metals from solids. FEMS Microbiol. Rev. 20 :605-617.
7. Lawrence, R. W. 1990. Biotreatment of gold ores. In: H. L. Ehrlich and C.L. Brierley (Ed.) Microbial Mineral Recovery. McGraw-Hill, Inc. N.Y. pp. 127-148.
8. Lawrence, R. W., and A. Bruynesteyn. 1983. Biological preoxidation to enhance gold and silver recovery from refractory pyrite ores and concentrates. CIM Bull. 76:197-110.
9. Monroy. M.G., M.A. Dziurla, B.-T. Lam, J. Berthelin and P. Marion. 1994. A laboratory study on the behavior of *Thiobacillus ferrooxidans* during pyrite bioleaching in percolation columns. In: E. Galindo and O.T. Ramirez (Ed). Advances in Bioprocess Engineering. Kluwer Academic Press. Netherlands. Pp. 509-517.
10. Monroy F. M. G., C. Mustin, P. de Donato, O. Barres, P. Marion, and J. Berthelin. 1995. Occurrences at mineral-bacteria interface during oxidation of arsenopyrite *by Thiobacillus ferrooxidans*. Biotechnol. Bioeng. 46:13-21.
11. Norris, P.R., Parrot, L.M., and R.M. Marsh. 1986. Moderately thermophilic mineral-oxidizing bacteria, in H. L. Ehrlich and D. S. Holmes (eds.). Biotechnology for the Mining, Metal-Refining, and Fossil Fuel Processing Industries, Biotechnology and Bioengineering Symp, No. 16, Wiley, New York. pp. 253-262.

12. Norman, P. F., and C. P. Snyman. 1988. The biological and chemical leaching of an auriferous pyrite/ arsenopyrite flotation concentrate: A microscopic examination. Geomicrobiol. J. 6:1-10
13. Orrantia-Borunda, E. 1997. Aislamiento y caracterización de cepas de *Thiobacillus ferrooxidans* con alta resistencia de arsénico y su utilización en la recuperación de oro a partir de concentrados de pirita y arsenopirita. Tesis de Doctorado. FCB/UANL. México.
14. Rawlings, D. E. and T. Kusano. 1994. Molecular genetics of *Thiobacillus ferrooxidans*. Microbiol. Rev. 58 :39-55
15. Shiratori, T., Inoue, C., Numata, M., and T. Kusano. 1991. Characterizacion and cloning of plasmids from the iron-oxidizing bacterium Thiobacillus ferrooxidans. Current Microbiol. 23:321-326
16. Silverman, M. P., and D. G. Lundgren. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. J. Bacteriol. 77:642-647.
17. Wiegel, J., and L. G. Ljungdahl. 1986. The importance of thermophilic bacteria in biotechnology. Crit. Rev. Microbiol. 3:39-108.