MINE, WATER & ENVIRONMENT. 1999 IMWA Congress. Sevilla, Spain

BIO-DESHYDROMETALLURGY

Nora H. Paños

LEACHING S.R.L.
Dr. R. Driollet 306 – Norte
5400 San Juan, República Argentina
Fax: + 54 264 4265395
e-mail: norap@unsj.edu.ar

ABSTRACT

Bio-deshydrometallurgy is the denomination assigned by LEACHING to its biological processes for oxidizing metals sulphides (insoluble) to sulphates or oxides (soluble). Bio-deshydrometallurgical processes are based on studies of physiological characteristics, conditions of growth and development of microorganisms related to the oxidation of metallic compounds. The essential fundamentals of these processes are based on the discovery of the involved microorganisms behaviour respect to water. In effect, in low water content media the microorganisms are stablely attached to metal sulphides. The microbial metabolism is activated increasing considerably the multiplication velocity. Bacterial generation times are about 20 minutes. The microorganisms multiply remaining temporally occluded into the solid bio-oxidized products. During and at the end of the bio-oxidation operation, the biooxidized products are obtained in solid state. In these conditions the microorganisms solve the barrier of large processing times and the problems derived from the dissolution of oxygen and carbon dioxide in the liquid phase of the known Bio-hydrometallurgical processes.

According to the prior features, the Bio-deshydrometallurgical processes have been patented by LEACHING in different countries (United States of America, European Economic Community, Japan,, Russian Federation, between others).

The objective of this communication is to present the fundamentals of an innovative technology for being applied to metals extraction from minerals ores or their concentrates, refractory gold treatment and coal desulphurization, with environmental, technical and economical advantages: low water consumption, particularly attractive when water availability is a limiting factor; they are clean processes of very low environmental impact; the short bio-oxidation times (hours or days) allow the fine regulation and control of the systems for a constant production; lower investment and operation costs than conventional metallurgical technologies.

For example, the main results of a pilot plant operation for fast bio-oxidation of different copper concentrates are: Bio-oxidation yield: 80 – 99 %, including chalcopyrite concentrates; Bio-oxidation time: 24-48 hrs; Bio-oxidation cost: U$S 30-50/tn of concentrate; Copper concentration in washing solutions of bio-oxidized concentrates: 40-80 g/l; Iron concentration in washing solutions of bio-oxidized concentrates: 100-500 p.p.m, including chalcopyrite concentrates; Direct electro-obtention. Current efficiency : 86-96%.

INTRODUCTION

Bio-hydrometallurgical processing of metals sulphides ores or concentrates has been in later years looked as an alternative to roasting or smelting, which produce large amounts of sulphur dioxide (SO_2), one of the main compound responsible for acid rain and require very large inputs of energy (the processes occur at about 1000 ºC) obtained through the combustion of fossil fuels. Furthermore, the exploitation of most and particularly new deposits don't justify the high investments cost of smelters.

Bio-hydrometallurgy has arise world-wide interest in the last years because of its potential advantages: low energy consumption, low chemical reagents consumption and low investments costs, being clean processes of low environmental impact (Biogeotechnology of Metals. Manual, 1988). Mining in the future must recognise that successful economic developments depends on the rational exploitation of resources while minimising the adverse environmental impacts of development projects.

The discovery of microorganisms capable to use metals sulphides, sulphur, some insoluble oxides and ferrous iron as energetic substrates, oxidizing them to soluble compounds made way to the possibility to apply biotechnology to: metals extraction from minerals or their concentrates, bio-oxidation of refractory gold and coal desulphurization. Bio-hydrometallurgical methods as Heap, in-Situ or Dump leaching have been applied to minerals, while Stirred tanks or Pachuca reactors have been developed particularly for concentrates treatment (Biogeotechnology of Metals. Manual, 1998; Biohydrometallurgical Processing, 1955). All of them are carried out in a three-phase system, consisting of a solid phase (the mineral component), a liquid phase (the leaching liquor carrying the microorganisms) and a gaseous phase (air sometimes enriched with carbon dioxide), being oxygen and carbon dioxide dissolved in the liquid phase for microbial utilisation, since the microflora is aerobic and autotrophic.

Even important efforts have been done in all the potential Bio-hydrometallurgical applications, only refractory gold bio-oxidation and copper recovery from low grade deposits or tails have been cost-effective applied and considered as "showcase" technologies (Lawrence and Pouling, 1995). The successful application of the Gencor biooxidation process BIOX (Van Aswegen et al., 1991; Dew, 1995) for pre-treatment of refractory gold ore concentrates has been a milestone, establishing credibility for use of bacterial processes (Lawrence and Pouling, 1995). However, as they have assumed (Dew,1995), the overall process performance in terms of gold recovery and required plant retention may limit the application of extreme operation conditions.

The relative scarcity of cost effective applications are due to some Bio-hydrometallurgical technologies barriers, which had not been overcame.

The requirement of large processing times, from several months to years in order to obtain acceptable recoveries had been attributed to an intrinsically low multiplication velocity of the responsible bacteria. The low leaching velocity requires operating with large mineral ores masses that, in general, are subject to climatic variations, preventing the precise control of the systems resulting in variable and unpredictable processing times or recoveries. Furthermore, Heap, in-Situ or Dump leaching demand high water consumption. Leaching solutions are continuously irrigated with important losses of water by evaporation, being a serious problem in mining regions characterised by water scarcity.

In the cases of Stirred reactors, it has been shown that bioleaching rates in S.T.R.'s of monometallic mineral sulphide concentrates such as sphalerite (Torma et al., 1970), chalcopyrite (Sakaguchi and Torma, 1976), pyrite from coal (Hoffman et al., 1981; Loi et al., 1994), or even sulphides from refractory gold (Dew, 1995), markedly decrease when the solids concentration in the pulp, defined as percent ratio of the dry solid to the mass of the liquid forming the suspension, exceeds 15-20 %. It has been strongly suggested that at solids concentrations of over 20%, collisions and sliding between solids particles occur resulting in the detachment and damage of microbial cells adhering to their surfaces.

By another way, in order to attain acceptable oxygen transfer rates and oxygen utilisation in the bio-reactor, the air supplied must be well dispersed by mechanical agitation. Nevertheless, the solids content of the pulp has a direct influence on the oxygen mass transfer rate (Escobar et al., 1995).

The prior effects of solid concentration has been observed regardless of bio-reactor type, be it an S.T.R., a Pachuca reactor or a shake flask (Mills et al., 1987). The development of the Biorotor (Loi et al., 1995) has been an effort for increasing the gas mass transfer coefficient in a Bio-hydrometallurgical process. The Biorotor allow up to 30% solids concentration.

Two of the most important consequences of the prior negative effects, are the large tanks volumes and the power requirements for stirring the pulp. Different authors have shown the potentiality of microbial coal desulphurization (Hoffman et al., 1981; Loi et al., 1994; Dugan and Appel, 1978), but the industrial application has not been possible due to the large processing times associated to the prior technical-economical barriers.

All the Bio-hydrometallurgical methods have common features. During the biooxidation operation, two phases clearly present are separable: the solid phase constituted by the mineral ore or concentrate to be treated and a liquid phase constituted by the leach liquors, while the microorganisms (one micron in size), are confined to live and take oxygen and carbon dioxide from the dissolved in the liquid phase.

The first stage of interaction of bioleaching bacteria with an insoluble inorganic substrate as metals sulphides, consists in the bacterial attachment to the surface, where upon the subs-

trate being oxidized is attacked biochemically (Karavaiko et al., 1988; Schaeffer et al., 1963). The attachment is specific to the mineral compounds which offer a source of energy, but such attachment not always occur in Bio-hydrometallurgical systems. The conditions which allow or facilitate an stable and efficient attachment, enabling the bacteria to transform the substrate and multiply quickly, had not been explained.

Bio-hydrometallurgy has not developed its own systems based on the knowledge of the physiology and specific requirements of the metals sulphides oxidizing bacteria, which are the real actors of the transformations, but has resorted to those used in another technologies as others microbial industrial processes or borrowed from chemical engineering.

The study of physiological characteristics, conditions of growth and development of the microorganisms related to the oxidation of metallic compounds, applied to the solution of the above mentioned technological problems, constitutes an important research area.

The objective of this paper is to present the microbial fundamentals and the main features of the Bio-deshydrometallurgical processes, internationally patented by LEACHING.

MICROBIAL GROWTH STUDIES

Preliminary studies in ferrous agarized medium

Thiobacillus ferrooxidans is the bacterium most commonly used in Biometallurgy. Although microorganisms like *T. ferrooxidans* use numerous insoluble sulphur compounds in addition to ferrous iron, the solubility of ferrous iron has encouraged its use in agarized media for laboratory cultures. The culture of *T. ferrooxidans* in solid agarized medium had presented problems in the past for those desiring to obtain colonies.

Several solid ferrous media have been designed so far. All these media support the development of colonies. However, the colonies are small, slow growing (between one and six weeks) and sometimes repetitive results are not obtained. These difficulties had been attributed to the low bacterial multiplication velocity. Besides, agar or the hydrolysis products of agarose, used as gelling agents, were thought to inhibit bacterial growth.

The following experiments and observations were done:

- Petri plates prepared with ISP ferrous medium (Manning, 1975) and agarose 0.5% (W:V), were inoculated with the strains ATCC 19859, and two strains of T. ferrooxidans called BA_1 and BA_2 , isolated from mineral ores samples coming from the Bajo de la Alumbrera deposit. The plates were incubated at 30 ºC and examined each six to eight hours by stereomicroscopy. For a period of approximately forty days, there was no evidence of growth. On the day the colonies appeared, the beginning of growth was observable by stereomicroscopy, and after a few hours, colonies 0.5-1 mm in diameter were clear to direct observation. If a forty-day period should be required to obtain colonies due to an intrinsically low bacterial multiplication velocity, it follows that growth must have been progressive during all the period.
- Petri plates placed on a slightly inclined plane were prepared with agarized ISP medium in order to obtain a thickness gradient of the agarized culture medium. Thus, the culture medium is thickest at one edge of the plate and thinnest at the diametrically opposed edge. The plates were inoculated by touching a location on the thicker edge with a loop holding a liquid inoculum, as it is indicated with an arrow in Figure 1. There was no evidence of development prior to the day which colonies were formed, and the colonies developed on the thinnest edge. In the case shown in Figure 1, some colonies were formed even on the thin film of medium deposited over the side wall. It must be taken account that the thinner a gel is, the quicker it is dehydrated. Furthermore, the inoculated bacterial cells have move from one edge to the other, prior to be attached and to develop colonies.

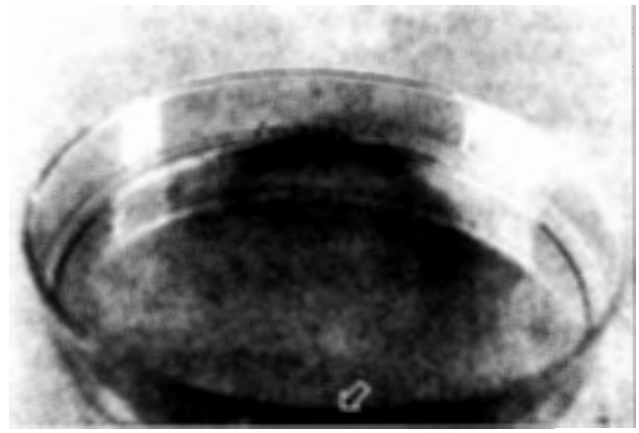

Figure 1. Colonies development on the deshydrated thinnest edge of a plate. The arrow indicates the inoculation place on the thickest edge of culture medium.

- The essential condition for colonies formation in a solid agarized medium is the cells attachment to the agarized medium. A great number of test were made varying agar or agarose concentrations and analysing the growth according the above guidelines. It was concluded that agar or agarose does not govern the attachment of these bacteria at the inoculation place as it happens with other bacteria.
- The conditions that may spontaneously vary with time in a solid agarized medium containing approximately 95 % of water are the loss of water by natural evaporation and consequently the increase of the component salts concentration in the medium. Test were carried out varying the component salts concentrations according to wide gradients, without observing a meaningful effect

on the colonies formation time, and without any effect on the cells attachment to the inoculation place. Everything indicated that cells attachment to the substrate requires a very low water content.

- In tests with plates 9 centimetres in diameter containing equal volumes of culture medium, the dehydration degree was increased by subjecting the plates to a laminar flow hood and/or keeping the plates at 30 ºC for the spontaneous loss of water prior to inoculation. A considerable decrease in the colonies appearance time was obtained. Using techniques combining the above strategies with the addition of chemical agents known as dehydrating agents, as polyethyleneglycol, it was possible to obtain colonies in twelve to twenty-four hours as the shown in Figure 2. Star shaped colonies of *T. ferrooxidans* had been described (Harrison, 1984), but for observing them it was required a 20X magnification in an stereomicroscope and were obtained after several days of incubation. In dehydrated medium the colonies were already 10 mm in size after incubation overnight. It was clearly suggesting a high multiplication velocity in media of low water content.

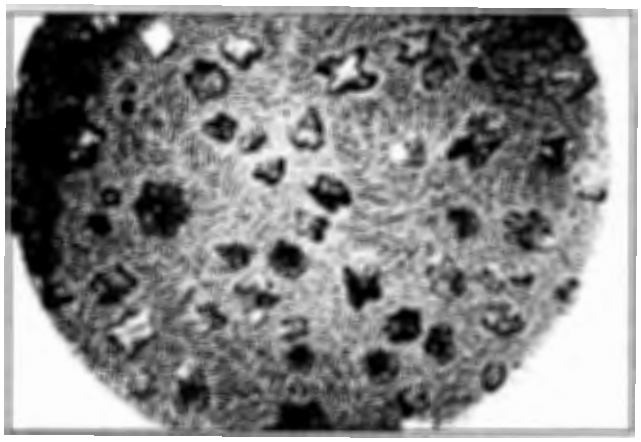

Figure 2. Star shaped colonies approximately one centimetre in diameter obtained in dehydrated agarized ferrous medium by incubation overnight.

- When working with inocula coming from a liquid medium, or suspended in a liquid medium prior to the inoculation, it was impossible to obtain a direct bacterial attachment to the inoculation place, even when the medium was highly dehydrated priory to be inoculated. These are typical features of gliding bacteria (Reichenbach, 1981). It must be taken into account that bacteria with metallic sulphides oxidation capacity have evolved in mineral environments where insoluble substrates are in low concentration and finely disseminated in mineral ores. Only the development of surface spreading mechanisms or surface translocation has allowed them to evolve.

Determination of generation times in liquid and dehydrated media

The slowness of bio-hydrometallurgical processes in the systems experimented so far, is essentially due to the low bacterial multiplication velocity. The bacterial colonies development in few hours times clearly indicates that when the bacterial cells are attached to a solid substrate of low water content, they multiply quickly.

The generation times (tg) of the ATCC 19859, BA_1 and BA_2 strains were determined in order to compare them in two systems. One was a conventional ferrous liquid medium shaken at 30 ºC. The development was followed up by extracting daily samples, and the number of cells was determined by dilution and recount of colonies in plates. During the exponential phase corresponding to the highest multiplication velocity, the minimum generation times were determined. They are indicated in Table I as generation times corresponding to free growth in a liquid medium. The other system correspond to the development in a medium of the same composition as the above, but solidified with agarose 0.35% (W:V) and dehydrated prior the inoculation. The mean generation times were determined considering that each colonies originates in one cell, and taking as the developing time, a period starting half an hour before the first evidence of growth was detected (by stereomicroscopy) until the moment when there were colonies clearly evident, which were completely isolated with a toothpick. Each colony was suspended in a measure volume of solution. Vortexing was carried in order to liberate the cells. The number of cells in each colony was determined by recount in plates and considered as an average of three different colonies. Table I indicates the mean generation times when the strains grow attached to a dehydrated solid medium.

Strains	Minimum tg. free growth in liquid medium	Mean tg. attached growth in solid dehydrated medium
ATCC 19859	10 hours and 20 min.	24 min. and 50 sec.
BA_1	8 hours and 27 min.	20 min.
BA_2	11 hours and 32 min.	28 min. and 15 sec.

Table I: Generation times (tg).

The results demonstrate that optimum microbial development corresponds to low water activity conditions or to a rather dry environment.

Microbial development and bio-oxidation of metals sulphides

The principles described previously respect to bacterial development in relation to water when ferrous iron was used as energetic substrate, were applied to bio-oxidation of metals sulphides by using them as the energetic substrates and being them as synthetic sulphides, natural specimens, concentrates or contained in metallic mineral ores or coal.

For example, a thin layer of dry and sterile synthetic copper sulphide was distributed in a Petri plate, 9 cm in diameter and adding drop by drop, the minimum quantity of acid solution for acidifying homogeneously the copper sulphide while introducing the less possible quantity of water. It was inoculated in the centre of the plate with 10 microliters of an inoculum of the BA_2 strain, prepared by dissolution of a copper sulphate crystal from a previous culture, in 0.06 N solution of sulphuric acid. The plate was incubated at 30 ºC in the open for allowing drying by natural evaporation. When the layer of copper sulphide acquired a dry appearance, evidences of development were observed, and a few hours later, blue crystals like colonies of copper sulphate were obtained. They were 0.5 centimetres wide by 1.5 to 2.5 centimetres long, as it is shown in Figure 3. These blue crystals are themselves bacterial colonies. By microscopic observations of the dissolved blue crystals in a solution, a dense bacterial population appears.

Figure 3. Blue bacterial colonies in copper sulphate developing in a layer of dehydrated acidified copper sulphide layer.

Applying the same criteria to minerals ores containing disseminated particles of metals sulphides, they are bio-oxidized in times of hours. Figure 4 show to the left triturated mineral ore from Campana Mahuida, an Argentine deposit, without treatment, and to the right the same mineral ore after bio-oxidation showing the colonies of copper sulphate coming from the original copper sulphides particles.

Metals sulphides as pyrite, arsenopyrite, chalcopyrite, covellite, chalcocite, galena, enargite, molibdenite, sphalerite, cobaltite, antimonite, etc., have shown may be fast bio-oxidized in dehydrated media with the previous features; since they are as metal mineral ores, their flotation concentrates or coal.

Scanning microscopy observations

The common feature between all the colonies of different strains obtained in dehydrated media of different compositions was "geometrical shapes that look likes crystals" and when

Figure 4. A mineral ore triturated to 1/4 inch, comprising chalcocite as the predominant specimen of copper, not subjected (left) and subjected (right) to biooxidation in dry conditions.

any of them is suspended in a liquid media in order to dissolved the bio-oxidation product, by microscopic observations a dense bacterial population is present.

As expected the bio-oxidized products, resulting from the bacterial metabolism acting in dehydrated media, are in solid state. In order to examine the distribution and the bacteria-solid product relationship in such colonies, scanning microscopy studies were carried out. Many interesting observations have been done, but here it is important to enphatize the two more general and important aspects. The first, is that different strains even growing in different substrates, multiply remaining temporally occluded in the solid bio-oxidized product they form. Figure 5 is a scanning microphotography of a colony priory slightly washed with an acidic solution, showing the bacteria in the inside. It should not have evolutive sense if the bacteria would not have mechanisms for abandon it. The photography of Figure 6 corresponds to the centre of a colony which after a time of colony formation began to exhibit, under direct observation, a granular appearance or degradation; while the scanning photographs show minor units of disintegrated cubic forms with their walls severely perforated. Different observations with this feature strongly suggest the bacteria remain occluded in the solid product they form. Afterwards, they perforate the solid product and abandon it.

Figure 5. A photography obtained by scanning microscopy showing the bacterial cells in the inner of the solid biooxidized products.

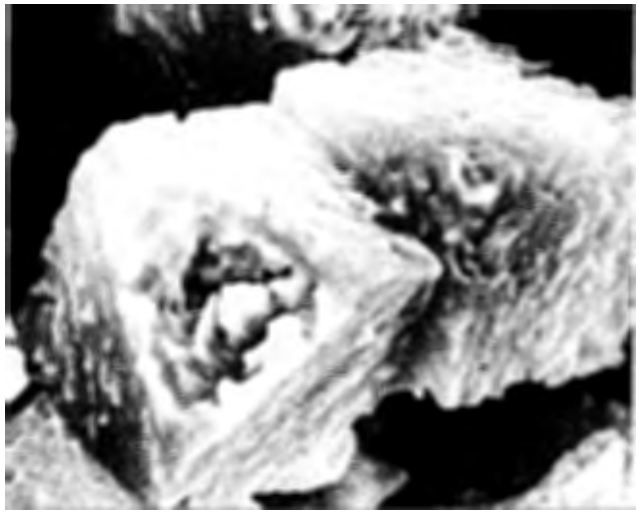

Figure 6. A photograph obtained by scanning microscopy showing minor units of the solid biooxidized product with their walls severely perforated.

BIO-DESHYDROMETALLURGICAL PROCESSING

The prior microbiological fundamentals of bioleaching bacteria have been the base to outline and optimise the features of Bio-deshydrometallurgical systems. In general they include the following operations, features and conditions.

Conditioning: The mineral ore or concentrate must be homogeneously acidified with the quantity of acid which is determined before-hand as the most convenient in order to neutralise them, prevent compaction and provide the proper acidity for the microorganisms, while introducing the smallest possible amount of water into the system. When triturated ores are treated, the acidification is done in the operation of agglomeration of thin around thick material. When concentrates are treated the acidification is done in an operation of pelletization. The acidified concentrate (pulp) is palletised forming a film around an inert element as quartz, plastic bolls, gravel etc. Agglomeration and pelletization are done for allowing a high specific surface in contact with air. It must be taking into account that in Bio-deshydrometallurgical processes, the microorganisms develop taken oxygen, carbon dioxide and nitrogen directly from the air. During the conditioning operation the material is inoculated with a fraction (1-5%) of biooxidized product coming from a prior operation.

Biooxidation: It is enabled the spontaneous or induced loss of water excess that may be present in the conditioned ore or concentrate, by natural evaporation (sun drying) or by drying with flowing air, until the thermodynamically available water is low enough for micro-organism being not suspended in a liquid film and being attached stablely to the substrate. So, the fast bio-oxidation is reached giving the bioozidized products in solid state, being them saturated in bacterial cells. Another way to decrease the thermodynamically available water is to add in the conditioning operation a dehydrating agent, as for example calcium or magnesium salts. In this manner, the requirements of drying may be reduced or eliminated according to the type and quantity of the employed dehydrating agent. In each case, the economy of the process must be considered in connection with the cost and quantity of the dehydrating agent to be employed, as well as the economic value of the ore or concentrate in question.

Washing: In order to separate the products obtained in solid state during the biooxidation operation, the material must be washed. This possibility offers several advantages. For example, it is possible to wash with a relation volume of washing solution to weight of bioozidized material determined before hand taking into account the metal content in the material, the bio-oxidation yield and the metal concentration one wants in the out solution. It has been possible to obtain solutions of copper concentration as high as 80 g/l. Over this concentration copper sulphate begin to be crystallised, which is attractive for copper sulphate production. Furthermore, it is possible to determine a priori the pHin of the washing solution for giving a selected pHout, in such a way of not to allow the dissolution of contaminating elements. For example, biooxidizing chalcopyrite concentrates in pilot plant, it has been obtained solutions of high copper concentration (up to 80 g/l) and iron content 100 – 500 ppm.

CONCLUSIONS

Although the bio-oxidation activity of different substrates by different bacterial species, and even by different strains of the same specie, is variable and determined by the prehistory of their existence (Groudev, 1985), the previous requirements which must be met for the efficient biological oxidation, such as a stable substrate-cell attachment and the destruction of the sulphide mineral lattice, are governed by similar conditions: water content low enough to prevent the suspension of the microorganisms in a liquid phase. At our understanding, there have been preconceptions for the Bio-hydrometallurgical processes development, as there is life only in systems having an abundance of water, without considering that these bacteria have evolved transforming mineral ores, which usually are not found in nature suspended in a liquid phase. Although mineral ores may look dry or dehydrated, they contain a percentage of water, at least what is in equilibrium with the environmental humidity.

Relatively few microorganisms have evolved with physiological adaptations enabling them the best growth in low water activity environments. Many terms have been used to describe these microorganisms: halophytic, osmophylic, osmotolerant, xerophylic, etc. (Hocking, 1988; Brown, 1976). Of these terms, xerophylic (from the greek: dry-loving) is perhaps the most appropiate for describing the microorganisms capable to biooxidate substrates as metals sulphides.

During the operation of biooxidation in the Bio-deshydrometallurgical processes patented by LEACHING, there is present only a solid phase, not being possible to separate a liquid phase nor even by drainage, and the microorganisms are taking oxygen and carbon dioxide directly from the air in contact with the solid phase in

an static system (not stirred). The microorganisms are stablely attached to their substrates developing with high multiplication and bio-oxidation velocity. These features overcome the main technical barriers of Bio-hydrometallurgical processing as: the low leaching velocity (months or years), the requirements of operating with large masses of minerals subject to climatic variations, high consumption of water (Heap, in-Situ or Dump leaching) and the problems of S.T.R.'s as the limitation of solids concentrations in the pulp (15-20%) with the consequently high volume of treatment in stirred systems (energy consumption), wherein collisions and sliding between solids particles result in damage of microbial cells and wherein oxygen and carbon dioxide transfer from the gaseous to the liquid phase is a limiting factor. The fact of overcoming the prior technical barriers results in a significant economical and environmental impact, notably increasing the application potentiality of biotechnological processes in mining. It has been shown, for example, by the results obtained in a pilot plant (Paños, N.H., 1998) applying a Bio-deshydrometallurgical process to copper extraction from concentrates, which main results for different copper concentrates are: Bio-oxidation yield: 80-99 % , Bio-oxidation time: 24-48 hours, Bio-oxidation cost: U$S 30-50/Tn of concentrate, Copper concentration in washing solutions: 40-80 g/l, Iron concentration in washing solutions: 100-500 ppm (including solutions of chalcopyrite concentrates). This process would allow the obtention of metallic copper in the origin country. So, Bio-deshydrometallurgy is arising as a new economical technology for mining in armony with nature.

REFERENCES

Bennett, J.C. and H. Tributsch, 1978. Bacterial Leaching Patterns on Pyrite Crystal Surfaces. J. Bacteriol., 134: 310-317.

Berry, V.K. and L.E. Murr, 1978. Direct Observations of Bacteria and Quantitative Studies of their Catalytic Role in the Leaching of Low-Grade, Copper-Bearing Waste. In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena. Edited by: Murr, L.E., Torma, A.E. and Brierley, J.A. Academic Press. New York. 103-136.

Biogeotechnology of Metals. Manual, 1988. Scientific Editors: G.I. Karavaiko (USSR), G. Rossi (Italy), A.D. Agate (India), SN. Groudev (Bulgaria), A.A. Avakyan (USSR). Centre for International Projects GKNT. Moscow.

Biohydrometallurgical Processing, 1995. Proceedings of the International Biohydrometallurgy Symposium IBS-95. Vol. I. Edited by T. Vargas, C.A. Jerez, JV. Wirtz and H. Toledo. University of Chile.

Brown, A.D., 1976. Microbial Water Stress. Bacteriol. Rev., 40(4) : 803-846.

Dew, D.W., 1995. Comparison of Perfomance for Continuous Bio-oxidation of Refractory Gold Ore Flotation Concentrates. In Ref.2., pp. 239-251.

Dugan, P.R. and W.A. Appel, 1978. Microbiological Desulfurization of Coal. In: Metallurgical Applications of Bacterial Leaching and related Microbiological Phenomena. Edited by: Murr, L.E., Torma, A.E. and Brierley J.A. Academic Press. New York. 223-250.

Escobar B., I. Godoy, E. Pardo and J.V. Wiertz, 1995. Bioleaching of Copper Concentrate by T. ferrooxidans at Increasing Pulp Density. In Ref. 2., pp. 273-281.

Groudev, S.N., 1985. Differences Between Strains of Thiobacillus ferrooxidans with Respect to their Ability To Oxidize Sulphide Minerals. In: Proceedings of International Seminar and Training Course: Biogeotechnology of Metals. Edited by: Karavaiko, G.I. (USSR) and Groudev S.N. (Bulgaria). Centre of International Projects GKNT. Moscow. pp. 83-96.

Harrison, A.P., Jr., 1984. The Acidophilic Thiobacilli and Other Acidophilic Bacteria that share their Habitat. Ann. Rev. Microbiol., 38 : 265-292.

Hocking, A.D., 1988. Strategies for Microbial Growth at Reduced Water Activities. Microbiol. Sci., 5 (9) : 280-284.

Hoffman, M.R., B.C. Faust, F.A. Panda, H.H. Koo. and H.M. Tsuchiya, 1981. Kinetics of the Removal of Iron Pyrite from Coal by Microbial Catalysis. Appl. Environs. Microbiol., 42 : 259-271.

Karavaiko, G.I., 1988. The Mechanism of Bacterial Oxidation of Fe^{2+}, S^{o}, and Sulfide Minerals. In Ref. 1., pp. 14-17.

Lawrence R.W. and R. Pouling, 1995. The Demand for Biotechnology in Mining in The 21st Century. In Ref. 2. pp. 185-195.

Loi, G., A. Mura, P. Trois and G. Rossi, 1994. Bioreactor Perfomance vs. Solid Concentration in Coal Biodepyritization. Fuel Process Technology, 40:251-260.

Loi G., P. Trois and G. Rossi, 1995. Biorotor: A New Development for Biohydrometallurgical Processing. In Ref. 2., pp. 263-271.

Manning, H.L., 1975. New Medium for Isolating Iron-Oxidizing and Heterotrophic Acidophilic Bacteria from Acid Mine Drainage. Appl. Microbiol., 30, (6) : 1010-1016.

Mills D.B., R. Bar and Kirwaa, 1987. Effect of Solids on Oxygen Transfer in Agitated Three-phase systems. AICHE Journal, 33 (9) : 1542-1549.

Paños, N.H., 1998. Fast Biooxidation of Copper Concentrates. Argentina Mining 98, Conference Proceedings. September 1998. Mendoza. Argentina.

Reichenbach, H., 1981. Taxonomy of the Gliding Bacteria. Ann. Rev. Microbiol.,35 : 339-364.

Schaeffer W.I., P.E. Holbert and W.W. Umbreit, 1963. Attachment of Thiobacillus thiooxidans to Sulfur Crystals. J. Bacteriol., 134 : 310-317.

Sakaguchi, S.M. and A.E. Torma, 1976. Microbiological Leaching of a Chalcopyrite Concentrate by Thiobacillus ferrooxidans. Biotechnol. Bioeng., 18, (8) : 1091-1101.

Torma A.E., C.C. Walden and R.M. Branion, 1970. Microbiological Leaching of ZnS Concentrate. Biotechnol. Bioeng., 12 : 501-517.

Van Aswegen, P.C., M.W. Godfrey, D.M. Miller and A.K. Jaines, 1991. Developments and Innovations in Bacterial Oxidation of Refractory Gold. Mine. Metal. Proc., 9 : 188-192.