AN INVESTIGATION INTO THE BACTERIAL LEACHING OF A GOLD-BEARING PYRITE/ARSENOPYRITE ORE

BY

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CONTENTS

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ABSTRACT

The main aim of this study was to develop an economically viable bacterial leaching process for a gold-containing pyrite/arsenopyrite ore. The effect of various parameters on, and the mechanism of, bacterial leaching were investigated.

Initially milled run-of-mine ore was examined. Batch tests and a continuous bacterial leach were carried out. Bacterial leaching was successful and 91-93% gold dissolution was attained in four days. The process was not economically feasible when compared to the standard flotation-roasting process.

In the batch tests a typical bacterial growth curve was apparent. This consisted of a 1-2 day lag phase, followed by a log phase and reached stationary phase after 5 days. Bacterial leaching required a retention time of 5 days, less than 30% solids, a particle size of 62% -74 μm, a pH of 1,6-2,2, a temperature within the 30-40°C range, additional ferrous iron and phosphate and less than 1 g/l As in solution. The subsequent cyanide leach required consumptions of approximately 15 Kg KCN/t ore and 25 Kg CaO/t ore and yielded 91-93% gold dissolution. In the continuous bacterial leach it was found that a four day retention time was needed, pH 1,8 was ideal and no nutrients were probably required. The cyanide leach required consumptions of approximately 10 Kg KCN and CaO/t ore and resulted in

91-93% gold dissolution. A reduction in particle size and sulphidic minerals was observed.

Control acid and acidic-ferric batch chemical leaches had no significant effect on gold dissolution.

Milled flotation concentrate was examined using batch tests and a continuous bacterial leach. Bacterial leaching resulted in 97-98% gold dissolution in 15 days i.e. approximately 91% of the gold in the run-of-mine ore. The process was economically viable when compared to the standard flotation-roasting process. It had an I.R.R. (Internal Rate of Return) of 50-60% and a pay-back period of 2,5 to 3 years.

In the batch tests a typical growth curve emerged. A 2-3 day lag phase was followed by a log phase and reached stationary phase after 10-12 days. Suitable conditions for bacterial leaching were a retention time of 10 days, a particle size of 88% -74 μm, less than 20% solids (optimum <10%), a pH of 1,6-2,2 (optimum pH 1,6), a temperature of 25-45°C (optimum 28°C), additional ferrous iron and less than 4 g/l As in solution. Consumptions of approximately 25 Kg KCN/t and 80 Kg CaO/t concentrate were required for the cyanide leach and resulted in 90% gold dissolution. In the continuous bacterial leach a ten day retention time was needed, pH 1,7-1,8 was found to be ideal and probably only 2 g/l Fe II was needed to be added in solution. Cyanide leach consumptions of approximately 20 Kg KCN/t concentrate (1 Kg KCN/t ore) and 50 Kg CaO/t concentrate (3 Kg CaO/t ore) were needed for 97-98% gold dissolution. A reduction in particle size and sulphidic minerals was observed.

Control acid and acidic-ferric batch chemical leaches had no significant effect on gold dissolution.

Pilot plant continuous vat bacterial leach of the milled flotation concentrate confirmed the laboratory testwork. The retention time was reduced to six days and an increased arsenic concentration of 10 g/l As was tolerated. It was found that potassium and possibly ammonia needed to be added to the leach, that the process was strongly exothermic and that the aeration efficiency was 38% (the air consumption was 2,62 times the theoretical air consumption). The cyanide consumption was 10 Kg KCN/t concentrate (0,5 Kg/t ore) and the lime consumption was 50 Kg CaO/t concentrate (3 Kg/t ore).

Bacteria were observed attached to the minerals. Etching in the vicinity of these bacteria occurred, with the exploitation of the mineral crystallography and weaknesses. This implied that either enzymes (direct mechanism of attack) and/or bacterial metabolites (indirect mechanism) were involved. Both were probably involved since acidic-ferric leaching also etched the minerals extensively, but had a different appearance. Galvanic interactions also played a role as the arsenopyrite was destroyed before the pyrite.

CHAPTER 1

GENERAL INTRODUCTION

Biohydrometallurgy is the technology by which metals are extracted from ores by processes using both water and microorganisms. It was developed from a need to tap low-grade ore resources as the higher-grade ores, which are processed by conventional hydro- and pyrometallurgical operations, dwindled. Costs, process control and pollution problems also contributed toward the surge in interest in microbiological leaching (Dutrizac and MacDonald, 1974; Torma, 1977; Brierley, 1978).

The recovery of copper from mine drainage water probably dates back to 1000 B.C. The Romans also took advantage of naturally leached copper, as did the 16th century Welsh at Anglesey, and on a large scale workers at the Rio Tinto mines in Spain in the 18th century. However, only in the last 35 - 40 years has the active role of bacteria in this leaching been realized (Kelly, Norris and Brierley, 1979; Brierley, 1982).

Bacterial leaching is employed in several ways, namely (i) dump leaching, which is in most cases an uncontrolled microbiological process (ii) heap leaching, where a smaller particle size, aeration and impermeable pads for solution recovery are used (iii) 'in situ' leaching and (iv) vat leaching. The first two methods have been used

predominantly for copper recovery, the third for uranium (with rare earths as by-products) and the latter for uranium, copper and zinc recovery from concentrates and high-grade ores. Leaching has also been used in the selective extraction of complex metal sulphides and in the desulphurization of coal (Kelly, Norris and Brierley, 1979; Brierley, 1982; Torma and Bosecker, 1982).

Thiobacillus ferrooxidans is considered to be the most important organism in microbiological leaching. It was discovered in the acid water drainage of a Pennsylvanian bituminous coal mine and in 1947 Cohen and Hinckle proved its metal dissolving ability. It is renowned for its ubiquity, yet during a survey of mine waters in Brazil it was shown that out of 1210 samples 776 contained thionic bacteria, but only 14 had T.ferrooxidans (Groudev, 1983). In addition, it has been shown that mixed cultures are more effective than pure cultures. For example, it was found that T.ferrooxidans and Thiobacillus thiooxidans are more effective in combination on many sulphide minerals, as are Leptospirillum ferrooxidans and T.thiooxidans, L.ferrooxidans and Thiobacillus organoparus, L.ferrooxidans and Thiobacillus acidophilus, T.acidophilus and T.ferrooxidans (Kelly, Norris and Brierley, 1979; Arkesteyn and de Bont, 1980; Brierley, 1982).

In addition to these mesophiles several thermophilic organisms have been recently discovered in leaching environments. They include several TH strains (thiobacillus-like thermophiles), Sulfolobus acidocaldarius, Sulfolobus brierleyi and Sulfobacillus thermosulfidooxidans (isolated from a copper-zinc sulphide deposit

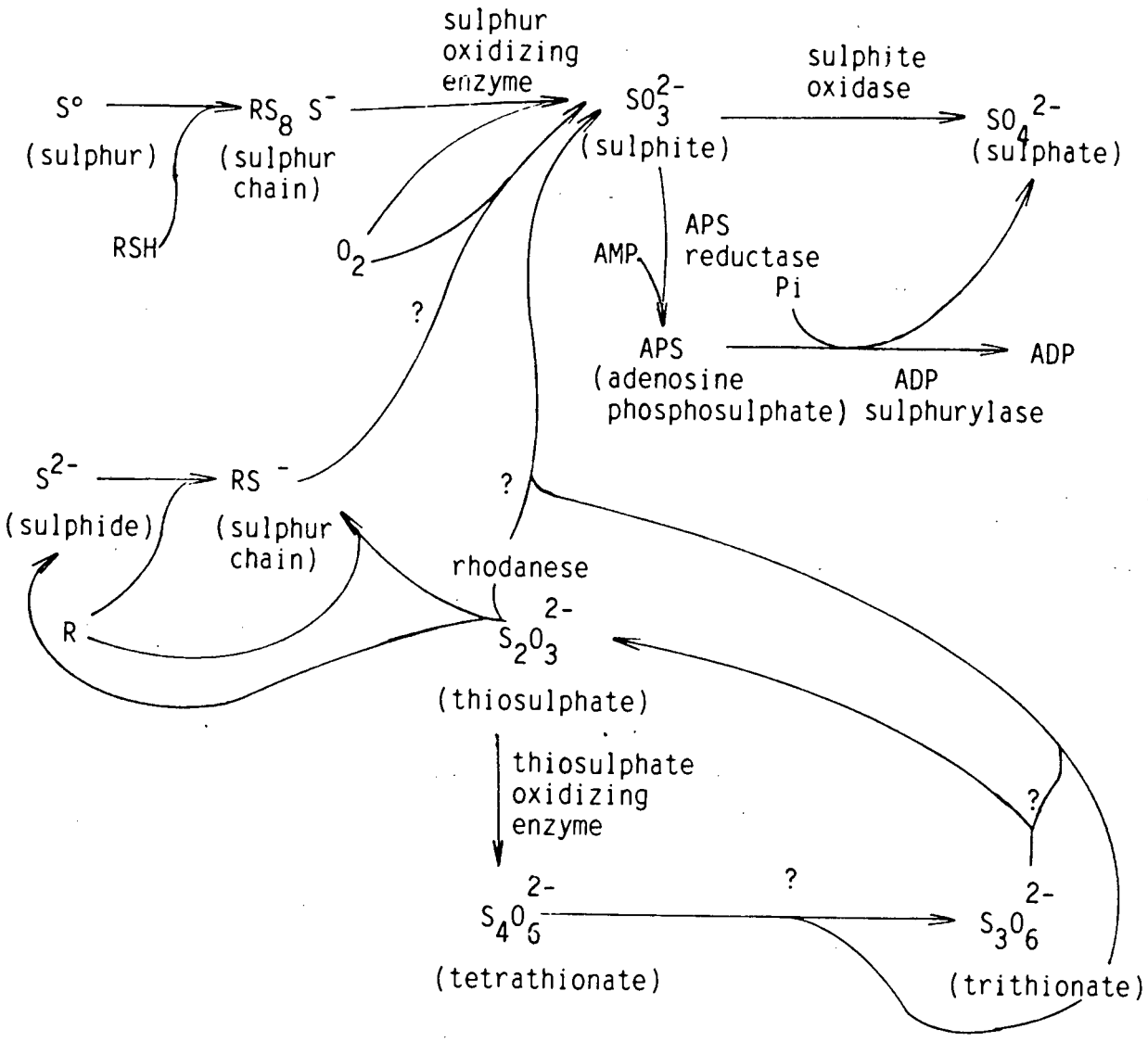
in Armenia). These organisms show promise in leaching, but their status has not yet been fully established (Brierley and Le Roux, 1977; Brierley, 1982; Torma and Bosecker, 1982; Marsh, Norris and Le Roux, 1983). To a lesser extent it has been found that a number of heterotrophic bacteria, fungi, algae and protozoa are involved (Dart and Stretton, 1980; Lundgren and Silver, 1980).

The majority of these organisms are autotrophic, aerobic and acidophilic. The mesophiles are capable of deriving all of their energy from the oxidation of either reduced inorganic sulphur or iron compounds while reducing oxygen to water. The thermophiles, on the other hand, normally require some organic matter for growth. S.thermosulfidooxidans, however, is autotrophic.

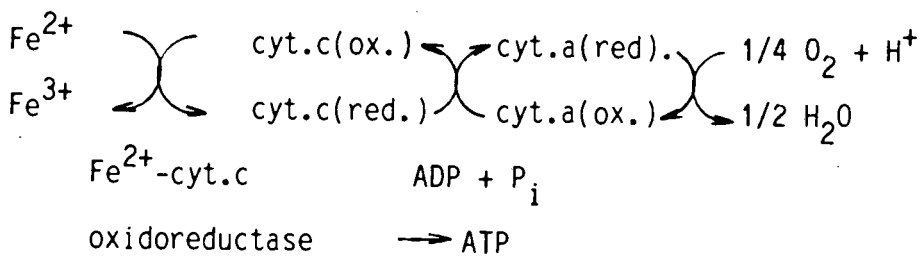
The thiobacilli are short, non-spore forming, Gram-negative rods (0,5 x 1,5 µm), while L.ferrooxidans is a Gram-negative, vibrio-shaped (0,2 - 0,4 x 0,9 - 1,1 µm) organism. At low pH, though, it becomes coccoid (0,4 - 0,8 µm in diameter). S.thermosulfidooxidans is Gram-positive, polymorphic and rod-shaped (0,6-0,8 x 1,0-3,0 µm), while the sulfolobi are Gram-negative cocci(0,8-1,0 µm in diameter) (Buchanan and Gibbons, 1974; Golovacheva, 1979; Pivovarova, Markosyan and Karavaiko, 1981).

These organisms obtain carbon by the fixation of carbon dioxide via the carboxylation of phosphoenol pyruvate and the Calvin-Benson cycle, both of which are coupled to oxidation. They obtain nitrogen from solution (NH_4^+ usually), but when necessary can fix atmospheric N_2 (Mackintosh, 1978; Lundgren and Silver, 1980).

The generally accepted mechanism of sulphur and iron oxidation by thiobacilli has been reported by Silver (1978):



The mechanism of iron oxidation is :



For this process to yield enough energy for ATP formation the ferrous iron must be complexed with an organic molecule so that the redox potential of the Fe II/Fe III couple is lowered. Coenzyme Q possibly acts as an intermediary electron carrier between the ferrous iron-sulphate-organic complex and the rest of the system.

There are many sources of sulphur and iron, sulphide minerals being one such source. T.ferrooxidans is reported to oxidize arsenopyrite, bornite, bravoite, chalcocite, chalcopyrite, cobaltite, covellite, enargite, galena (slightly), manganese oxide, marcasite, marmatite, millerite, molybdenite (slightly), orpiment, pentlandite, pyrite, pyrrhotite, sphalerite, stannite, stibnite, tetrahedrite, uranium oxide and violarite among others (Corrans, Harris and Ralph, 1972; Bruynesteyn et al, 1979).

The actual mechanism whereby sulphide minerals are oxidized microbially has been studied extensively and has generated much controversy. Although some authors would disagree it is now generally believed that both "direct" and "indirect" leaching can occur. The mode of action of the organism can be due to (i) oxidation of the mineral by acidic-ferric sulphate, with the bacteria oxidizing the resultant ferrous iron, making the process cyclical (ii) direct action on the iron and/or sulphide moiety of the mineral (iii) a combined action of the above. How the process occurs depends on various factors such as the solubility of the mineral, crystal structure and lattice energy, the electrode potentials of the minerals present, the E_h of the solution, the formation of coatings on the minerals (Karavaiko and Pivovarova, 1977; Berry, Murr and

Hiskey, 1978; Torma and Sakaguchi, 1978; Kelly, Norris and Brierley, 1979).

Although much work has been carried out on microbiological leaching in the past 40 years much of it has been of a fundamental nature. Although heap leaching for copper recovery and uranium solubilization are being successfully exploited, not many economically viable processes have been developed in the fields of 'in situ' and vat leaching. Due to the practicality of vat leaching on a continuous basis, success in this field is important. To date only two cases have been documented - B.C.Research (McElroy and Bruynesteyn, 1978) calculated that a chalcopyrite concentrate copper leaching process was competitive but not economically superior and Torma (1978) developed a viable process whereby a lead sulphide concentrate was up-graded and zinc, copper and cadmium were recovered as by-products.

The subject of this thesis is to establish whether an economically viable process for the recovery of gold from a pyrite/arsenopyrite ore by bacterial leaching can be developed.

CHAPTER 2

BACTERIAL LEACHING OF THE MILLED RUN-OF-MINE ORE

Summary: The milled run-of-mine ore was analysed chemically and mineralogically and found to have characteristics likely to support bacterial growth and activity. It also contained 9,75 g Au/t ore.

Although the ore contained arsenic a bacterial culture capable of tolerating 1 g/l As was prepared and used for batch tests. In batch tests it was found that the bacterial growth curve consisted of a 1-2 day lag period, a log phase and reached stationary phase after 5 days. The maximum gold dissolution of 93-94% was obtained in 9 days, although close to 90% was attainable in 5-6 days. 107,4 Kg H_2SO_4/t ore was consumed and a reduction in particle size and sulphidic minerals also occurred during the leach.

A reduction in the particle size from 62% to 96% -74 µm had no effect on gold dissolution. Pulp densities up to 30% solids resulted in good bacterial leaching and gold dissolution. A pH range of 1,6 to 2,2 was satisfactory, as was a temperature in the 30-40°C range. Tests indicated that the only nutrients likely to be in less than optimum concentration in a bacterial leach of the milled run-of-mine ore were ferrous iron and phosphate.

Control acid and acidic-ferric batch tests had no significant effect on gold dissolution.

A three month continuous bacterial leach of the ore was carried out, initially under the conditions based on results from the batch work. The pH and nutrients were altered successively in an attempt to optimize these two parametres. A 15% weight loss was observed (in batch it was virtually nil). A reduction in particle size and sulphidic minerals was observed.

Reduction of the amount of ammonium sulphate added to the bacterial leach (from 4,40 to 2,22 Kg/t ore) had no effect, while removal of ferrous sulphate addition caused an increase in gold dissolution and a drop in cyanide (but not lime) consumption. Increasing the pH of the bacterial leach (from 1,82 to 1,97) caused a drop in gold dissolution, while a decrease in pH (from 1,82 to 1,74) caused a slight decrease in gold dissolution and a large drop in cyanide consumption.

It was concluded that (i) a bacterial leach of the milled run-of-mine ore was possible; (ii) a retention time of four days was needed to produce a 91-93% gold dissolution upon cyanidation; (iii) further ore milling was unnecessary; (iv) 30% solids could be used; (v) pH 1,8 should be used, the acid consumption being about 71 Kg H_2SO_4/t ore; (vi) 35°C was the optimum temperature, but 30°C was also satisfactory; (vii) no nutrients probably needed to be added; (viii) the cyanide and lime

consumptions for gold solubilization would be approximately 10 Kg/t ore.

An economic feasibility study, however, showed this process to be non-competitive.

2.1 INTRODUCTION

In the field of biohydrometallurgy vat leaching of ores and concentrates is of recent interest. Vat leaching has been more widely attempted with concentrates than ores. Lawrence and Bruynesteyn (1983) have reported work on pyritic ores (containing approximately 10% sulphur and iron) and Qiu et al (1980) on an arsenic-containing sulphide ore. Arsenic containing ores particularly have been little studied, probably due to the problem of arsenic toxicity associated with the solubilization of the mineral during bacterial leaching. Some workers have found that T.ferrooxidans could only tolerate up to 1 g/l As (Ehrlich, 1964; Lundgren and Silver, 1980). However, other workers have reported higher arsenic tolerance - Polkin et al (1975) up to 3 g/l As and Atkins (1978) up to 4,9 g/l As. In solution arsenic can exist as either As III or As V, or a mixture of these two species. The former is more toxic to bacteria and hence, the arsenic form is very relevant. Neither these nor the present study examined this. In this study only total arsenic was determined. It is apparent that the bacterial leaching of a pyrite/arsenopyrite ore is problematic and needs further study.

Since ore is continually produced, the most practical process on a mine would be on a continuous basis. The following advantages would be attainable: (i) greater production and product consistency; (ii) reduced manpower and downtime (for cleaning, emptying and so on); (iii) steady services demand; (iv) simpler process automation; (v) better process control. The disadvantages would be : (i) a need for highly qualified staff; (ii) more expensive leach vessels and ancillary equipment; (iii) difficulty in altering the type of ore treated (Dean et al, 1976).

Of all the continuous-type processes vat leaching is possibly the best. A tubular fermenter is difficult to control and requires a constant feed of microorganisms (if flocs are used) or requires microbial hold-up control (if films are used), while a fluidized bed fermenter is both difficult to control and the flowrate is limited by washout. In a stirred tank the major variable is the flowrate (Atkinson and Mavituna, 1983).

Although this argument excludes the large-scale use of a batch process, batch work is still necessary to provide a qualitative sense of the continuous process configuration and preliminary quantitative design estimates. These findings would still need to be checked by laboratory or pilot plant experiments prior to large-scale design (Bailey and Ollis, 1977).

The main factors affecting a bacterial leach are temperature, pH, E_h, particle size, pulp density (substrate concentration),

nutrients, aeration, light and inoculum (Duncan, Trussel and Walden, 1964; Pinches, 1975; Lundgren and Silver, 1980). Most of these parameters need to be examined, although little can be done about the E_h and exposure to light is usually not a factor. In addition, the size and type of inoculum are not applicable to continuous bacterial leaching. A mixed culture adapted to growth on the ore was used as it is the proven and accepted method (Polkin et al, 1975; Atkins, 1978; Norris and Kelly, 1978; Lawrence and Bruynesteyn, 1983; Marchant, 1985). Gold dissolution rather than bacterial counts was used as a gauge of the effectiveness of bacterial ore leaching. There are two main reasons for this; firstly, gold dissolution is of interest as gold is the product of the process. Secondly, although bacterial counts reflect the growth and activity taking place, there is no reliable method for obtaining them. Several methods have been used, namely the determination of the protein content, nitrogen content, rate of autotrophic carbon dioxide fixation, manometric oxygen uptake, the MPN method (most probable number), Coulter counter, photometrical ATP determination, fluorescent antibody techniques, growth on solid media, determination of dry weight and the rate of iron oxidation. All these methods, however, have drawbacks due to dissolution products interfering with the chemical analysis of the cell constituents, cell sorption to solid particles, chemical inhibition and lack of discrimination between different bacterial species (Brierley, 1978; Hiltunen et al, 1981; Groudev, 1982a).

The economic feasibility of the process is also highly relevant,

since little work on ores has been documented and no successes have yet been reported.

2.2 MATERIALS AND METHODS

Details of the methods, media and solutions are listed in Appendices A and B respectively.

2.2.1 Analysis of the Milled Run-of-Mine Ore

The ore was analysed for a large number of elements by ICAP (Inductively Coupled Argon Plasma). Wet chemistry methods were used to analyse for total iron, total sulphur and arsenic. The gold and silver content was determined by fire assay. A mineralogical examination and screen analysis were also performed.

2.2.2 Bacterial Strain Development and Identification

2.2.2.1 Adaptation to Test Conditions

The bacterial culture used in this work was adapted to growth in an aerated slurry of milled ore and 9K medium (pH 1,8, 30°C). Small amounts of ore and fresh 9K medium were added regularly to the culture. The inoculum was used when there was approximately 20% solids (determined by specific gravity), 1 g/l As in solution (determined by X-ray) and the added ferrous iron was oxidized within 16 hours (determined by titration with standard

potassium dichromate solution).

Sterility was not enforced as the plant would be non-sterile.

2.2.2.2 Identification of the Bacteria Present

The adapted culture solution was serially diluted with sterile distilled water and 0,1 ml plated onto Nutrient agar, Waksman's and 9K media solidified with 2% Oxoid agar-agar. The plates were incubated at 30°C for up to four weeks. Isolated colonies were inoculated onto the different types of agar plates and into the corresponding liquid media. They were incubated at 30°C for up to four weeks. The pH was monitored sporadically and cultures at different initial pHs used. Gram stains and wet slide preparations were made from selected colonies and solutions and examined microscopically. Identification was done on the basis of morphology and energy source (Fig 2.1).

For total counts the nitrogen content method of Gormely and Duncan (1974) was used.

2.2.3 Batch Tests - Bacterial Leach Procedure

Milled run-of-mine ore (350 g) and 9K medium (1,40 l) were placed in a 2 l beaker, which gave a slurry of approximately 20% pulp density. The stirred slurry was aerated and kept at 30°C in a water bath. The pH was adjusted to pH 1,8 with concentra-

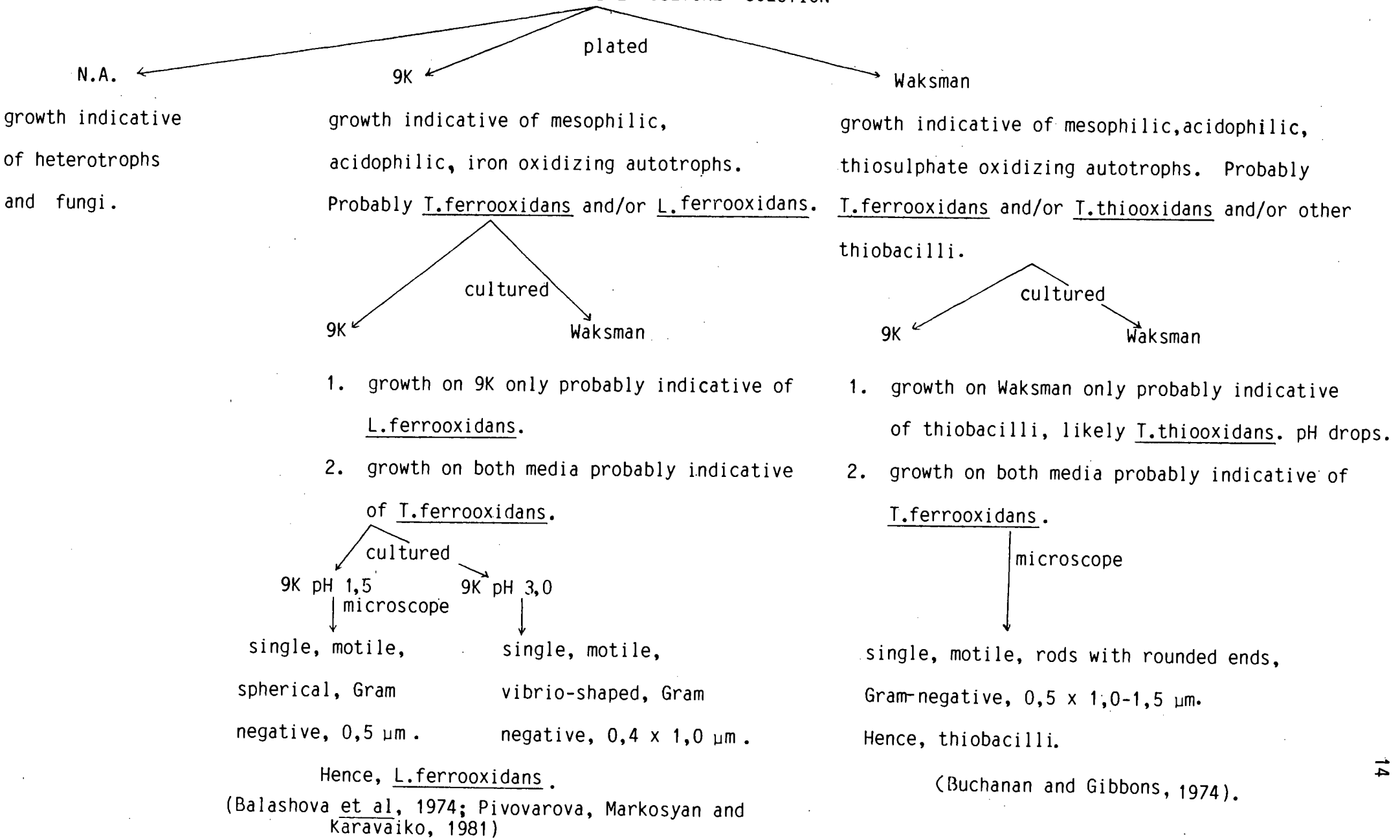


Fig. 2.1: Flow chart for the identification of the bacteria present in the adapted culture. N.A. = Nutrient agar.

ted H_2SO_4. A 10% slurry inoculum (150 ml) prepared as in 2.2.2 was added and the mixture maintained at pH 1,8 by addition of either concentrated H_2SO_4 or 130 g/l CaO slurry. After five days the slurry was filtered, the filter cake washed, repulped, refiltered and rewashed. The final filter cake was dried at 60°C (to avoid decomposition) and weighed. The filtrate was collected and analysed for ferrous iron, ferric iron (by titration with standard sodium thiosulphate solution), arsenic and pH. The dried ore was analysed for total iron, total and pyritic sulphur, arsenic and gold. Mineralogical and screen analyses were also carried out.

2.2.4 Batch Tests - Sterile Control Leach Procedure

Acid and acidic-ferric control leaches were run in parallel with the bacterial leach. Instead of 9K medium being used to make up the slurry either tap water or a ferric sulphate (7 g/l Fe III) solution acidified to pH 1,8 was used. To inhibit bacterial activity 100 p.p.m. thymol was used.

2.2.5 Continuous Bacterial Leach Procedure

2.2.5.1 Inoculum Preparation

The initial inoculum ($\pm$ 2 l) was progressively increased until a volume of 30 l was reached. All conditions were kept at the optimum for bacterial growth and activity except the temperatu-

re. For practical reasons this was 20°C (room temperature).

The inoculum was distributed among the leach vessels and the system turned on.

2.2.5.2 Apparatus and Test Procedure

The continuous bacterial leach apparatus used is schematically illustrated in Fig 2.2.

The system was as follows:

- four 7,5 l water jacketed leach vessels were used. In plant engineering terms this is the minimum number to satisfactorily reduce short-circuiting. There was an inlet and outlet spout on opposite sides of the vessel. A baffle plate the width of the leach vessel and reaching 2/3 downward was positioned close to the inlet of the vessel, thus reducing short-circuiting. Two narrow, oblong baffles on the inside of the vessel aided mixing. There was a fitted lid to reduce evaporation and material loss by spillage.

- dry, milled ore was fed to the first vessel at a rate of 1,05 g/min $\pm$ 2% by a 12 Kg capacity double-screw feeder manufactured by K-Tron Corp. (T-20 model) with the series 6300 control unit.

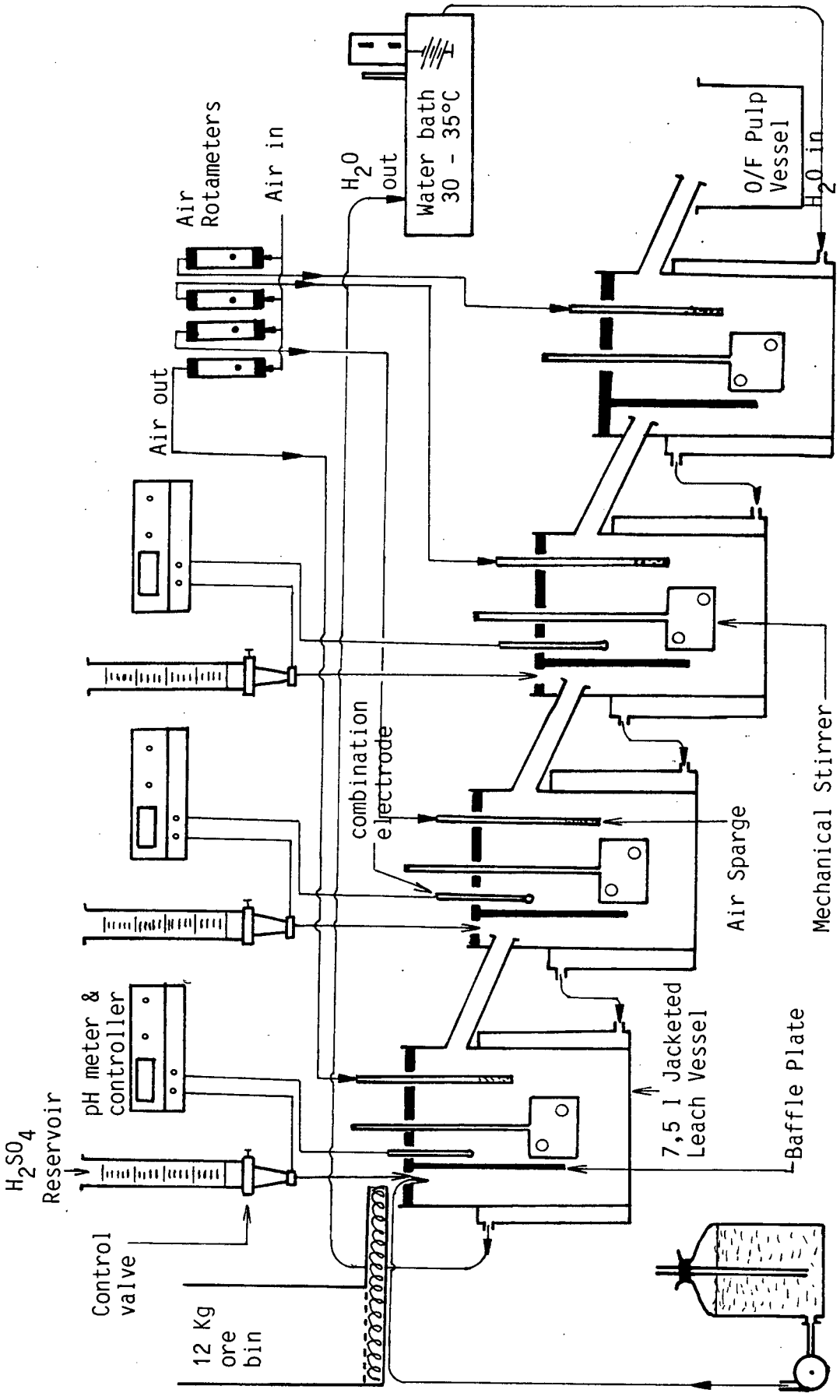


Figure 2.2: Diagrammatic illustration of the continuous bacterial leach apparatus for the milled run-of-mine ore.

- a variable speed peristaltic pump allowed for feed solution to enter the first vessel at a rate of 4,40 ml/min from a 20 l reservoir containing a 10 g/l $FeSO_4.7H_2O$ solution at pH 1,8-2,0.

- a given volume of 70 g/l $(NH_4)_2SO_4$ solution was added to the first vessel each day.

- four variable speed stirrers with flat 30 x 40 mm paddles kept the slurry in suspension within the vessels.

- the vessels were aerated at about 1,3 l/min. Four Aalborg FMO 13-88C (0,2 - 5,8 l/min range) air rotameters and air sparges were used.

- three pH control units were used. Each consisted of a combination-type pH electrode, a pH meter and a titrator. This regulated the addition of a 500 g/l H_2SO_4 solution to vessels 1, 2 and 3.

- the leach slurry temperature was kept at 33°C $\pm$ 2°C using a thermostat-controlled water bath. A magnet pump was used to circulate the water through the leach vessel jackets.

- the slurry from leach vessel no. 1 flowed on into vessels nos 2, 3 and 4, arranged in series. Slurry from vessel no. 4 was collected in a 10 l container. This represented an overall slurry retention time of approximately 4 days.

- the final overflow slurry was removed every 24 h. After settling the solution was siphoned off.

- approximately 10% of the thickened slurry was recycled to the first leach vessel each day.

- Guargum flocculant was added to the remaining slurry, which was then filtered. The filter cake was washed with pH 2 water, repulped, refiltered and rewashed. The final filter cake was dried at 60°C and weighed.

- the filtrate and combined washings were collected, weighed and measured, then analysed for ferrous and ferric iron, arsenic, NH_4^+ (by ICAP) and pH.

- the dried residue was analysed for total iron, total sulphur, arsenic and gold. Mineralogical and screen analyses were also done. The feed ore was also analysed as above.

- a sample from each leach vessel was taken daily. The pH and redox potential were measured and the ferrous and ferric iron content of the solution determined. The specific gravity of the slurry was measured to give an indication of the pulp density.

2.2.6 Cyanidation Test Procedure

Dried bacterial leach residue (150 or 400 g) and enough tap water to give a pulp density of 40% (225 or 600 ml) were placed in a 1-2 l beaker. The slurry was stirred and aerated. An approximate 10% lime slurry was used to adjust the ore slurry to about pH 11,5. This pH was checked every 0,5 - 1,0 h and lime was added to maintain the desired pH. After 7-68 h the pH had stabilized (i.e. it no longer rapidly decreased with time). A predetermined amount of granular KCN was added and the leach continued for another 24 h.

The leach slurry (now at a pulp density of 33%) was filtered, the filter cake washed, repulped, refiltered and rewashed. The final filter cake was dried at 60°C, weighed and assayed for gold. The first filtrate was collected and analysed for residual cyanide and lime by titration with standard silver nitrate and standard oxalic acid solutions, respectively.

2.3 RESULTS

2.3.1 Analysis of the Milled Run-of-Mine Ore

The elemental analysis of the ore is given in Table 2.1 and the mineralogical and screen analyses in Tables 2.2 and 2.3. There was sufficient iron and sulphur, as pyrite and a little arsenopyrite, to support bacterial growth (thiobacilli and leptospirilli) and the only inhibitory compound was arsenic. It is probable that all soluble nutrient requirements of the organisms would be satisfied.

Table 2.1: Chemical analysis of the run-of-mine ore.

Element	Concentration (%)
Gold	0,000975(9,75 g/t)
Silver	0,000060(0,60 g/t)
Arsenic	0,35
Iron	6,23
Sulphur	1,76
Calcium	2,49
Nitrate	0,034
Potassium	2,20
Chlorine	0,082
Phosphorous	0,013
Magnesium	3,40
Sodium	0,17
Aluminium	10,67
Boron	0,003
Copper	0,008
Manganese	0,12
Nickel	0,033
Molybdenum	0,002
Titanium	Trace
Silicon	26,22

Table 2.2: Mineralogical composition of the milled run-of-mine ore.

Mineralogical Component	Percent of Total
Pyrite	3,40
Arsenopyrite	0,77
Sphene	0,60
Rutile	0,22
Hematite	0,03
Sphalerite	<0,01
Free gold	-
Gangue	94,98

Table 2.3: Tyler screen analysis of the milled run-of-mine ore.

Size (µm)	Percent of Total
+208	1,93
+147	7,73
+104	12,72
+74	14,89
-74	62,73

2.3.2 Bacterial Strain Identification

The adapted bacterial culture grew well in the presence of the ore and 1 g/l As in solution. The total bacterial count was approx. $1,2 \times 10^{10}$ cells/ml slurry. Visually there were $2\text{-}5 \times 10^{8}$ cells/ml solution. When the solution was plated on different solid media the counts were slightly lower (Table 2.4). The culture consisted of T.ferrooxidans, sulphur-oxidizing thiobacilli (probably T.thiooxidans), L.ferrooxidans and some fungi and heterotrophs.

Table 2.4: Bacterial counts on different solid media.
N.A. = Nutrient agar; TNC = too numerous to count.

Medium	Titre of Bacteria (cells/ml)	Titre of Fungi (cells/ml)
N.A.	-	10
9K	$\pm 10^{4}$	10
Waksman	$> 10^{7}$	10

2.3.3 Batch Tests - Bacterial Leaches

All tests were done in duplicate or triplicate.

2.3.3.1 Arsenic Tolerance

It had been found that the bacterial culture to be used in this work could only tolerate 1 g/l As. This observation, made while preparing

inoculum, was confirmed when the culture was inoculated into 9K medium supplemented with varying amounts of arsenic (as As_2O_3) (Table 2.5).

Table 2.5: Arsenic tolerance of the bacterial culture in 9K medium supplemented with As_2O_3.

Arsenic Concentration (g/l)	Lag Period (days)	Period for Complete Fe II Oxidation (days)
0,5	3	5
1,0	4	6
1,5	25	27
2,0	23	25
2,5	42	44

2.3.3.2 Effect of Leaching Time

The effect of bacterially leaching this ore for up to 9 days was examined. During the leach ferrous iron in solution rapidly dropped to less than 0,3 g/l, while the ferric iron and arsenic increased to a constant value within 5 days. The lag period was 1-2 days (Fig. 2.3). During this lag period the pH increased due to acid consumption required by the bacteria for iron oxidation.

Although arsenic was leached out of the ore other indications of bacterial attack of the minerals was lacking due to reprecipitation of

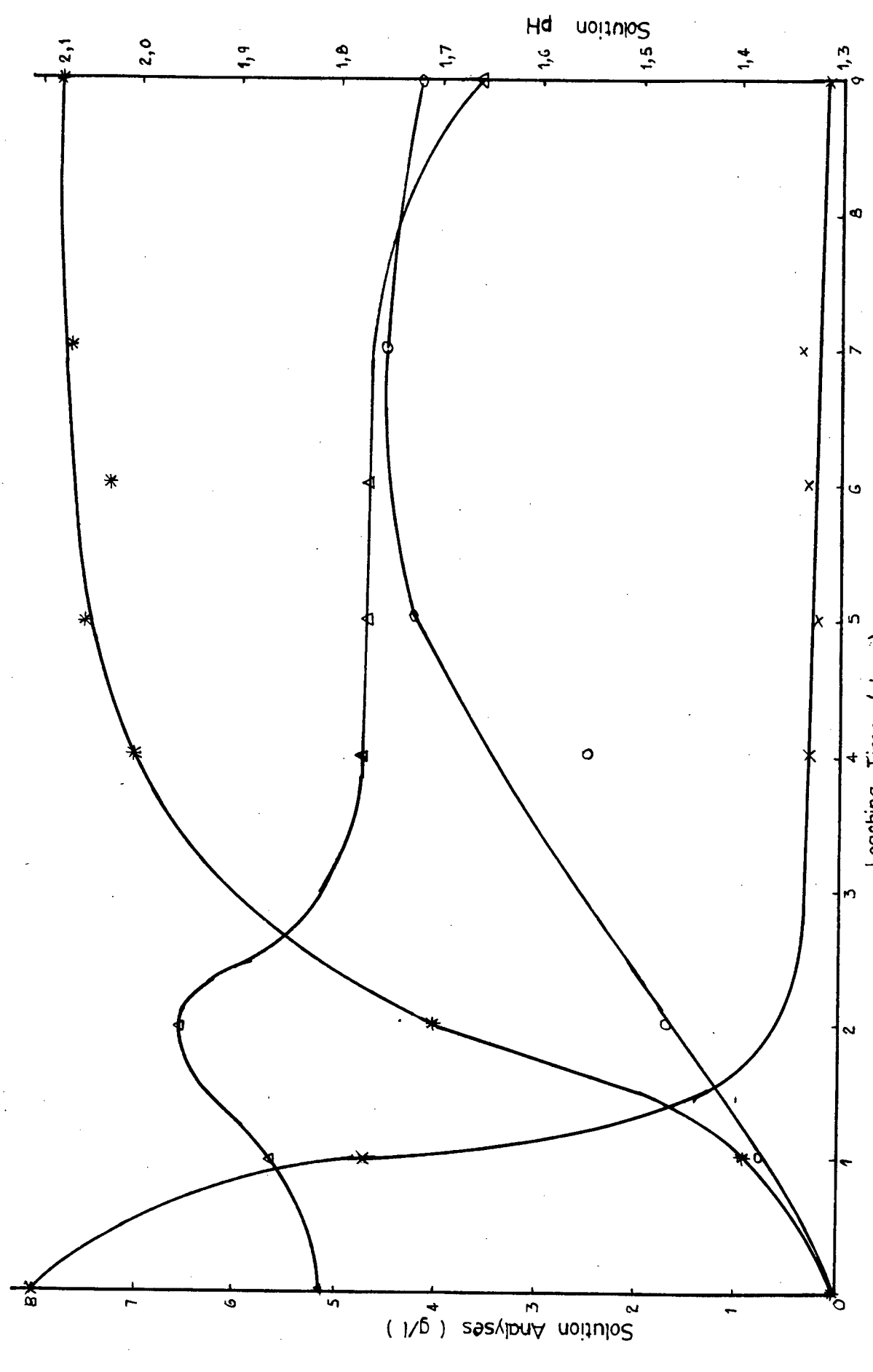


Fig.2.3: Solution changes during the bacterial leaching of the milled run-of-mine ore. Arsenic $x10^{-1}$ (-*-); ferrous iron (-x-); ferric iron (-o-); pH (-Δ-).

iron and sulphur (Table 2.6). However, it was apparent that mineral attack had occurred, since gold dissolution upon cyanidation increased with time, stabilizing within 5 days (Fig. 2.4). This indicated that a 5 day bacterial leach was sufficient.

Table 2.6: Analysis of bacterial leach milled run-of-mine residues. Gold values are corrected for weight change (shown in brackets).

Bacterial Leach Period (days)	Analysis of Leach Residue			
	Au(g/t)	Total S(%)	Total Fe(%)	As(%)
0	9,40(0%)	5,13	5,98	0,49
2	9,65(+0,6%)	6,40	7,01	0,39
4	9,49(-1,1%)	5,29	6,61	0,17
6	9,31(+1,1%)	5,54	7,23	0,11
9	9,94(+1,4%)	5,33	6,17	0,17

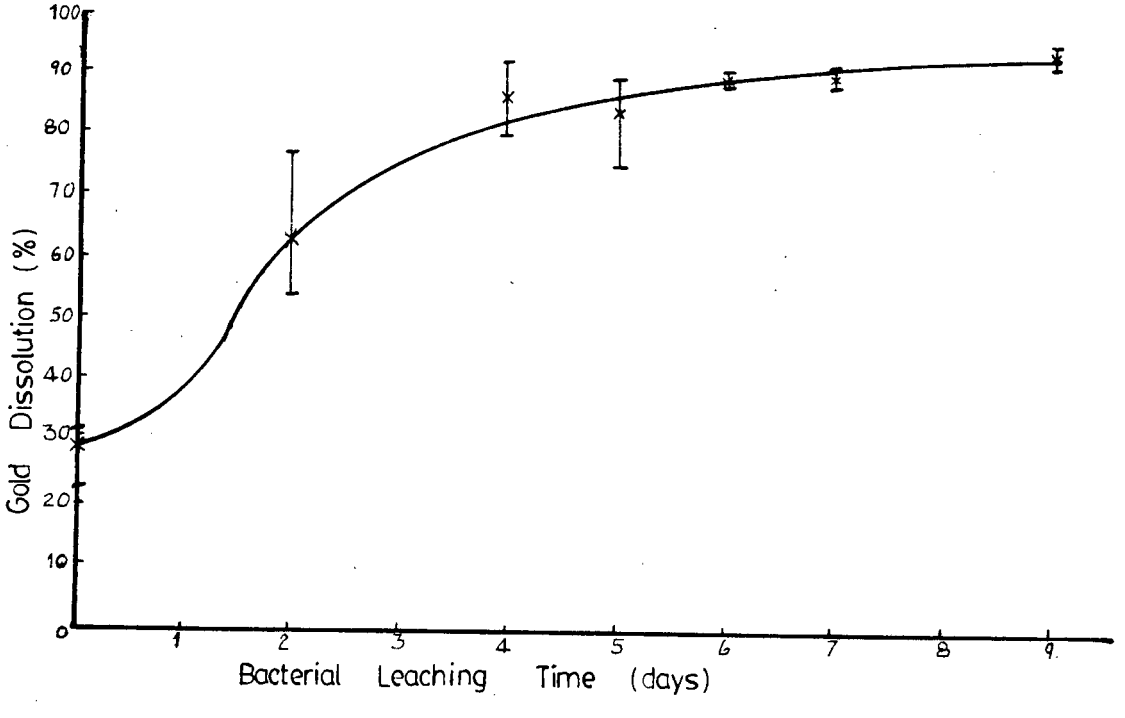


Fig. 2.4: Effect of bacterial leaching on gold dissolution from the milled run-of-mine ore. Points are the mean of several assays and vertical bars indicate the deviation.

The cyanide leach required to dissolve out the gold from the bacterially leached ore showed some unusual characteristics. The cyanide consumed in the process roughly doubled when compared with the untreated ore control and the lime consumed increased several fold. The longer the pre-aeration time prior to the addition of cyanide the lower the cyanide and the higher the lime consumption (Table 2.7).

No significant changes in the mineralogy of the ore were found due to bacterial leaching. This was due to the large amount of gangue minerals present and the consequent low amounts of gold-bearing minerals. Tyler screen analysis also revealed little change: within each particle size class examined there was approx. 10% loss, except for the -44 μm class which showed an approx. 10% gain. These changes were not due to attrition, since particle size reduction was not observed in control leaches (section 2.3.4).

2.3.3.3 Effect of Particle Size

Two ranges of particle sizes were used: the untreated ore and ore milled to 96% -74 μm (Table 2.8).

From these results it was apparent that milling had no effect. The solution and ore analyses were almost identical, as were the cyanide leach results. This can be seen by comparing Table 2.9 with Figure 2.3 and Tables 2.6 and 2.7 - the latter apply to the untreated ore and the former to the rod-milled ore.

Table 2.7: Cyanidation details of the bacterially leached run-of-mine ore.

Bacterial Leach	Cyanide Leach					
Period (days)	Head Au(g/t)	Pre-aeration Time (h)	Reagents Consumed KCN (Kg/t)	Reagents Consumed CaO(Kg/t)	Residue Au (g/t)	Gold dissolution (%)
0	10,60	7	7,57	1,76	7,91	25,4
	9,40	26	2,82	5,45	6,50	30,8
2	9,70	7	20,21	5,96	3,38	65,2
	9,60	26	5,62	24,73	3,50	63,5
4	10,60	7	23,44	21,84	2,18	79,4
	9,60	26	5,02	28,34	0,80	91,7
5	10,21	7	18,81	10,98	1,67	83,6
6	10,75	7	22,50	24,81	1,30	87,9
	9,20	26	4,68	32,55	0,92	90,0
7	10,72	7	22,24	21,97	1,16	89,2
9	9,80	26	5,26	33,10	0,56	94,3

Table 2.9 : Effect of particle size (96% -74 µm) on the bacterial and cyanide leaches of run-of-mine ore. The values given for the bacterial leach product have been corrected for weight change.

Bacterial Leach				Cyanide Leach			
Time	Solution	Product		Reagents Consumed		Residual	Gold dissolu-
(days)	Total As (g/l)	As (%)	Au(g/t)	KCN(Kg/t)	CaO(Kg/t)	Au (g/t)	tion (%)
0	-	0,38	10,92	9,08	2,75	7,60	30,4
2	0,35	0,26	10,26	22,72	6,13	4,22	58,9
4	0,73	0,20	10,92	18,78	7,04	1,57	85,6
9	0,72	0,10	10,34	21,96	7,98	1,02	90,1

Table 2.8: Screen analysis of the two ores used in the study of the effect of particle size on bacterial leaching of run-of-mine ore.

Screen Mesh Size(μm)	Percent of Total	
	Untreated ore	Milled ore
+295	0,42	0,06
+208	1,66	0,12
+147	7,40	0,54
+104	12,60	1,26
+ 74	16,12	2,26
+ 44	16,22	32,93
- 44	45,58	62,83

2.3.3.4 Effect of Pulp Density

The inoculum used in this study had 20% solids, thus making the bacterial leaching effects comparable. This was compensated for in the making up of the pulps.

As the substrate concentration increased it was expected that the ferric iron and arsenic concentrations in solution would increase. This, however, was not the case. Rather, it appeared that there was a saturation concentration of these ions in solution and any excess was reprecipitated (Table 2.10). Bacterial leaching, however, did not seem to be affected as gold dissolutions were obtained with up to 30% solids (Fig 2.5).

Table 2.10: Ferric iron and arsenic concentrations in solution during bacterial leaching of pulps of milled run-of-mine ore at different densities.

Pulp Density (% solids)	Fe III in solution(g/l)	As in solution(g/l)
20,8	2,0	0,54
24,4	2,7	0,61
30,0	2,5	0,63
43,5	0,2	0,07

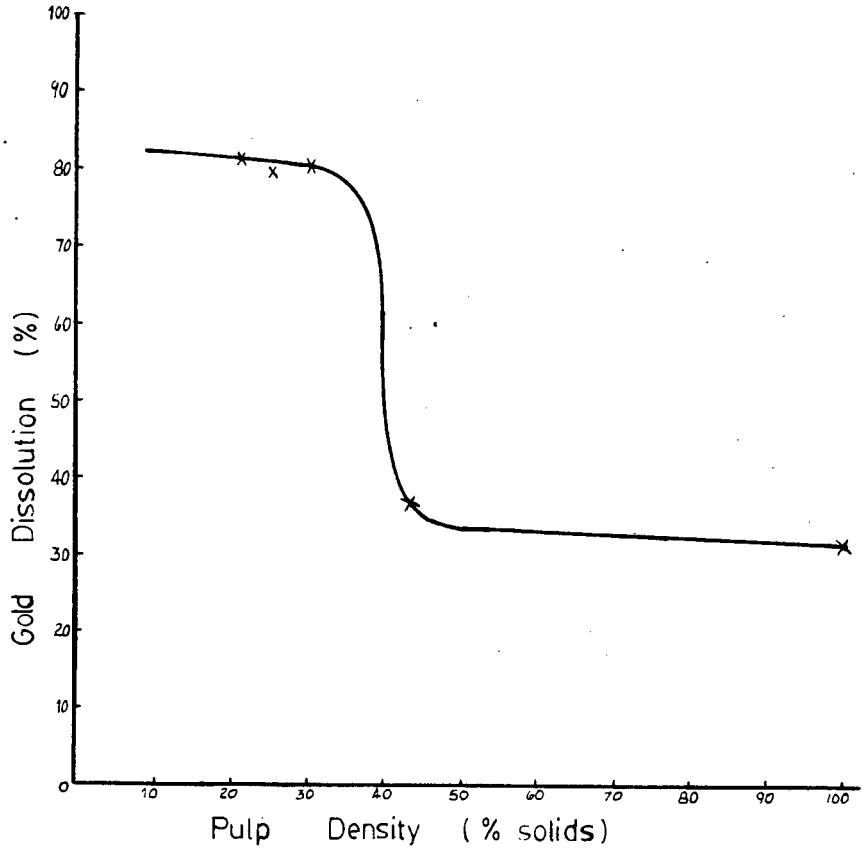


Fig.2.5: Effect of pulp density on gold dissolution from milled run-of-mine ore by bacteria.

Lime and cyanide consumptions for the batch leach residues were similar, even for the unsuccessful leach using 43,5% solids. This result was unexpected.

2.3.3.5 Effect of pH

In the pH range of 1,6 to 2,2 bacterial leaching in terms of gold dissolution was not affected very much, although pH 2,2 gave the best results (Table 2.11). As the pH increased ferric iron and to some extent arsenic in solution dropped due to precipitation rather than a lessening of bacterial leaching. The increase in iron oxides in the residue caused an increase in lime consumption during cyanidation. This expense, however, is counterbalanced by a decreasing acid consumption during bacterial leaching.

2.3.3.6 Effect of Temperature

The optimum temperature was found to be 35°C. In addition to a high mineral breakdown and gold dissolution it was found that cyanide consumption for gold dissolution was low, but was accompanied by a high lime consumption for neutralization of cyanicides (Table 2.12). Increasing or decreasing the temperature reduced gold dissolution and mineral decomposition. In the 30°-40°C range the effect of temperature was limited, but at 20°C bacterial activity was greatly reduced (Fig 2.6).

Table 2.11: Effect of pH on the bacterial and cyanide leaches of milled run-of-mine ore. Bacterial leach gold residue values have been corrected for weight change.

	Bacterial Leach					Cyanide Leach			
	pH	H_2SO_4 consumed(Kg/t)	Solution FeIII(g/l)	Solution As(g/l)	Product Au(g/t)	Reagents Consumed KCN(Kg/t)	Reagents Consumed CaO(Kg/t)	Residual Au (g/t)	Gold Dissolution(%)
	unleached control	-	-	-	9,80	2,32	5,22	6,87	29,9
unadjusted									
(±	1,8)	91,6	6,9	0,44	10,20	11,72	29,89	0,88	91,4
	1,6	167,3	8,2	0,46	10,33	10,43	15,92	1,35	86,9
	1,8	108,9	5,6	0,50	9,87	7,93	24,69	1,13	88,5
	2,0	73,4	3,4	0,51	9,33	10,73	33,93	1,30	86,1
	2,2	57,4	2,0	0,38	9,20	9,40	40,24	0,75	91,8

Table 2.12: Effect of temperature on the bacterial and cyanide leaches of milled run-of-mine ore. Bacterial leach gold residue values have been corrected for weight change.

	Bacterial Leach		Cyanide Leach			
Temperature (°C)	As in solution (g/l)	Product Au (g/t)	Reagents Consumed KCN (Kg/t)	CaO (Kg/t)	Residual Au (g/t)	Gold Dissolution (%)
Unleached control	-	9,40	2,38	3,23	7,08	24,7
20	0,55	9,40	5,76	15,23	3,50	62,8
25	0,83	9,90	8,85	20,30	1,56	84,2
30	0,77	9,60	8,91	29,88	0,88	90,8
35	0,94	9,70	4,33	44,96	0,79	91,9
40	0,91	9,20	5,46	38,85	0,83	91,0

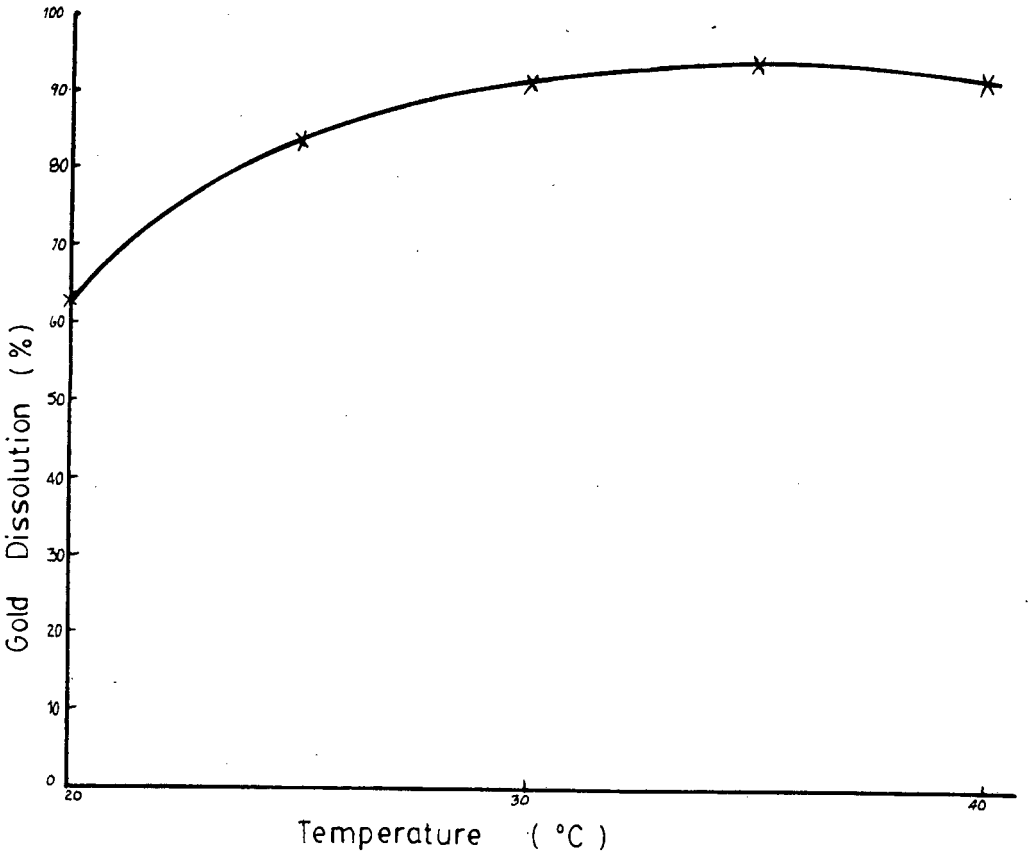


Fig. 2.6: Effect of temperature on gold dissolution from milled run-of-mine ore by bacteria.

2.3.3.7 Nutrient Requirements

Since the ore contained virtually all the 9K medium nutrients in some quantity (Table 2.1) and the addition of chemicals to a process decreases its economic feasibility it was important to determine whether the ore could provide the necessary nutrients. It was also possible that some of those present in 9K medium were not essential or affected bacterial leaching only marginally.

A comparison of the bacterial leach solution before and after a 5 day leach was made (Table 2.13). It was apparent that iron (Fe II), potassium, ammonia and chlorine were the only nutrients depleted in the course of the leach.

Bacterial leach tests in which each of the 9K medium components in turn was removed revealed that ferrous iron and phosphate were the only two nutrients to have a noticeable effect on the leach (Table 2.14). From the cyanide leaches it was noted that lime consumption dropped when ferrous iron was not present in the bacterial leach.

Table 2.13 : Nutrient concentrations in milled run-of-mine ore bacterial leach solution.

	Analysis of Leach Solution (mg/l)	
Chemical	Before Leach	After Leach
Iron	10 050 (Fe II)	6 112(Fe III)
Sulphur	6 549	11 287
Calcium	2	496
Potassium	97	0,5
Phosphorous	18	142
Nitrate	5	2 400
Ammonia	841	0
Magnesium	49	3 302
Chlorine	48	0

2.3.3.8 Summary of Minor Analyses and Investigations

Throughout much of this work attention was given to the acid consumption, particle size changes, mineralogical changes and chemical composition changes of the ore that took place during the bacterial leaching of the milled run-of-mine ore. Calculated averages from tests yielding high gold dissolutions showed that the average acid consump-

Table 2.14: Nutrient requirements and effects on the leaching of milled run-of-mine ore. Bacterial leach product values have been corrected for weight changes. The "*" indicates the two nutrients that were linked as K_2HPO_4 in 9K medium, each nutrient being proportionally replaced in the form of Na_2HPO_4 or K_2SO_4, respectively, as needed.

	Bacterial Leach				Cyanide Leach			
Absent	Solution		Product		Reagents Consumed		Residual	Gold Dis-
Nutrient	FeIII (g/l)	As(g/l)	As (%)	Au (g/t)	KCN (Kg/t)	CaO (Kg/t)	Au (g/t)	solution (%)
Unleached control	-	-	0,44	9,76	3,88	6,21	7,04	27,9
None	4,4	0,76	0,11	10,00	13,14	27,20	0,85	91,5
Iron	2,0	0,76	0,13	9,90	14,13	10,46	1,67	83,1
Ammonia	3,4	0,80	0,09	9,93	9,70	23,42	1,28	87,1
Nitrate	2,8	0,73	0,08	9,78	10,56	29,56	1,30	86,7
Potassium*	3,5	0,84	0,15	9,87	9,33	26,46	1,05	89,4
Magnesium	3,0	0,74	0,16	9,67	9,70	29,04	1,28	86,8
Phosphate*	3,6	0,78	0,21	9,90	8,86	30,31	1,76	82,2
All	2,3	0,86	0,16	10,20	15,09	8,60	1,84	82,0

tion was 107,4 Kg H_2SO_4/t ore. A reduction in particle size was also noted (Table 2.15), with most of the arsenopyrite and some pyrite being destroyed while the gangue remained unaltered (Table 2.16).

Table 2.15 : Tyler screen analysis of the head and tail of the bacterial leach of milled run-of-mine ore.

Screen Mesh Size (µm)	Percent of Total	
	Head Before Leach	Tail After Leach
+295	0,41	0,22
+208	1,55	1,10
+147	7,48	5,96
+104	12,69	11,36
+ 74	16,08	13,28
+ 44	13,80	12,06
- 44	47,99	56,02

Table 2.16: Mineralogical examination of the head and tail of the bacterial leach of milled run-of-mine ore.

Mineral	Percent of Total	
	Head Before Leach	Tail After Leach
Pyrite	2,0	1,5
Arsenopyrite	0,3	0,1
Sphalerite	trace	-
Titanium Oxide	trace	-
Gold	<0,1	+
Gangue	97,7	98,4

Chemical analyses (see Table 2.17) confirmed the breakdown of sulphidic minerals. However, whereas the mineralogical examinations indicated a 67% destruction of arsenopyrite and a 25% destruction of pyrite, the chemical analyses showed 58% and 65% respectively.

Table 2.17: Chemical analysis of the head and tail of the bacterial leach of milled run-of-mine ore.

	Analysis of Sample (%)	
Chemical	Head Before Leach	Tail After Leach
Fe	6,40	6,94
S	2,31	3,38
Pyritic S	1,52	0,54
As	0,45	0,19

2.3.4 Batch Tests - Control Leaches

The effect of acid (pH 1,8) and acidic-ferric (pH 1,8, 7 g/l Fe III) leaching on the ore for up to 5 days was examined. Neither had any significant effect on gold dissolution (Table 2.18).

Table 2.18: The effect of an acid and acidic-ferric solution on the milled run-of-mine ore.

Type of Leach	Leach Period (days)	Gold Dissolution (%)
Unleached control	-	29,1
Acid	2	31,0
	5	28,1
Acidic-Ferric	2	33,5
	5	36,8

2.3.5 Continuous Bacterial Leach Tests

From the batch testwork it was concluded that :

(i) a 5-6 day residence time was optimum. Since the lag period was approx. 1,5 days, and this is essentially eliminated in a continuous system, a residence time of 4 days was considered suitable for the continuous bacterial leach.

(ii) besides being uneconomical further ore milling was not beneficial.

(iii) although a 30% pulp density was satisfactory, other reports consistently referred to much lower values. It was, therefore, considered safer to work at 20-25% solids in the continuous bacterial leach.

(iv) the bacterial leach pH did not affect gold dissolution, but did affect both the lime consumption and the stabilization period for cyanidation. These increased at higher pH and cyanide consumption was expected to follow the same trend. Since acid consumption during the bacterial leach decreased with increasing pH the most economical pH to work at needed to be reinvestigated. Initially the pH would be set at 1,8.

(v) 30°C was the minimum temperature for successful bacterial leaching.

(vi) no extra nutrients would probably be needed, but the results were not entirely conclusive. A limited reinvestigation would be done, keeping in mind that workers have unanimously considered an iron and nitrogen source to be beneficial.

(vii) recycle is not necessary in a batch test, but it is in a continuous test where bacterial washout is possible. A 10% recycle of solids (with a large number of bacteria attached) would, therefore, be used.

The continuous bacterial leach of the milled run-of-mine ore lasted 3 months. The first two weeks allowed for mechanical and design problems to be corrected as well as allowing steady state to develop throughout the system. The subsequent 2,5 months were divided into five runs (of approximately two weeks duration), each of which allowed the examination of the effect of a particular parameter on the bacterial leach.

Each set of data was an average of approx. 15 days' readings. Data related to the conditions pertaining to a particular run were those observed during the test period, but the data relating to the processes' overflow were those recorded four days later (thus taking into account the 4-day residence time). Cyanidations were done on 2-3 day composites.

Run 1 was carried out under the conditions determined from the batch tests. The three parameters to be studied were the ferrous sulphate concentration in the feed (41,4 Kg/t ore in run 1), the ammonium sulphate concentration in the feed (4,40 Kg/t ore) and the pH (pH 1,81). In run 2 the amount of ammonium sulphate feed was halved (to 2,22 Kg/t ore). In run 3 the pH was increased (to pH 1,97), but the ammonium sulphate concentration remained halved. In run 4 the pH was returned to pH 1,8 and ferrous sulphate feeding was stopped. In run 5 the pH was dropped (to pH 1,74) while keeping nutrient feeding as for run 4. These parameter changes are clearly shown in Table 2.19.

Table 2.19: Parameter changes made during the continuous bacterial leach of milled run-of-mine ore.

Run No.	Feed $FeSO_4.7H_2O$ (Kg/t)	Feed $(NH_4)_2SO_4$ (Kg/t)	pH
1	41,4	4,40	1,81
2	41,1	2,22	1,82
3	41,1	2,24	1,97
4	-	2,22	1,85
5	-	2,24	1,74

The process operated smoothly, although only 86,0% of the feed was recovered in the overflow (Table 2.20). Approximately 85,1% of the solution was recovered when it was taken into account that the filter cake contained approx. 25% w/w solution; 85,7% of the ore was recovered. Loss of solution was mainly due to evaporation from the leach vessels, while residue loss was due to bacterial ore breakdown and loss of fines during decantation prior to filtration. Runs 2 and 4, however, showed good overflow and solution accountabilities (>90%), while in the other runs approx. 80% was recovered. Residue values, on the other hand, were very constant.

The leach vessel temperatures were found to increase from 31°C in the first vessel to 34°C in the last one.

On analysis of the head (ore) and tail (residue and filtrate) of the bacterial leach it was found that the results were similar to those obtained in the batch testwork (Table 2.21). The total sulphur in the residue was more than in the head, most probably due to the precipitation of basic sulphates. When ferrous iron was not added to the process this excess of sulphur was reduced, thus confirming the previous observation. Total iron in the residue dropped slightly, indicating mineral solubilization. This was more noticeable when no ferrous iron was fed into the system, because less Fe III precipitated out. Arsenic values were low, indicating arsenopyrite breakdown during the leach. This arsenic solubilization was not as accentuated in runs 1 and 3 for two different reasons: in the former the process was not yet working at its best (the gold dissolution was only 84%), whereas in the latter arsenic and iron were coprecipitating due to

Table 2.20: Test conditions during the continuous bacterial leach of milled run-of-mine ore. Residence time : 4,1 days. Inflow pulp density : 23% solids. Temperature : 33°C. Recycle : 7,0-7,5% solids. Air inflow : 1,3 l/min. Values in brackets are the minimum and maximum values observed.

Condition	Run 1	Run 2	Run 3	Run 4	Run 5
Ore feed (Kg/d)	1,56(1,53-1,59)	1,53(1,52-1,60)	1,53(1,50-1,56)	1,52(1,44-1,60)	1,52(1,45-1,61)
Solution feed (l/d)	6,49(5,76-6,91)	6,48(5,79-6,79)	6,48(5,78-6,82)	6,48	6,48
H_2SO_4 consumed(Kg/t)	33,4(26,1-47,1)	67,4(40,4-92,4)	29,9(24,1-40,7)	74,2(57,8-113,2)	98,9(79,0-114,6)
Overflow (l/d)	5,43(4,86-6,64)	7,08(6,22-9,00)	5,72(5,17-6,30)	6,51(4,90-8,06)	5,74(5,40-6,08)
Filtrate (l/d)	4,59(4,42-6,00)	5,94(5,53-6,18)	4,61(3,18-5,58)	5,47(4,31-6,18)	4,79(4,31-5,22)
Residue (Kg/d)	1,33(1,01-1,57)	1,34(1,08-1,72)	1,30(1,18-1,50)	1,26(0,98-1,52)	1,25(0,89-1,76)
Recycle: solids(Kg/d)	0,114(0,109-0,129)	0,123(0,114-0,133)	0,118(0,112-0,126)	0,122(0,107-0,135)	0,123(0,110-0,131)
solution(l/d)	0,05(0,00-83,40)	0,08(0,07-0,09)	0,08(0,07-0,09)	0,07(0,06-0,09)	0,08(0,07-0,09)

Table 2.21: Analyses of head and tail of the continuous bacterial leach of the flotation concentrate. Values in brackets are the minimum and maximum values observed.

Sample Analyses	Run 1	Run 2	Run 3	Run 4	Run 5
Head : Au (g/t)	9,67	9,34	9,43	9,58	9,12
		(9,04-9,57)	(9,40-9,50)	(9,10-10,12)	(9,09-9,14)
S (%)	1,80	1,82	1,69	1,83	2,00
		(1,73-1,94)	(1,61-1,73)	(1,77-1,94)	(1,78-2,34)
Fe (%)	6,10	5,94	5,98	6,27	7,29
		(5,78-6,03)	(5,91-6,01)	(5,78-7,99)	(6,17-7,99)
As (%)	0,33	0,55	0,75	0,31	0,27
		(0,36-0,94)	(0,34-0,94)	(0,26-0,35)	(0,26-0,27)
Residue: Au (g/t)	8,10	9,48	8,43	10,17	8,88
	(6,50-9,60)	(8,14-9,88)	(7,85-9,04)	(9,32-11,06)	(7,92-9,98)
S (%)	2,68	2,39	2,65	2,16	2,27
	(2,22-3,14)	(2,18-2,74)	(2,47-2,90)	(2,04-2,36)	(2,09-2,52)
Fe (%)	5,54	4,76	5,47	4,55	4,42
	(5,43-5,94)	(4,52-5,37)	(5,33-5,71)	(4,40-4,67)	(4,27-4,55)
As (%)	0,22	0,07	0,19	0,09	0,08
	(0,20-0,26)	(0,06-0,09)	(0,15-0,23)	(0,05-0,12)	(0,07-0,12)
Filtrate:Fe II (g/l)	0,3	0,2	0,3	0,2	0,1
	(0,0-0,8)	(0,1-0,4)	(0,1-0,7)	(0,0-0,8)	(0,0-0,2)
FeIII(g/l)	1,8	4,5	2,6	3,9	4,1
	(1,0-2,5)	(3,0-5,7)	(2,0-3,2)	(3,3-4,5)	(3,7-5,5)
As (g/l)	0,47	0,74	0,50	0,58	0,74
	(0,39-0,58)	(0,64-0,91)	(0,32-0,53)	(0,46-0,68)	(0,66-0,81)
NH_3 (g/l)	0,07	0,06	0,03	0,09	0,10
	(0,05-0,08)	(0,03-0,09)	(0,01-0,06)	(0,07-0,10)	(0,08-0,12)

the higher pH (pH 1,97).

The filtrates had low Fe II, high Fe III and As, and low NH_3 concentrations, indicating a good bacterial leach. Except during run 1 very little of the added ammonia was utilized - of the 0,13 g/l NH_3 added only approx. 0,05 g/l was used. Again, runs 1 and 3 seemed less successful than the others.

Mineralogical and Tyler screen analyses of the head and tail (Tables 2.22 and 2.23) revealed that approx. 53% of the pyrite and 25% of the arsenopyrite were destroyed. Gangue minerals were not decomposed. The overall particle size diminished to a small extent, since only the sulphide minerals were destroyed.

Table 2.22: Mineralogical examination of the head and tail of the continuous bacterial leach of milled run-of-mine ore.

Mineral	Percent of Total	
	Head Before Leach	Tail After Leach
Pyrite	1,7	0,8
Arsenopyrite	0,4	0,3
Titanium Oxide	trace	<0,1
Sphalerite	trace	trace
Hematite	trace	trace
Zircon	trace	trace
Gold	0,5	0,3
Gangue	97,4	98,6

Table 2.23: Tyler screen analysis of the head and tail of the continuous bacterial leach of milled run-of-mine ore.

Screen Mesh Size (μm)	Percent of Total	
	Head Before Leach	Tail After Leach
+ 295	0,23	0,16
+ 208	2,38	0,67
+ 147	12,32	6,11
+ 104	13,14	13,34
+ 74	11,61	11,99
+ 44	13,52	16,20
- 44	46,80	51,53

During the continuous bacterial leach analyses of the leach vessel contents were carried out. It was found that the Fe II concentration dropped along the system, while the Fe III concentration rose. The E_h, therefore, also increased. The pH and pulp density fluctuated. The pH in particular was difficult to control (see Table 2.24).

The major criterion for success of the bacterial leach was gold dissolution. The cyanide leach confirmed that runs 1 and 3 were the least successful (84,2% and 87,7% gold dissolution, respectively). The other runs gave a very satisfactory gold dissolution of 90-93%. For the bacterial leach at pH 1,97 the cyanide leach results (run 3) were similar to those of the pH 1,8 leach (run 2), except for the lower gold dissolution (see Table 2.25). Reduction in the amount of ammonium sulphate added to the bacterial leach had no effect (cf runs 1 and 2), while removal of ferrous sulphate addition (cf runs 2 and

Table 2.24: Analyses of leach vessel contents during the continuous bacterial leach of milled run-of-mine ore. Values in brackets are the minimum and maximum values observed.

Sample Analyses		Run 1	Run 2	Run 3	Run 4	Run 5
pH: vessel	1	1,78 (1,70-1,93)	1,69 (1,56-1,75)	1,82 (1,75-1,83)	1,80 (1,64-2,48)	1,71 (1,65-1,82)
"	2	1,88 (1,76-2,22)	1,83 (1,65-2,06)	2,04 (1,86-2,13)	1,94 (1,87-2,03)	1,80 (1,64-2,00)
"	3	1,80 (1,72-2,12)	1,89 (1,73-1,92)	2,03 (1,90-2,12)	1,88 (1,82-1,97)	1,72 (1,65-1,87)
"	4	1,77 (1,67-2,00)	1,89 (1,77-1,98)	1,99 (1,91-2,08)	1,81 (1,72-1,92)	1,73 (1,60-1,80)
E_h(mV) : vessel	1	-	-	-	478 (446-513)	492 (482-522)
"	2	-	-	-	470 (425-511)	456 (436-465)
"	3	-	-	-	538 (510-571)	505 (483-532)
"	4	-	-	-	550 (530-566)	524 (493-552)
% solids:vessel	1	25,3 (22,0-33,0)	19,5 (17,2-22,0)	22,0 (17,1-24,2)	20,9 (19,6-23,1)	23,1 (20,8-25,3)
"	2	33,8 (26,5-39,5)	29,7 (27,5-30,7)	31,8 (26,5-34,7)	39,6 (35,7-44,4)	39,6 (31,8-43,7)
"	3	29,7 (26,5-34,7)	24,2 (20,8-26,5)	26,5 (22,0-30,7)	20,9 (18,2-23,1)	26,5 (19,6-31,8)
"	4	24,2 (24,2-27,5)	22,0 (19,6-26,5)	23,0 (19,6-25,3)	15,9 (13,0-17,1)	20,8 (19,6-24,2)
FeII (g/l):vessel	1	2,3 (2,0-2,5)	0,8 (0,4-1,5)	0,7 (0,2-1,1)	0,3 (0,1-0,4)	0,3 (0,2-0,4)
"	2	1,1 (0,8-1,3)	0,7 (0,3-0,9)	0,7 (0,3-0,9)	0,3 (0,1-0,5)	0,4 (0,2-0,5)
"	3	0,1 (0,1-0,3)	0,2 (0,1-0,4)	0,3 (0,1-0,5)	0,1 (0,0-0,1)	0,1 (0,1-0,3)
"	4	0,1 (0,0-0,1)	0,1 (0,1-0,2)	0,2 (0,1-0,4)	0,0 (0,0-0,1)	0,1 (0,0-0,2)
FeIII (g/l):vessel	1	0,8 (0,4-1,2)	1,6 (1,2-1,7)	1,8 (1,3-2,2)	2,1 (1,5-2,5)	2,5 (1,7-3,0)
"	2	1,2 (0,7-1,5)	1,4 (1,1-1,9)	1,6 (1,2-2,2)	2,2 (1,5-2,6)	2,5 (1,8-2,9)
"	3	1,6 (1,5-2,0)	1,7 (1,1-2,3)	1,9 (1,5-2,4)	3,1 (2,5-3,4)	3,4 (3,0-3,7)
"	4	2,0 (1,6-2,2)	2,3 (1,4-2,7)	2,0 (1,6-2,5)	3,3 (2,6-3,7)	4,0 (3,6-4,4)

Table 2.25: Cyanide leach of the continuously bacterially leached milled run-of-mine ore. Values in brackets are the minimum and maximum values observed.

	Run 1	Run 2	Run 3	Run 4	Run 5
Pre-aeration Time (h)	26-48	43-49	27-49	43-68	31-68
Reagent Consumption: KCN (Kg/t)	16,54	14,80	13,47	10,83	6,02
	(14,8-18,9)	(7,3-19,7)	(7,3-19,8)	(4,6-16,1)	(5,1-7,0)
CaO(Kg/t)	10,69	7,92	11,57	8,62	10,40
	(4,0-21,7)	(4,7-9,4)	(6,0-16,0)	(4,2-13,3)	(9,1-12,3)
Head Au (g/t)	8,10	9,48	8,43	10,17	8,88
	(6,5-9,6)	(8,8-9,9)	(7,8-9,0)	(9,3-11,1)	(7,9-10,0)
Residue Au (g/t)	1,28	0,99	1,04	0,67	0,79
	(1,0-1,5)	(0,8-1,2)	(1,0-1,2)	(0,5-0,8)	(0,7-1,0)
Gold dissolution (%)	84,2	89,6	87,7	93,4	91,1
	(83,0-86,0)	(88,0-91,3)	(86,0-88,3)	(91,1-95,4)	(89,1-92,4)

4) not only caused an increase in gold dissolution but also a drop in cyanide, but not lime, consumption. Decreasing the bacterial leach pH further (to pH 1,74 - run 5) caused a large drop in cyanide consumption and also a slight lowering of the gold dissolution.

In addition, on completion of the continuous bacterial leach, an overall material balance was done. It was found that a small weight loss had taken place and that most of the gold, iron and arsenic could be accounted for. The biggest discrepancy was with regard to arsenic.

The process had few mechanical problems. Among these were no solution feed, too much acid addition, not enough acid addition and no air flow. The only factor that had a noticeable effect on the leach was the absence of air.

2.4 DISCUSSION AND CONCLUSIONS

The chemical leaches (with acidic and acidic-ferric solutions) were totally unsuccessful. Duncan and Drummond (1973) observed that pH 2 water had no effect on sulphides even though, theoretically, intimate contact of sulphuric acid with the sulphide can bring about metal dissolution. In this study pH 1,8 water was also found to have no effect. It has also been reported that 50 g/l Fe III only mildly tarnishes some pyrite surfaces, while a Fe III solution at pH 1,6 was found to only dissolve 3% of the pyritic iron in four weeks with no improvement in a further eight weeks (Duncan, Landesman and Walden, 1967; Duncan and Drummond, 1973; Le Roux, North and Wilson, 1973).

On the other hand, Keller and Murr (1982) observed clear pyrite etching within seven days using Fe III solutions of 2 g/l or more, at 30°C. Groudev (1982b) reported that the solubilization of pure arsenopyrite was greatly enhanced by Fe III. In this study gold dissolution increased by 7,7%, indicating a real but insignificant attack on the minerals. The ferric iron was converted to ferrous during the leach and possibly if the ferric iron were regenerated the process would be more successful. However, it is thought that the reason why pyrite does not respond particularly favourably to Fe III leaching is due to the formation of a protective sulphur layer on the mineral (Dutrizac and MacDonald, 1974). From these results it was obvious that a simple chemical leach could not contribute to an increased gold solubilization from the milled run-of-mine ore.

Bacterial leaching, on the other hand, was successful. T.ferrooxidans, a sulphur-oxidizing thiobacillus (possibly T.thiooxidans) and L.ferrooxidans were identified, but it is possible that other bacteria were present e.g. T.acidophilus, since it is regularly isolated from T.ferrooxidans cultures grown on iron and pyrite. T.acidophilus prefers organic substrates, but can grow oligotrophically on sulphides and/or may use the organic matter liberated by T.ferrooxidans (Arkesteyn and de Bont, 1980; Harrison, Jarvis and Johnson, 1980). The presence of T.thiooxidans would not be surprising since pyrite oxidation has been shown to be enhanced by it. It is thought that the biological effect is surface-active or physico-chemical through the generation of an organic metabolite (Groudev, 1981; Wakao et al, 1983). L.ferrooxidans has also been reported growing with

thiobacilli on pyrite and chalcopyrite (forming 10% of the culture) and is thought to be able to degrade pyrite in pure culture (Balashova et al, 1974; Kelly, Norris and Brierley, 1979; Norris, 1983).

Generally, the bacteria are distributed between the leach solution and the mineral surfaces with the proportions varying, although most of the organisms are attached. On zinc sulphide 77% were attached; on chalcopyrite 95-98%; on pyrite 86%, 60-80% and 98-99% have been reported, with at least 5×10^8 cells/ml in solution (McGoran, Duncan and Walden, 1969; Le Roux, North and Wilson, 1973; Groudev, 1979; Roy and Mishra, 1981). In this study 96-98% of the bacteria were attached, with $2\text{-}5 \times 10^8$ cells/ml solution.

Although sterility is normally maintained in bacterial leach tests it was not considered necessary, since contaminants would have to be both autotrophic and arsenic resistant. No problems regarding loss of an active culture occurred and the culture adapted progressively with time.

When studying the basic characteristics of the bacterial leach of the milled run-of-mine ore a typical growth curve was apparent : a 1-2 day lag phase, log phase and a stationary phase developed after 5 days. Analysis of the solution demonstrated Fe II oxidation with concomitant Fe III formation as well as arsenic solubilization, thus indicating ore degradation. If the ferrous and ferric iron values obtained in the continuous bacterial leach vessels were plotted against time the graph would be very similar to Fig. 2.3 (the graph

obtained from the batch testwork). The main difference was a greatly reduced lag phase in the former. These solution changes were accompanied by an increasing E_h (480 mV to 550 mV). With metal sulphide oxidation an increase in E_h to about 515 mV is usually observed. The redox potential of the Fe III/Fe II system (+747 mV) should be expected toward the end of the leach, but is rarely achieved due to jarosite precipitation once an E_h of about 500 mV is reached (Torma 1977; Lundgren and Silver, 1980). The precipitation of jarosite, basic iron sulphates, ferric arsenate etc. probably did occur. Ferric concentrations in solution were not as high as expected and chemical analysis of the leach residues often showed increases in iron and sulphur, presumably because 9K was initially present in the medium.

Precipitation of leach products results primarily from the formation of iron arsenate and the hydrolysis of both it and ferric sulphate (Pinches, 1975). The pH and the ions present in solution both play a role in this regard. Ivarson, Ross and Miles (1979) used T.ferrooxidans and modified 9K medium to study the effect of K^+, Na^+ and NH_4^+ individually and in pairs on acid production and basic ferric sulphate crystallization rates. They found that both showed similar patterns and that $K^+ > NH_4^+ > Na^+$.

The effect of the bacterial mineral attack on gold dissolution was enormous (the gold dissolution increased from 30% to 90%). For a given bacterial leach time gold dissolution results could vary greatly. This variability decreased with time (Fig. 2.4). Variations in inoculum size affected the length of the lag phase, but

disappeared with time. A maximum gold dissolution of 93-94% was observed. This was predicted from a computer-assisted analysis carried out by Gencor staff. The 6-7% unavailable gold was encased in the gangue minerals.

The cyanide leach lime consumption increased with bacterial leaching time, because more and more acid was added and, therefore, had to be neutralized. At 11 Kg/t the cyanide consumption was also high (an average plant consumes less than 1 Kg/t ore), but could be reduced by either adding an excess of lime at the start of the leach or by increasing the pre-aeration time (see Table 2.7).

Once the feasibility of the bacterial leach had been proven the most important parameters likely to affect it were examined. Particle size was investigated since it has been reported in both batch and continuous leaches that an increase in surface area due to a decrease in particle size caused a rise in oxidation rate of the ore (Razzell and Trussel, 1963; Napier, Wood and Chambers, 1967; Chang and Myerson, 1982; Lawrence and Bruynesteyn, 1983). Torma and Bosecker (1982) advocated a particle size less than 32 µm, but Duncan, Walden and Trussel (1966) reported that when less than 37 µm particle size ceased to be limiting to the bacterial leach of chalcopyrite ore and some other factor became rate-controlling. Since milling of the run-of-mine ore was expensive it was decided to mill it to 65% -44 µm. Gold dissolutions remained unaltered. Normally milling alone liberates some gold. As this was not the case it would appear that the milling was "preferential" in that the softer gangue minerals were the ones contributing to the drop in size, not the harder

sulphide minerals (<5% of the ore). Lundgren and Silver (1980) commented that a drop in particle size of low-grade ores could increase the surface area of the gangue relative to the substrate, this being equivalent to substrate dilution.

The initial choice of pulp density was made based on two things. Firstly, the bacterial culture could tolerate only 1 g/l As and, if one assumed total ore degradation and arsenic solubilization, a 20% solids pulp density would result in only 0,7 g/l As in solution. Secondly, 20% solids was the highest value quoted in the literature (Lawrence and Bruynesteyn, 1983). Generally, however, the highest metal extractions by T.ferrooxidans have been found to be at low pulp densities (Torma and Sakaguchi, 1978). Le Roux, North and Wilson (1973) reported that 6% solids was optimal when working with pyrite. Roy and Mishra (1981) considered that 3-5% solids was optimum, but that 5-10% was acceptable. High pulp densities, however, were not tested. This study clearly demonstrated that up to 30% solids could be used. Batch tests at 30% solids were very satisfactory; in the continuous bacterial leach the inflow was approximately 23% solids, but up to 40% solids occurred in some leach vessels (e.g. vessel 2 in runs 4 and 5) and 30% solids was common.

Ferric iron and arsenic in solution were expected to increase at increasing pulp densities, but there seemed to be a saturation concentration above which any excess precipitated. Hence, it was not the arsenic concentration in solution that was limiting. It was most likely mass transfer of nutrients, CO_2 and particularly O_2.

The ideal pH would be in the pH 1,5-3,0 range, but an exact value could not be obtained from the literature. Polkin et al (1975) found a pH of 2,0-2,3 was ideal, Pinches (1975) showed that pyrite was leached best at pH 2,75-3,0, but poorly at pH 1,8. Arsenopyrite leached best at pH 2,0, metal leaching dropping as the pH rose. For pyrite oxidation by T.ferrooxidans specifically, a pH of 1,8 was optimum but acceptable leach rates between pH 1,3 and 4,0 were reported (Corrick and Sutton, 1965). Bruynesteyn and Vizsolyi (1981) found that a pH of 1,6-1,7 was best, but that rates were good between 1,2-2,0 and an optimum pH of 2,0-3,0 was reported by Roy and Mishra (1981) and by Kandemir (1983). For ferrous iron oxidation by T.ferrooxidans an optimum pH of 2,4 has been reported (Schnaitman, Korczynski and Lundgren, 1969). These authors report that with adaptation oxidation can be very satisfactory even at pH 1,25. The effect of pH in the range 1,6-2,2 was therefore examined. The batch bacterial leaches did not allow an optimum pH to be pinpointed as results were all rather similar. During the continuous leach it was found that pH 1,80-1,85 was clearly best. At pHs 1,97 and 1,74 gold dissolution dropped by 1,9% and 2,3% respectively when compared to pH 1,84.

As expected, acid consumption decreased with increasing pH. In the batch testwork acid consumption at pH 1,6 was approximately three times and at pH 1,8 approximately twice that observed at pH 2,2. In the continuous testwork the same trend was seen, but the actual values were much lower than those obtained in batch tests; 30 vs 73 Kg H_2SO_4/t ore at pH 2,0 and 71 vs 109 Kg/t at pH 1,8. This probably had to do with the use of several leach vessels resulting in the

better utilization of both the added and bacterially generated acid. Acid had to be added because although the bacteria generate acid from sulphur oxidation the sulphide content of the ore was low (2-4% of the ore) and the natural pH of the ore slightly alkaline (pH 7,49).

As the pH increased iron and, to some extent, arsenic precipitated, as would be expected from the chemistry of these elements. Jarosite, iron arsenate and such compounds are known cyanicides and at the bottom end of the pH range (pH 1,74) cyanide consumption for gold solubilization was significantly lower.

The optimum temperature for the bacterial leaching of the milled run-of-mine ore was found to be 35°C. In the 30-40°C range results were similar, but at 20°C bacterial activity was greatly reduced. Metal sulphide ore leaching is usually good in the 25-45°C range, 35°C being optimum when using T.ferrooxidans (Lundgren and Silver, 1980; Torma and Bosecker, 1982). With low-grade pyrite the acceptable range was found to be 25-40°C, 35°C being the optimum (Corrick and Sutton, 1965; Lawrence and Bruynesteyn, 1983). The present study is in exact agreement with these figures. Razzell and Trussel (1963), working on the T.ferrooxidans leaching of a chalcopyrite ore specified that 35°C was ideal and reported a sharp drop in oxidation (to the same as the uninoculated control) at below 25°C and above 40°C. It would appear that there is a noticeable difference between T.ferrooxidans metabolism and leaching temperature optima. The former is 20-35°C (usually accepted as 28°C) with more than 35°C causing a marked decline (Torma, 1977; Murr and Brierley, 1978).

Temperature also had an effect on the cyanide leach. As the temperature increased so did the lime consumption. At higher temperatures many chemical reactions are accelerated, which resulted in more cyanicides forming.

Nutrients are another important parameter in the bacterial leaching of ore. It has been reported that Fe II oxidation was able to take place in acid water to which only ferrous sulphate had been added. The rest of the inorganic nutrients were presumably provided by the air (e.g. NH_3) or were present as trace impurities in the ferrous sulphate or water (Schnaitman, Korczynski and Lundgren, 1969; Rawlings, 1981). For ore leaching, however, no nutrients may have to be added as, in most cases, all are available from the material to be leached. Phosphate and ammonia are the least likely to be present in sufficient quantity although phosphorous may be present from the water or apatite and nitrogen from blasting. However, it has been recommended that 0,5 g/l K_2HPO_4, 3 g/l $(NH_4)_2SO_4$, 0,2% CO_2, lots of air and some Fe II be added (Bruynesteyn et al, 1979; Lundgren and Silver, 1980; Torma and Bosecker, 1982). The need for the addition of at least some nutrients, in small amounts, has been shown in several studies using pyrite and either T.ferrooxidans or mixed cultures. Roy and Mishra (1981) observed inhibition of pyrite oxidation, compared to growth in 9K medium, in the absence of nitrogen (<1% oxidation), phosphorous (45%) and potassium (63%). Lloyd (1967) added 0,3 g/l K_2HPO_4 and not more than 3,35 g/l $(NH_4)_2SO_4$ - similar to what Torma and Bosecker (1982) recommended. Although iron was found not to be essential it was seen as being helpful (at approx.

2 g/l) for both pyrite and arsenopyrite oxidation. This is thought to be due to the extra Fe III generated (Corrick and Sutton, 1965; Polkin et al, 1975; Groudev, 1981; Chang and Myerson, 1982).

From the batch testwork reported here only ferrous iron and phosphate addition were beneficial to the leach, although not essential. A bacterial leach in tap water gave the same gold dissolution (of 82%) as a phosphate-limited one and slightly less than a ferrous-limited one (83%). The addition of these nutrients was, therefore, beneficial but not essential. A solution analysis at the end of the leach indicated that certain other nutrients had been depleted, but there had apparently been enough present to ensure good mineral attack.

Although some workers have proposed the need for phosphate, others claim it is deleterious to pyrite oxidation. It is thought to react with the iron atoms at active sites in the pyrite crystal, hence preventing microbial attack (Corrick and Sutton, 1965; Beck, 1969; Le Roux, North and Wilson, 1973).

In the continuous leach the goal was to eliminate nutrient addition completely. At the end of the experiment only 0,5 g/l $(NH_4)_2SO_4$ was added and it was highly likely that even this amount was not needed. It was interesting that the absence of nutrient addition caused a drop in cyanidation lime consumption.

A change in grading and compositional analyses of the ore occurred during the leach. There was a reduction in particle size which was not due to attrition, as this did not occur in the control leaches.

The largest particle size fraction (295 µm) showed the greatest reduction (50-70% loss in weight). This effect decreased with particle size and the -44 µm fraction showed a particle mass gain (10-20%). This was not unexpected, since when the larger particles were eroded they would fall into the next fraction. Only sulphide minerals were degraded in the leach, with about two thirds of the pyrite and arsenopyrite being decomposed.

From this work it was concluded that (i) the bacterial leach of the milled run-of-mine ore was successful; (ii) a retention time of four days was needed, with 7-10% recycle, yielding 91-93% gold dissolution upon cyanidation; (iii) further ore milling was unnecessary; (iv) 30% solids could be used; (v) the pH should be approximately pH 1,8, with a corresponding acid consumption of about 71 Kg H_2SO_4/t ore; (vi) 35°C was the optimum temperature, but 30°C was also very satisfactory; (vii) probably no nutrients needed to be added; (viii) the cyanide and lime consumptions for the solubilization of the gold would be approximately 10 Kg/t ore.

Based on these findings an economic feasibility study was carried out. A bacterial leaching plant for the run-of-mine ore could not compete with the conventional flotation-roasting process (Livesey-Goldblatt, Norman and Livesey-Goldblatt, 1983).

CHAPTER 3

BACTERIAL LEACHING OF THE FLOTATION CONCENTRATE

Summary: The milled flotation concentrate was analysed chemically and mineralogically and found to have characteristics likely to support bacterial growth and activity. It also contained 146 g Au/t concentrate.

Although the concentrate contained arsenic a bacterial culture capable of tolerating 4 g/l As was adapted and used for batch tests. In batch tests it was found that the bacterial growth curve consisted of a 2-3 day lag period, a log phase and reached stationary phase after 10-12 days. The maximum gold dissolution of 98% could be obtained in 15 days, although over 90% recovery was attainable in 10 days. The best method for batch leaching was using 9K medium, pH control and intermittently removing leach solution. 62,3 Kg H_2SO_4/t concentrate and 68,1 Kg CaO/t concentrate were consumed. A reduction in particle size and sulphidic minerals also occurred during the leach.

A reduction in particle size from 68% to 88% -74 µm was essential. Inadequate milling delayed the leach by approximately 8 days. Pulp densities up to 20% solids allowed for good bacterial leaching and gold dissolution, the optimum, however, being 10% solids or less. A pH in the range of 1,6-2,2 was satisfactory, as was a temperature in the 25-45° C range. Tests indicated that the only chemical addition like to be necessary in a bacterial leach of the milled flotation concentrate was ferrous iron. It was found that the presence of

flotation reagents in the leach was not detrimental and that the optimum air flow was approximately 1,43 l air/min Kg.

Control acid and acidic-ferric batch tests had no significant effect on gold dissolution.

A two and a half month continuous bacterial leach of the concentrate was carried out, initially under the conditions based on results from the batch work. The pH and nutrients were altered successively in an attempt to optimize these two parameters. A 43% weight loss was observed, which reflected bacterial mineral breakdown as well as loss of fines during decantations. A reduction in particle size and sulphidic minerals was observed.

Increasing the bacterial leach pH (from 1,81 to 2,07) had no effect. Reducing the ferrous sulphate addition by 50% (to 160 Kg/t) and the ammonium sulphate and di-potassium hydrogen phosphate additions by 20% (to 8,5 and 6,3 Kg/t, respectively) resulted in a drop in cyanide and lime consumptions as well as the pre-aeration time during the cyanide leach. This was probably also affected by the use of lime as an acid neutralizing agent during the bacterial leach.

Microscopic examination of epoxy resin mounts of the flotation concentrate revealed that bacterial and acidic-ferric leaching both etched the pyrite and arsenopyrite, but their effects were different. Galvanic interactions caused the arsenopyrite to be destroyed first, followed by the pyrite. Bacterial attachment of thiobacilli and leptospirilli was seen.

It was concluded that (i) a bacterial leach of the milled flotation concentrate was possible; (ii) a retention time of ten days was needed, yielding 97-98% gold dissolution upon cyanidation; (iii) milling to 88% -74 µm was necessary; (iv) 12% solids could be used; (v) pH 1,7-1,8 was most suitable, with a corresponding lime consumption of about 120 Kg/t concentrate; (vi) 28°C was the optimum temperature; (vii) only 2 g/l Fe II needed to be added; (viii) for gold dissolution 18 Kg KCN/t concentrate and 50 Kg CaO/t concentrate were needed.

A feasibility study showed this process to be practical and economically viable.

3.1 INTRODUCTION

The bacterial leaching of concentrates is of recent interest. Studies have generally been aimed at the development of a vat leach, using a variety of minerals including pyrite, arsenopyrite and mixtures of these. In some cases pure minerals and in others concentrates have been used.

Several studies have been reported using pyrite and T.ferrooxidans. Le Roux, North and Wilson (1973) found that 12-17 weeks were needed to achieve 99,8% pyritic iron dissolution; Wakao et al (1982) required 3-4 weeks for 90-95% pyrite oxidation; Hiltunen et al (1981) found that in 5 weeks 73% of the pyritic iron dissolved. Other studies have involved several bacterial cultures, such as T.ferrooxidans, T.thiooxidans, Thiobacillus TH1, L.ferrooxidans, mixtures of

these and natural mixed cultures. Norris and Kelly (1978) found that pyritic iron dissolution was greatest using the two natural mixed cultures or L.ferrooxidans mixed with a mesophilic thiobacillus; T.ferrooxidans and T.TH1 alone were half as effective; while T.thiooxidans and L.ferrooxidans alone did not oxidize the pyrite at all. Later Norris (1983), in similar testwork, found that a natural culture and pure L.ferrooxidans gave the highest pyritic iron dissolutions; T.ferrooxidans alone and mixed with T.thiooxidans were half as effective; while T.thiooxidans alone was ineffective. These experiments were continued for 2-3 weeks before maximum dissolution occurred.

Some workers have used pyritic concentrates for their studies. Atkins (1978) reported T.ferrooxidans as requiring approximately 75 days to completely oxidize a 10-12% solids slurry of a pyritic concentrate. Lawrence and Bruynesteyn (1983) reported that a mixed culture required 33 days to oxidize a 17% solids slurry to yield 91% gold dissolution. Lawrence and Gunn (1985) reported that T.ferrooxidans required only 5 days to leach a 16% solids slurry to yield 92% gold and 75% silver recovery. It was done in batch and confirmed in a continuous bacterial leach test.

Bacterial leaching of arsenopyrite is much faster. Within a week about 80% of the cobalt and 84% of the gold in arsenopyrite concentrates were made available and similar results were obtained with a tin-containing arsenopyrite concentrate (Karavaiko, Kuznetsov and Golonizik, 1977; Qiu et al, 1980).

T.ferrooxidans leaching of pyrite/arsenopyrite concentrates has also been studied. In 4-7 days maximum leaching yields have been achieved, with approximately 90% of the arsenic and gold being dissolved out. These concentrates contained up to 9,3% arsenic. To overcome arsenic toxicity either low substrate concentrations were used or leach solution was removed and replaced during the process (Kulebakin, 1974; Pinches, 1975; Polkin et al, 1975; Yudina et al, 1979). And recently Marchant (1985), in batch and continuous tests, bacterially leached a 10% solids slurry of a pyrite/arsenopyrite concentrate and in 40 h achieved about 90% gold and silver recovery.

Hence, it was thought that a system for the flotation concentrate used in this study could be developed, with a retention time of less than one week. As with the milled run-of-mine ore, a continuous vat leach would be the final goal. Other workers have got similar systems to work using chalcopyrite, copper-zinc or galena concentrates (Polkin et al, 1977; McElroy and Bruynesteyn, 1978; Torma, 1978; Lawrence, Bruynesteyn and Hackl, 1981). A continuous leach of pyrite from coal has been reported, as has that of a gold-bearing pyrite/arsenopyrite concentrate (Polkin et al, 1975; Myerson and Kline, 1984).

Since the flotation concentrate contains approximately 5% As it was necessary to develop a bacterial culture that was arsenic tolerant. Generally either 0K or 9K plus the mineral(s) was used for the adaptation process. Success in adapting T.ferrooxidans to a substrate and heavy metal ion concentrations has been well established e.g. 12 g/l uranium, 160 g/l iron, 55 g/l copper, 72 g/l

nickel, 120 g/l zinc and 4,9 g/l arsenic (Khalid and Ralph, 1977; Torma, 1977; Atkins, 1978; Torma and Bosecker, 1982; Norris, 1983). The adaptation mechanism is not clear, but is probably connected to the induction of enzymes, the production of surface active agents, alteration of the cell surface etc. The behaviour is genetically determined, but influenced by the environment (Groudev and Genchev, 1978; Groudev, 1979).

What happens during the bacterial leaching of a pyrite/arsenopyrite is summarized by the following reactions (Murr and Berry, 1976; Brierley, 1978; Kelly, Norris and Brierley, 1979; Wakao et al, 1982; Livesey-Goldblatt, Norman and Livesey-Goldblatt, 1983; Lawrence and Gunn, 1985):

$4FeAsS + 13O_2 + 6H_2O \xrightarrow{\text{Bacterial}} 4H_3AsO_4 + 4FeSO_4$

$2FeS_2 + 7O_2 + 2H_2O \xrightarrow{\text{Bacterial}} 2FeSO_4 + 2H_2SO_4$

$4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{\text{Bacterial}} 2Fe_2(SO_4)_3 + 2H_2O$

$2FeAsS + Fe_2(SO_4)_3 + 6O_2 + 4H_2O \xrightarrow{\text{Chemical}} 2H_3AsO_4 + 4FeSO_4 + H_2SO_4$

$2H_3AsO_4 + Fe_2(SO_4)_3 \xrightarrow{\text{Chemical}} 2FeAsO_4 + 3H_2SO_4$

$2FeAsS + 2H_2SO_4 + O_2 \xrightarrow{\text{Chemical}} 2FeAsO_4 + 4S^\circ + 2H_2O$

$FeS_2 + Fe_2(SO_4)_3 \xrightarrow{\text{Chemical}} 3FeSO_4 + 2S^\circ$

$2S^\circ + 3O_2 + 2H_2O \xrightarrow{\text{Bacterial}} 2H_2SO_4$

$2FeS_2 + 2H_2SO_4 + O_2 \xrightarrow{\text{Chemical}} 2FeSO_4 + 4S^\circ + 2H_2O$

$Fe_2(SO_4)_3 + 6H_2O \xrightarrow{\text{Bacterial}} 2Fe(OH)_3 + 3H_2SO_4$

$Fe(OH)_3 + H_2SO_4 \xrightarrow{\text{Chemical}} Fe(OH)(SO_4) + 2H_2O$

$3Fe(OH)(SO_4) + NaOH + 2H_2O \xrightarrow{\text{Chemical}} NaFe_3(SO_4)_2(OH)_6 + H_2SO_4$

Some of the effects are direct and some indirect. That both types occur is likely, since when pyrite is placed in a dialysis bag during bacterial leaching oxidation is reduced but not eliminated. Direct bacterial attack has been proven by the bacterial dissolution of iron-free minerals such as chalcocite, covellite, molybdenite, orpiment and tetrahedrite. In addition, when the ferroxidase system is absent (in UV mutants or by sodium azide inhibition) mineral oxidation still occurs; and from analyses and comparison to predicted values derived from the reactions ferric iron leaching can only account for 52,9% of pyrite dissolution. Indirect bacterial attack has been illustrated in uranium oxidation and by the drop in the leaching rate of pyrite from which acid-soluble iron has been removed. Most of this decrease was reversible when Fe III was added (Silverman, 1967; Gaidarjiev, Groudev and Genchev, 1975; Arkesteyn, 1979; Kandemir, 1983). Pinches (1975) suggested that attack is always indirect, because a coating forms on the mineral and the attached bacteria are consequently separated from it by a permeable or semi-permeable barrier.

What is certain is that microorganisms attach to the mineral surface, suggesting direct leaching. The bacterial metabolite responsible for the leaching action may be involved in ferrous iron oxidation, the solubilization of molecular sulphur and/or the direct attack of the sulphide (Lundgren and Silver, 1980). Work done with T.ferrooxidans and pure metal sulphides has shown that the highest rate of substrate oxidation was achieved with the highest solubility product of its sulphide. This strongly suggests that substrate dissociation is a prerequisite, though not necessarily an exclusive one, for metal

sulphide oxidation (Torma et al, 1974; Torma and Sakaguchi, 1978). The dissociated metal and sulphide migrate to the boundary layer and the bacteria oxidize these. The equilibrium of the reaction

$$MS \rightleftharpoons M^{2+} + S^{2-}$$

moves to the right-hand side and causes more metal sulphide dissociation. Bacterial attachment would guarantee proximity to these ions which are their energy source (Tuovinen and Kelly, 1974; Torma and Bosecker, 1982).

In addition, galvanic effects and interactions need to be taken into account. It has been observed that pyrite with hole conductivity (anodic) was oxidized by T.ferrooxidans continuously and more intensely than that with electron conductivity (cathodic). Three chalcopyrites, when leached by T.ferrooxidans, had leachability rates which were directly proportional to the largest electrode potential (EP)-E_h difference. This was due to the bacteria attaching to the mineral surface and transporting electrons from the anodic area of the mineral to some constituent of the bacterial electron chain which served as a cathode (Karavaiko and Pivovarova, 1977; Groudev, 1980). In the absence of bacteria the EP-E_h difference is small and leaching was negligible. With a mixture of minerals there are galvanic interactions and there was some corrosion, even in the absence of bacteria. The rest potential of pyrite is 630 mV, chalcopyrite 520 mV and sphalerite -240 mV. The pyrite, therefore, dissolved the slowest in a mixture of these minerals because the other mineral acted anodically while the pyrite was passivated. When the anodic curve of one mineral crosses the cathodic curve of another any elec-

trolyte could yield a corrosion potential and a current could flow. The electrons flowed into the pyrite, oxygen absorbed to it and therefore passivated it. Pyrite behaved similarly with arsenopyrite, covellite, galena and molybdenite (Karavaiko, Kuznetsov and Golonizik, 1977; Karavaiko and Pivovarova, 1977; Berry and Murr, 1978; Berry, Murr and Hiskey, 1978; Mehta and Murr, 1982). Chalcopyrite behaved similarly with pentlandite and pyrrhotite (their rest potentials are 390 and 360 mV, respectively), although when these two touched there was little leaching (Natarajan and Iwasaki, 1983; Natarajan, Iwasaki and Reid, 1983). In the presence of bacteria Fe III was generated, this increased the electrolyte potential, the EP-E_h difference therefore also increased and the galvanic effect was enlarged (Mehta and Murr, 1982). The bacteria can also act as a cathode.

The end result of these various forms of leaching was mineral etching and ultimately total mineral dissolution. A lot of work on this aspect has been done on pyrite, using T.ferrooxidans. The pyrite after leaching has usually been observed to be tarnished and have jagged edges. But more important are the pits and grooves that are formed, these being in the region of contact between the mineral and the attached bacteria. Although thiobacilli are seen attached this is not very common. Some workers claim that these pits show no particular orientation or distribution, while others suggest they reflect both the cubicity of the crystal and crystal weaknesses or imperfections. It is thought that T.ferrooxidans may be able to select between more or less favourable sites for energy extraction (Duncan and Drummond, 1973; Bennett and Tributsch, 1978; Keller and Murr,

1982; Southwood and Southwood, 1985). In studies with pyrite and chalcopyrite bacterial attachment and pitting along grain boundaries and weakneses was again observed. In addition the patterns were found to differ with differing crystallographic orientations (Berry and Murr, 1978; Berry, Murr and Hiskey, 1978). Similar patterns were reported for galena (Tributsch, 1976). With arsenopyrite a coating, formed by mineral replacement, was seen (Pinches, 1975). These patterns differed from those produced by a purely chemical (acidic-ferric) leach. Chemical etches reflected the cubic structure of the pyrite, were regular and monodirectional. Jarosite and elemental sulphur deposition were also noted (Bennett and Tributsch, 1978; Keller and Murr, 1982).

In these studies bacterial attachment and etching of the flotation concentrate was examined, since the microscopic examination of the minerals might shed some light on the mechanism of bacterial (and chemical) leaching.

One other aspect of this work was to use the data gathered during the leach of the flotation concentrate to carry out an economic feasibility study. To date only McElroy and Bruynesteyn (1978) and Torma (1978) have done such a study on a concentrate. The former, using a chalcopyrite concentrate, found the bacterial process had no cost advantage when compared to copper smelting. The latter, using a lead sulphide concentrate, reported that the bacterial process yielded a profit of $\$6{,}3 \times 10^5$ for an investment of $\$7{,}3 \times 10^6$, which was acceptable. This did not include any possible revenue from smelting the lead by-product. Lawrence, Bruynesteyn and Hackl (1981) claim that chalcopyrite, sphalerite and galena leaches were all economically viable, but gave few details.

3.2 MATERIALS AND METHODS

Details of the methods, media and solutions are listed in Appendices A and B, respectively.

3.2.1 Analysis of the Flotation Concentrate

The concentrate was analysed for a large number of elements by I.C.A.P. Wet chemistry methods were used to analyse for total iron, total and pyritic sulphur and arsenic. The gold and silver content was determined by fire assay. A mineralogical examination and screen analysis were also done.

3.2.2 Milling of the Flotation Concentrate

The concentrate was wet milled in a laboratory-scale iron rod mill until it was approximately 90% -74 μm.

3.2.3 Bacterial Strain Development and Identification

3.2.3.1 Adaptation to Test Conditions

The bacterial culture was adapted to growth in an aerated slurry of milled concentrate and 9K medium (pH 1,8, 30°C). Small amounts of concentrate and fresh 9K medium were added regularly to the culture. It was used as inoculum when there was approximately 10% solids, 4

g/l As in solution and the added ferrous iron was oxidized within 16 hours.

Sterility was not enforced as the plant would be non-sterile.

3.2.3.2 Identification of the Bacteria Present

The adapted culture solution was serially diluted with sterile distilled water and 0,1 ml plated onto Nutrient agar, Waksmans' and 9K medium solidified with 2% Oxoid agar-agar. The plates were incubated at 30°C for up to four weeks. Isolated colonies were inoculated onto the different types of agar plates and into the corresponding liquid media. They were incubated at 30°C for up to four weeks. The pH was monitored sporadically and cultures at different initial pHs used. Gram stains and wet slide preparations were made from selected colonies and solutions and examined microscopically. Identification was done on the basis of morphology and energy source (as in Fig. 2.1).

For total counts the nitrogen content method of Gormely and Duncan (1974) was used.

3.2.4 Batch Tests - Bacterial Leach Procedure

Milled flotation concentrate (350 g) and 9K medium (3,50 l) were placed in a 5 l beaker, which gave a slurry of approximately 10% pulp density. The stirred slurry was aerated and kept at 30°C in a water bath. The pH was adjusted to pH 1,8 with concentrated H_2SO_4.

A 10% slurry inoculum (360 ml) prepared as in 3.2.3 was added and the pH thereafter maintained at pH 1,8 by addition of either concentrated H_2SO_4 or 130 g/l CaO slurry.

At specific intervals the slurry was allowed to settle, the solution was decanted off and fresh 9K medium was added as replacement (volume for volume).

After 10 days the slurry was filtered, the filter cake washed, repulped, refiltered and rewashed. The final filter cake was dried at 60°C and weighed. The filtrate and decanted solutions were collected and analysed for ferrous iron, ferric iron, arsenic and pH.

The dried ore was analysed for total iron, total and pyritic sulphur, arsenic and gold. Mineralogical and screen analyses were also carried out.

3.2.5 Batch Tests - Sterile Control Leach Procedure

Acid and acidic-ferric control leaches were run in parallel with the bacterial leach. Instead of 9K medium being used to make up the slurry either tap water or a ferric sulphate (7 g/l Fe III) solution acidified to pH 1,8 was used. There were no decantations. Bacterial activity was inhibited through either the addition of 100 p.p.m. thymol or autoclaving.

To maintain the autoclaved samples sterile conical flasks were incubated in a Gallenkamp orbital shaker at 30°C and 150 r.p.m. instead of as in 3.2.4.

3.2.6 Continuous Bacterial Leach Procedure

3.2.6.1 Inoculum Preparation

The initial adapted inoculum ($\pm$ 2 l) was progressively increased in volume until about 100 l had been produced. All conditions were kept at the optimum for bacterial growth and activity except the temperature. For practical reasons this was 20°C (room temperature).

The inoculum was distributed among the leach vessels and the system turned on.

3.2.6.2 Apparatus and Test Procedure

The continuous bacterial leach apparatus used is shown in Fig. 3.1 and schematically illustrated in Fig. 3.2.

Fig. 3.1: Operating continuous bacterial leach unit for the milled flotation concentrate.

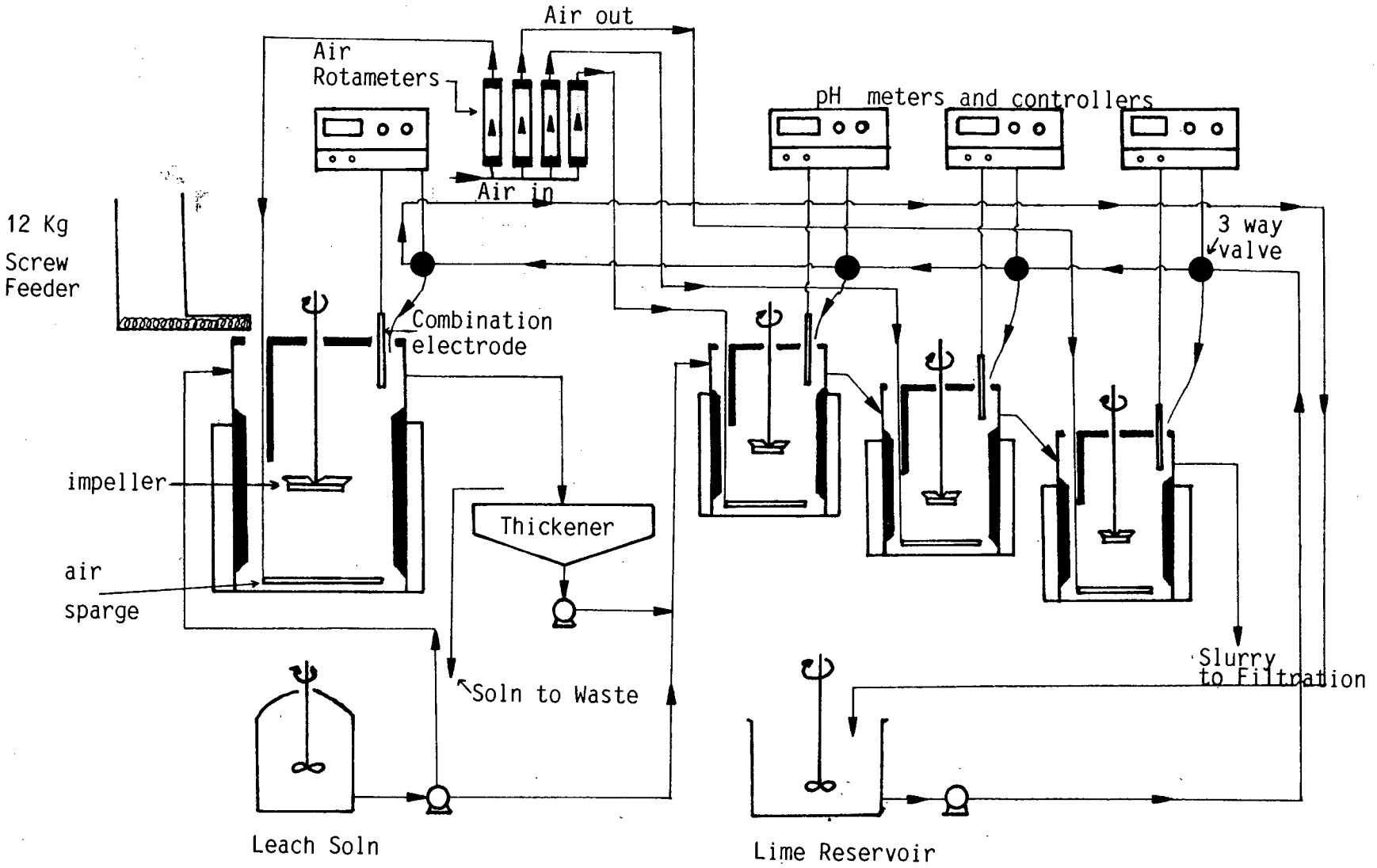


Figure 3.2: Diagrammatic illustration of the continuous bacterial leach apparatus for the milled flotation concentrate.

The system was as follows:

- five 20 l water jacketed leach vessels were used initially - see Fig. 3.3. Later the first two vessels were replaced with a similar 40 l vessel. This was intended to help prevent bacterial washout. The baffles aided mixing, the partition reduced short-circuiting and the lid reduced evaporation and material loss by spillage.

- dry, milled concentrate was fed to the first vessel at a rate of 0,63 g/min $\pm$ 2% by a 12 Kg capacity double-screw feeder manufactured by K-Tron Corp (T-20 model) with the series 6300 control unit.

- a variable speed peristaltic pump allowed for feed solution to enter the first vessel at a rate of 4,90 ml/min from a 25 l reservoir containing a solution of 10 g/l $FeSO_4.7H_2O$, 0,5 g/l $(NH_4)_2SO_4$ and 0,37 g/l K_2HPO_4 at pH 1,8-2,0.

- five (or four) variable speed stirrers with four-bladed PVC impellers with the blades angled at 45° kept the slurries in suspension within the vessels.

- the vessels were aerated at about 6,0 l/min. Five (or four) Aalborg FM 102-05 S (0,0 - 6,0 l/min range) air rotameters and air sparges were used.

- three (or four) pH control units were used. Each consisted of a combination-type pH electrode, a pH meter and a titrator. This allowed alkali (250 g/l ammonia, 250 g/l sodium hydroxide or 100-150 g/l

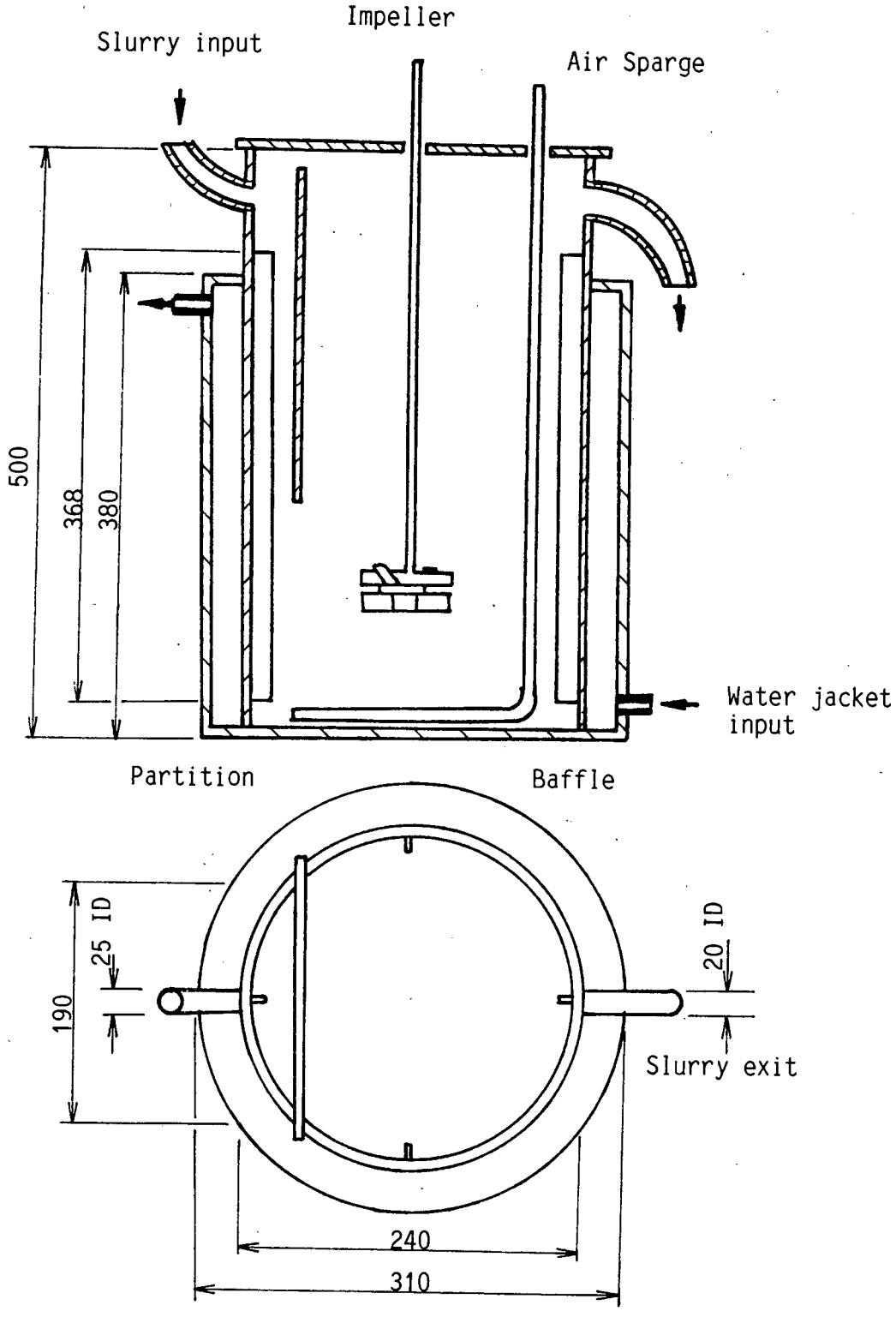


Figure 3.3: Diagrammatic illustration of a leach vessel used for the continuous bacterial leach of the milled flotation concentrate.

lime) to be added to vessels 1, 3 and 4 in the five vessel system and to all vessels in the four vessel system.

- the leach slurry temperature was kept at 29°C ± 2°C using a thermostat-controlled water bath. A magnet pump was used to circulate the water through the leach vessel jackets.

- in the five vessel system the slurry from leach vessel no. 1 overflowed into vessel no. 2 and from there into a "settling vessel". In the four vessel system the first 40 l leach vessel overflowed directly into a "settling vessel".

- every 24h (± 1 h) the settling vessel solution (which was high in arsenic and ferric iron) was siphoned off. Fresh nutrient solution from the reservoir was added as replacement. The slurry was thoroughly mixed and transferred to a "feed vessel" (of 12 l capacity).

- the contents of the feed vessel were fed to three further vessels arranged in series at a rate of 5,1 ml/min using a variable speed peristaltic pump. To maintain the feed vessel slurry in suspension a variable speed stirrer with a smaller version of the PVC impeller was used.

- from the last vessel the slurry overflowed into a final settling vessel. This represented an overall slurry retention time of approximately 12 days.

- the final overflow slurry was removed every 24 h(± 1 h). After

settling the solution was siphoned off.

- approximately 10% of the thickened slurry was recycled to leach vessel no. 1 each day.

- Magnafloc 351 flocculant was added to the remaining slurry, which was then filtered. The filter cake was washed, repulped, re-filtered and rewashed. The final filter cake was dried at 60°C and weighed.

- the filtrate, the combined washings and the intermediate decanted solutions were collected, weighed and measured, then assayed for ferrous and ferric iron, arsenic and pH.

- the dried residue was analysed for total iron, total and pyritic sulphur, arsenic and gold. Mineralogical and screen analyses were also carried out. The feed concentrate was analysed in the same manner.

- a sample from each leach vessel was taken daily. The pH and redox potential were measured and the ferrous and ferric iron content of the solution determined. The specific gravity of the slurry was measured as an indication of the pulp density.

3.2.7 Cyanidation Test Procedure

Dried bacterial leach residue (150 or 300 g) and enough tap water to give a pulp density of 33% (300 or 600 ml) were placed in a 1 l bea-

ker. The slurry was stirred and aerated. An approximate 10% lime slurry was used to adjust the ore slurry to about pH 11,5. This pH was checked every 0,5-1,0 h and lime was added to maintain the desired pH. After 21-74 h the pH has stabilized (i.e. it no longer rapidly decreased with time). A predetermined amount of granular KCN was added and the leach continued for another 24 h.

The leach slurry (now at a pulp density of 25%) was filtered, the filter cake was washed, repulped, refiltered and rewashed. The final filter cake was dried at 60°C, weighed and assayed for gold. The first filtrate was collected and analysed for residual cyanide and lime.

3.2.8 Microscopic Examination of the Concentrate

3.2.8.1 Bacterial Leach Tests

Unmilled flotation concentrate was mounted in epoxy resin blocks. The milled concentrate was not used, because the fineness of the particles made microscopic examination and mineral identification difficult.

These mounts were examined with a light microscope. When a representative field of view was found it was photographed and circled using a diamond scratcher. The mounts were placed in a porcelain dish with 720 ml 9K medium and 80 ml bacterial inoculum. The pH was adjusted and the solution aerated at room temperature (20°C) for various time periods. The mounts were then thoroughly washed with distilled wa-

ter, treated with a 50% solution of HCl (to remove the jarositic coating), rewashed with distilled water and stored away from dust.

On microscopic examination the same field of view was found and photographed.

For the scanning electron microscope the leached mounts were attached to a stub using conducting paint, sputtered with gold (20 nm thick coat) and examined with a Cambridge 54 stereoscan unit at 20 KeV. Photographs were taken as required.

3.2.8.2 Sterile Ferric Leach Tests

Epoxy resin mounts of the unmilled flotation concentrate were made and treated as in 3.2.8.1 with one exception - to the porcelain dish 800 ml of ferric sulphate (7 g/l Fe III) solution and 100 p.p.m. thymol was added instead of the 9K medium and bacterial inoculum.

3.3 RESULTS

3.3.1 Analysis of the Flotation Concentrate

The elemental analysis of the concentrate is given Table 3.1 and the mineralogical and screen analyses in Tables 3.2 and 3.3.

There was ample iron and sulphur, as pyrite and some arsenopyrite, to support bacterial growth (thiobacilli and leptospirilli). The only inhibitory compound present in significant quantities was arsenic. The large amount of gold present made the leaching of this concentrate a very attractive economic proposition.

Table 3.1 : Chemical analysis of the flotation concentrate.

Element	Concentration(%)
Gold	0,014560(145,60 g/t)
Silver	0,000580(5,80 g/t)
Arsenic	6,31
Iron	31,20
Sulphur	31,71
Calcium	0,22
Nitrate	-
Potassium	0,79
Chlorine	0,001
Phosphorous	0,05
Magnesium	0,90
Sodium	0,25
Aluminium	1,93
Boron	0,24
Copper	0,09
Manganese	0,02
Nickel	0,25
Molybdenum	0,003
Titanium	0,16
Silicon	16,40

Table 3.2: Mineralogical composition of the flotation concentrate.

Mineralogical Component	Percent of Total
Pyrite	49,19
Arsenopyrite	10,27
Sphene	0,02
Rutile	0,08
Hematite	-
Sphalerite	-
Free gold	<0,01
Gangue	40,44

Table 3.3: Tyler screen analysis of the flotation concentrate.

Size (µm)	Percent of Total	
	Before Milling	After Milling
+ 208	2,33	3,51
+ 147	5,67	2,61
+ 104	9,25	2,38
+ 74	15,00	3,21
- 74	67,75	88,29

3.3.2 Bacterial Strain Identification

The adapted bacterial culture grew well in the presence of the concentrate and 4 g/l As in solution. The total bacterial count was

approximately 1,6 x 10^{10} cells/ml slurry. Visually there were 1-3 x 10^{8} cells/ml solution. When the solution was plated on solid media the counts were considerably lower (Table 3.4).

The culture consisted of T.ferrooxidans, sulphur-oxidizing thiobacilli (probably T.thiooxidans), L.ferrooxidans and some heterotrophic bacteria and fungi.

Table 3.4: Bacterial counts on different solid media. N.A. = Nutrient agar; TNC = too numerous to count.

Medium	Titre of Bacteria (cells/ml)	Titre of Fungi (cells/ml)
N.A.	10	$\pm$ 10^3
Waksman	$\pm 10^3$	$\pm$ 10^3
9K	10^4	$\pm$ 10^2

3.3.3 Batch Tests - Bacterial Leaches

All tests were done in duplicate or triplicate.

3.3.3.1 Arsenic Tolerance

During inoculum preparation it had been observed that the bacterial culture to be used in this work could tolerate only 4 g/l As. This was confirmed when the culture was inoculated into 9K medium supplemented with varying amounts of arsenic (as AS_2O_3) (Table 3.5).

Table 3.5: Arsenic tolerance of the bacterial culture in 9K medium supplemented with As_2O_3.

Arsenic Concentration (g/l)	Initial Culture		Subculture	
	Lag Period (days)	Period for Fe II Oxidation (days)	Lag Period (days)	Period for Fe II Oxidation (days)
1,0	14	15	8	10
2,0	14	15	8	9
3,0	14	15	7	9
4,0	14	15	9	15
5,0	>42	-	-	-

Batch tests using 9K medium or water, with or without pH adjustment, were carried out to see what would happen during a bacterial leach. No decantations were performed and the solutions were assayed for ferrous and ferric iron, and arsenic. It was found that there was a characteristic pattern of solubilization. The amount of ferric iron and arsenic in solution was greatest when 9K medium and no pH adjustment was used, less when water and no pH adjustment was used and was least when 9K medium was used and maintained at $\pm$ pH 1,8 (Fig. 3.4 and Fig. 3.5). Only in the latter case was the arsenic below 4 g/l, yet the bacteria remained viable in all cases. During the leach the ferrous iron in solution rapidly dropped to less than 0,5 g/l, the ferric iron displayed two phases of increase and the arsenic increased to a constant value. The first phase of ferric increase coincided with the drop in ferrous concentration and the second started about 5 days later. The lag period was 2-3 days, and stationary phase was reached after 10-12 days.

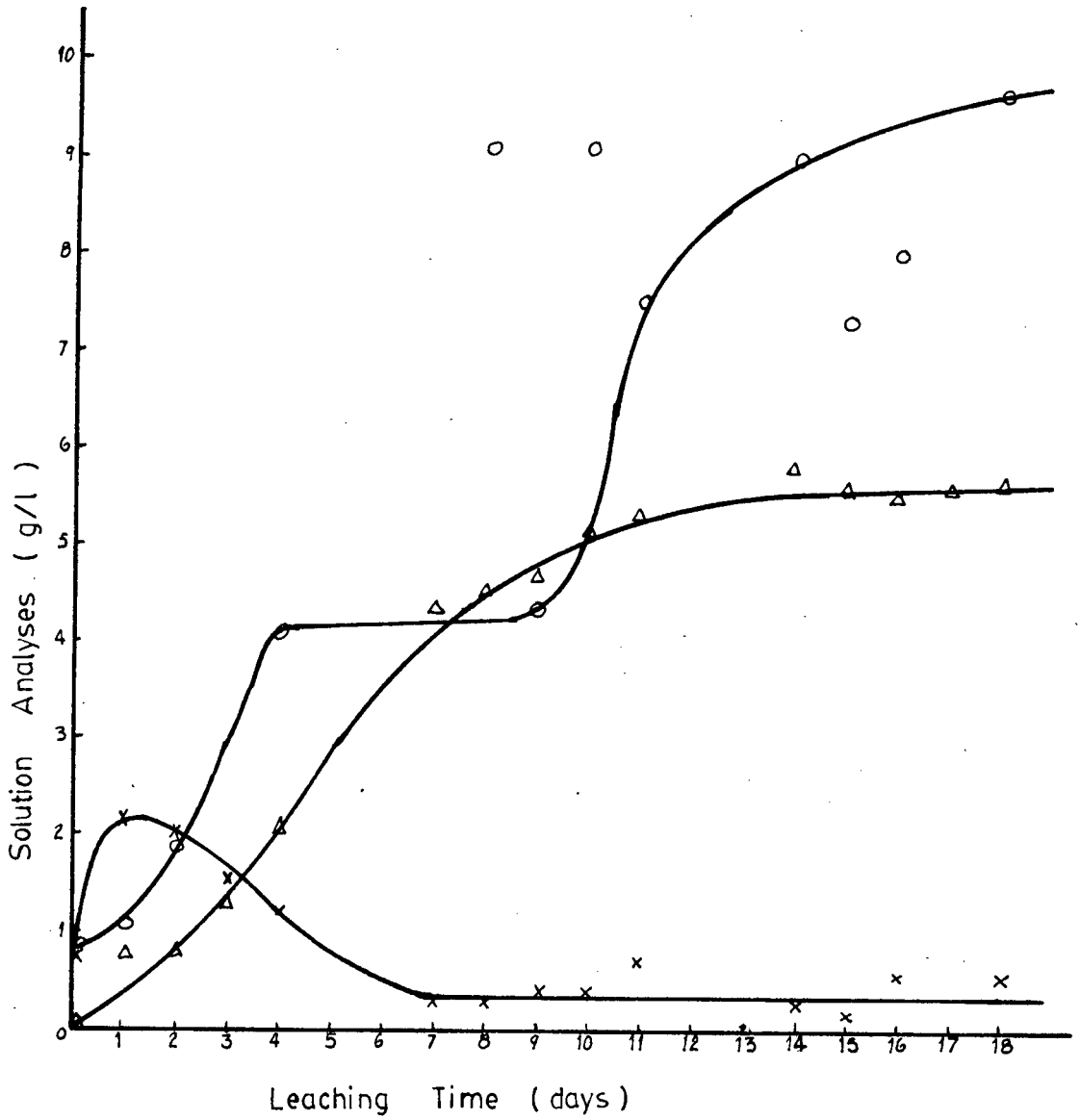


Fig.3.4: Solution analyses during the bacterial leach in water of the flotation concentrate. There were no pH adjustments made during the leach. Arsenic (Δ-Δ); ferrous iron (x-x); ferric iron (o-o).

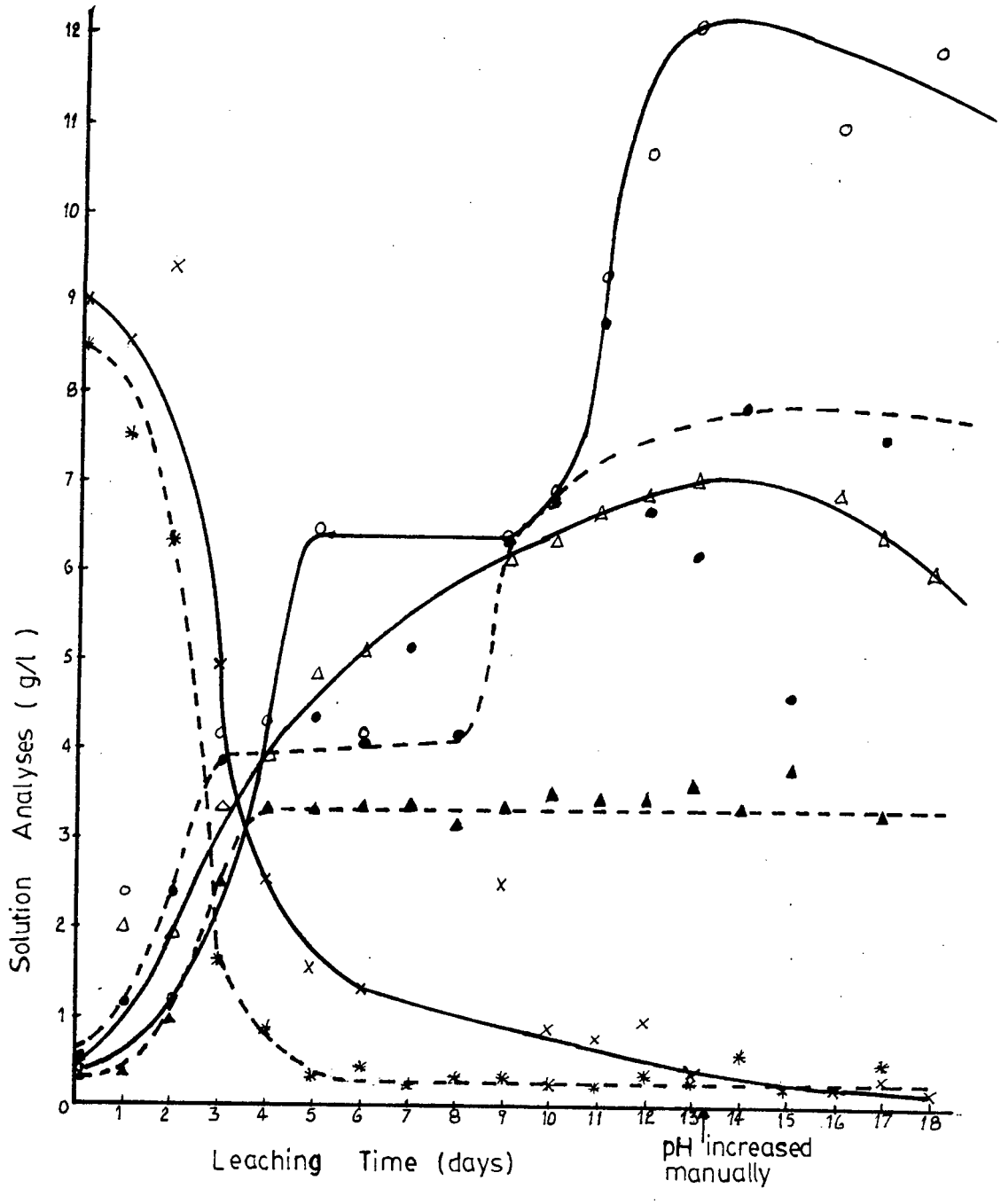


Fig.3.5: Solution analyses during the bacterial leach in 9K medium of the flotation concentrate. With no pH adjustments: arsenic (Δ -Δ); ferrous iron (x-x); ferric iron (o-o). With pH control: arsenic (▲ --▲); ferrous iron (*--*); ferric iron (●--●).

When no pH adjustments were made the pH increased slightly initially (to pH 1,9), then dropped to pH 1,1 or less. When the pH was controlled iron and arsenic precipitated out of solution (Fig. 3.5). Gold dissolutions confirmed that a leach in 9K medium was more effective than in water. This was also indicated by the iron and arsenic concentrations in solution. On the other hand, when pH control was exercised, gold dissolution dropped and arsenic and ferric iron levels in solution were lower.

3.3.3.2 Effect of Leaching Time

The effect of bacterial leaching this concentrate for up to 17 days was examined, as well as solution removal (decantation). Changes in the solution during the bacterial leach are illustrated in Fig. 3.5.

From the residue analyses (Table 3.6) it was apparent that mineral leaching had taken place, since pyritic sulphur and arsenic were leached out of the concentrate. Iron and total sulphur values did not change much due to their reprecipitation. This was more accentuated when there were no decantations and caused some arsenic coprecipitation.

A third piece of evidence that mineral attack occurred was the increase in gold dissolution (upon cyanidation) with time (Fig. 3.6). This stabilized within about 10 days and confirmed that a 10 day bacterial leach was all that was needed.

Table 3.6: Analysis of the bacterial leach flotation concentrate residues. All values are corrected for weight change. "*" denotes those tests during which no decantations were performed.

Bacterial Leach Period (days)	Analysis of Leach Residue				
	Au(g/t)	Total Fe(%)	Total S (%)	Pyritic S(%)	As(%)
0*	129,3	27,30	27,69	21,85	4,56
0	139,3	27,90	26,90	25,83	5,05
3*	127,0	26,91	26,75	18,00	2,15
4	143,7	29,73	27,30	22,12	2,12
6*	127,6	25,84	26,99	14,30	1,70
7	140,4	28,99	24,57	12,26	1,04
10*	129,2	23,99	26,62	13,90	1,47
10	139,1	28,09	22,86	7,53	0,76
15	137,8	30,01	20,51	7,99	0,48
17*	128,7	21,92	26,20	11,68	1,71

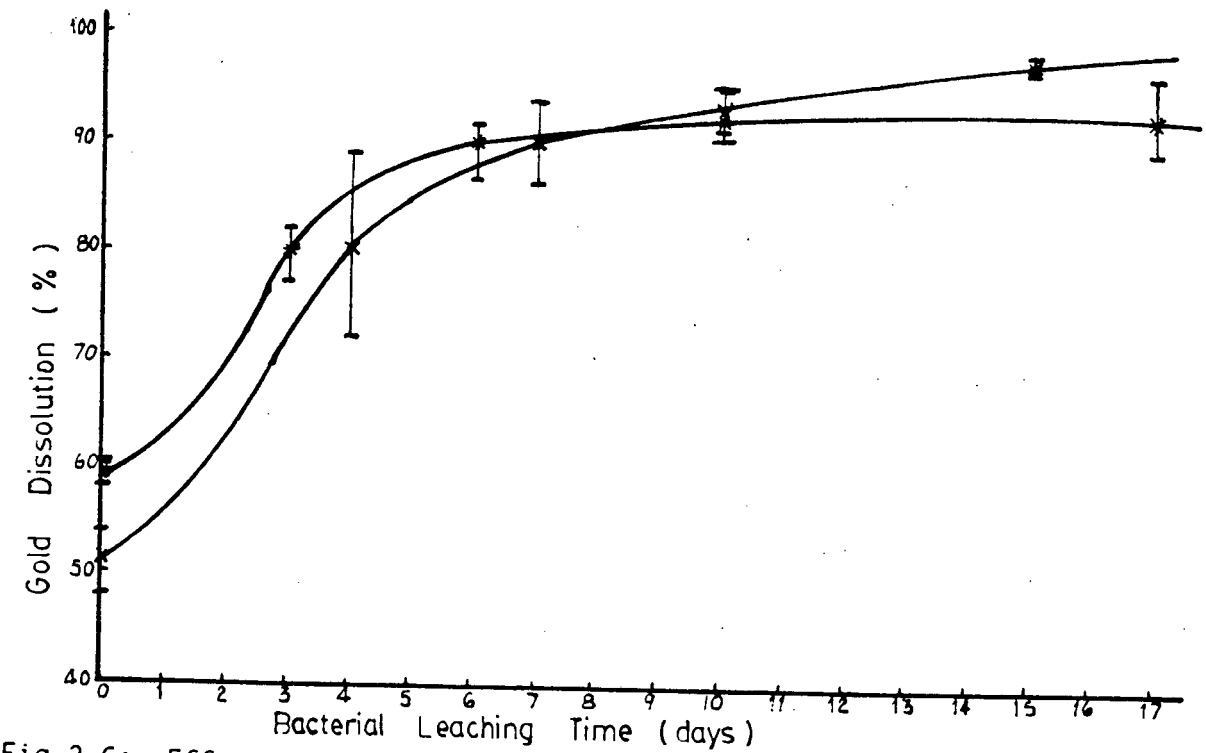


Fig.3.6: Effect of bacterial leaching on gold dissolution from the flotation concentrate. With decantations (x-x); without decantations (*-*). Points are the mean of several assays and vertical bars indicate the deviation.

Due to the greater precipitation of solutes toward the end of the leach tests where no decantations were done, gold solubilization was blocked. Nearly total gold dissolution could be expected from the concentrate, but without decantations this would be precluded.

The cyanide leach required to dissolve the gold from the bacterially leached concentrate showed some unusual characteristics. The cyanide consumed in the process roughly trebled when compared with the control (untreated concentrate) and the lime consumed increased with bacterial leaching time (Table 3.7). Consumptions were slightly lower in the tests without decantations.

Upon mineralogical examination it was found that the pyrite content of the concentrate dropped from the original 40-47% to 22-26%, while the arsenopyrite dropped from approximately 9% to less than 1%. Tyler screen analysis revealed that when the solution was removed during the bacterial leach all particle size classes diminished, with a concomitant increase of -44 µm particles (from 82% to 89%). When solution was not removed the +208 µm particle class increased, while the other classes behaved as above. This particle size reduction was not due to attrition, since it was not observed in control leaches (section 3.3.4).

3.3.3.3 Effect of Particle Size

Two ranges of particle sizes were used : the unmilled concentrate and milled to 88% -74 µm (Table 3.3).

Table 3.7: Cyanidation details of the bacterially leached flotation concentrate.

"*" denotes those tests during which no decantations were performed.

Bacterial Leach	Cyanide Leach				
Period (days)	Head Au(g/t)	Reagents KCN(Kg/t)	Consumed CaO(Kg/t)	Residue Au (g/t)	Gold Dissolution (%)
0*	129,3	7,29	11,33	53,05	59,0
0	139,3	8,52	11,05	68,20	51,0
3*	127,0	21,84	31,17	25,00	80,3
4	143,7	24,72	23,97	28,00	80,5
6*	127,6	14,73	56,27	12,20	90,4
7	140,4	26,73	58,61	13,37	90,5
10*	129,2	12,54	79,03	9,20	92,9
10	139,1	24,83	92,49	8,90	93,6
15	137,8	23,81	133,29	3,25	97,6
17*	128,7	23,82	44,36	9,00	93,0

Although all the analyses and mineralogical examinations followed the same pattern (cf. Table 3.8 with Tables 3.6 and 3.7), it was clear that the unmilled concentrate required an additional 8 days leaching to achieve similar gold dissolutions.

3.3.3.4 Effect of Pulp Density

The inoculum for this study had 10% solids, thus making the bacterial leaching effects comparable. This was compensated for in the making up of the pulps.

As the substrate concentration increased it was expected that the ferric iron and arsenic concentrations in solution would increase. This was seen, the increase being directly proportional to the additional substrate present (Table 3.9). From the bacterial leach product analyses and gold dissolutions it was concluded that the effectiveness of the leach dropped with increasing pulp densities. The cut-off point, at which no bacterial activity could be detected, was between 20 and 30% solids. But effective leaching only occurred at pulp densities of less than 10% solids (Fig. 3.7).

On cyanidation of the bacterial leach residues all cyanide consumptions were found to be similar, even for the unsuccessful leach using 32,3% solids. This result was not expected.

Table 3.8: Effect of particle size (68% -74 μm) on the bacterial and cyanide leaches of the unmilled flotation concentrate. The values given for the bacterial leach product have been corrected for weight change.

Bacterial Leach					Cyanide Leach			
Time	Solution	Product			Reagents	Consumed	Residue	Gold Dis-
(days)	Total As(g/l)	Pyritic S(%)	As(%)	Au (g/t)	KCN(Kg/t)	CaO(Kg/t)	Au(g/t)	solution(%)
0	-	31,33	6,10	155,2	9,90	12,43	100,2	35,4
3	1,06	31,75	4,70	155,0	18,79	13,34	86,8	44,0
6	1,47	31,83	4,25	157,7	20,89	15,07	79,4	49,7
10	2,85	29,86	2,76	163,4	19,70	51,25	53,3	67,4
17	3,69	25,03	1,53	152,1	16,89	108,01	24,8	83,4

Table 3.9: Effect of pulp density on the bacterial and cyanide leaches of the flotation concentrate. The values given for the bacterial leach product have been corrected for weight change.

Bacterial Leach					Cyanide Leach			
Pulp Density (% solids)	Solution	Product			Reagents Consumed		Residue	Gold Dissolution (%)
	Total As (%)	Pyritic S (%)	As (%)	Au (g/t)	KCN (Kg/t)	CaO (Kg/t)	Au (g/t)	
-	-	31,30	5,26	157,4	18,84	14,14	83,20	47,1
6,2	3,05	24,00	1,00	162,4	21,28	116,70	10,40	93,6
10,1	5,08	25,69	0,98	146,8	32,55	93,99	14,28	90,3
14,9	6,95	31,00	1,77	165,4	34,92	32,82	25,75	84,4
19,2	9,12	26,98	1,75	151,7	29,44	30,06	27,07	82,1
32,3	1,86	31,80	5,22	161,5	29,71	14,03	78,85	51,2

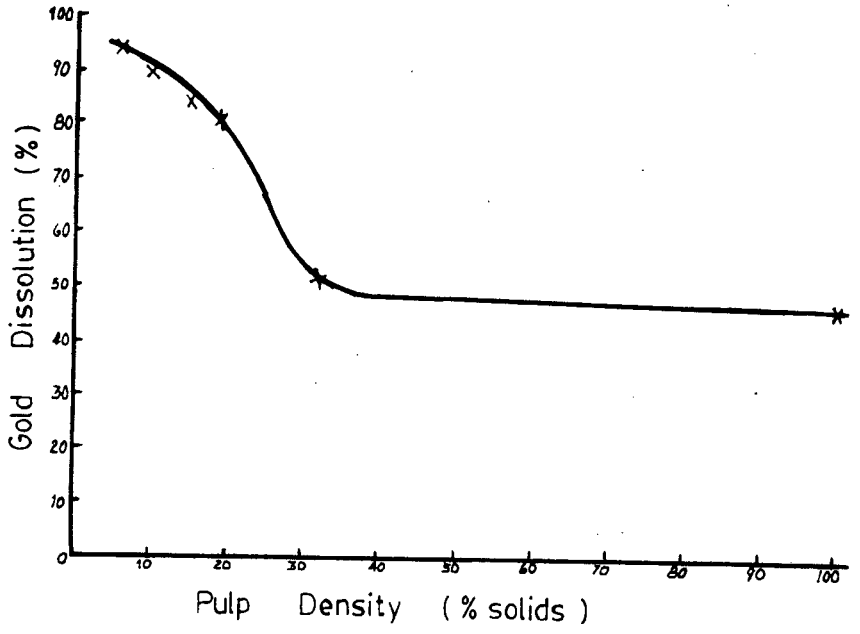


Fig. 3.7: Effect of pulp density on gold dissolution from the flotation concentrate by bacteria.

3.3.3.5 Effect of pH

In the pH range of 1,6 to 2,2 bacterial leaching in terms of gold dissolution was not affected very much, although pH 1,6 gave the best results (Table 3.10). As the pH increased the initial acid consumption dropped (from approximately 97 Kg/t to 33 Kg/t) and the lime consumption for neutralization rose (from approximately 60 Kg/t to 152 Kg/t). When the pH increased ferric iron and arsenic in solution dropped due to precipitation. This effect was not due to a significant lessening of bacterial leaching. The increasing amount of iron oxides in the residue caused an increasing cyanide consumption.

3.3.3.6 Effect of Temperature

The optimum temperature was found to be about 28°C. An increase or decrease in the temperature reduced gold dissolution. However, in the 25°-45°C range the effect of temperature was limited, a significant drop being observed only at lower temperatures (20°C)(Fig.3.8).

Table 3.10: Effect of pH on the bacterial and cyanide leaches of the flotation concentrate. The values given for the bacterial leach product have been corrected for weight change.

	Bacterial Leach					Cyanide Leach			
	Solution		Product			Reagents Consumed		Residue	Gold Dissolution (%)
pH	Total As(g/l)	FeIII(g/l)	Pyritic S (%)	As (%)	Au(g/t)	KCN(Kg/t)	CaO(Kg/t)	Au (g/t)	
unleached control	-	-	23,02	4,01	117,0	5,86	7,14	59,4	49,2
1,6	3,68	6,68	12,21	0,66	112,0	14,15	77,31	11,8	89,5
1,8	3,25	4,35	17,49	1,01	113,6	16,97	67,79	14,4	87,3
2,0	1,71	1,30	12,51	2,34	109,3	22,69	80,60	14,0	87,2
2,2	0,56	0,83	14,02	3,27	101,9	25,96	51,41	14,8	85,5

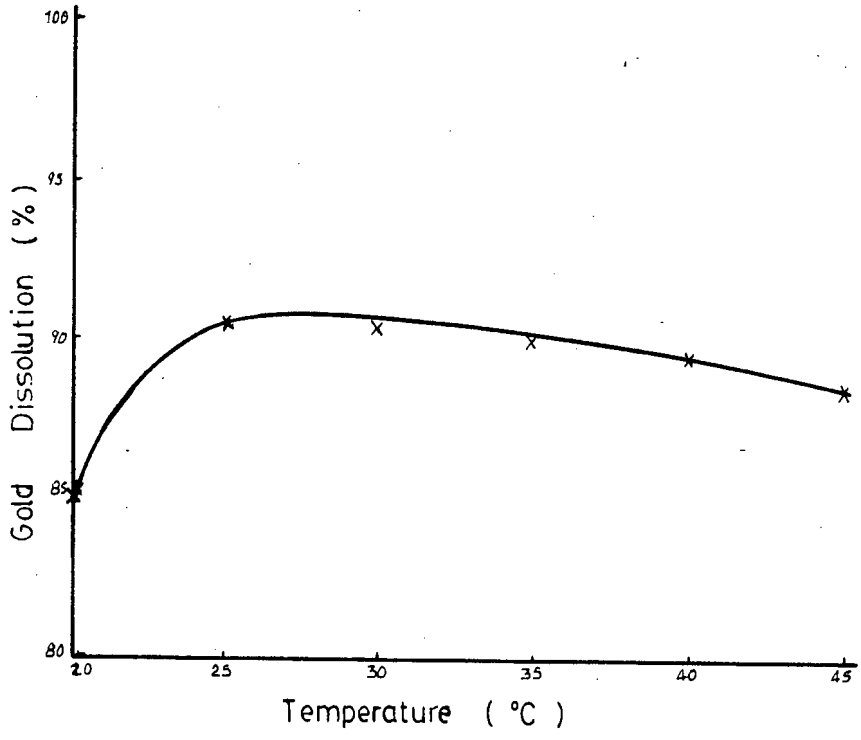


Fig. 3.8: Effect of temperature on gold dissolution from the flotation concentrate by bacteria.

As the temperature increased above 30°C ferric iron and arsenic in solution precipitated progressively more (Table 3.11). It was also noted that the cyanide and lime consumptions during cyanidation were highest when gold dissolutions were greatest, and vice-versa.

3.3.3.7 Nutrient Requirements

As the concentrate contained most of the 9K medium nutrients in some quantity, with the exception of a nitrogen source (Table 3.1), and since the addition of chemicals to a process always decreases its economic feasibility, it was important to establish whether the concentrate could provide the necessary nutrients. It was also possible that some of those present in 9K medium were not essential or affected bacterial leaching only marginally.

Table 3.11: Effect of temperature on the bacterial and cyanide leaches of the flotation concentrate. The values given for the bacterial leach product have been corrected for weight change.

	Bacterial Leach					Cyanide Leach			
Temperature	Solution		Product			Reagents Consumed		Residue	Gold Dissolution(%)
(°C)	Total As(g/l)	FeIII (g/l)	Pyritic S (%)	As(%)	Au(g/t)	KCN(Kg/t)	CaO(Kg/t)	Au (g/t)	
Unleached control	-	-	30,05	5,24	136,1	7,81	13,40	63,2	53,6
20	2,87	4,2	21,63	1,04	139,2	27,65	55,05	21,2	84,8
25	3,24	5,5	20,85	0,65	137,1	38,52	84,34	13,0	90,5
30	4,52	5,6	22,25	0,98	146,8	32,55	92,88	14,3	90,3
35	2,06	2,0	14,52	2,16	130,7	21,57	24,10	13,3	89,8
40	0,82	1,3	19,96	3,40	128,8	24,34	28,56	13,5	89,5
45	0,43	1,0	19,96	3,87	124,3	22,33	29,28	14,3	88,5

A comparison of the bacterial leach solution before and after a 10-day leach was made (Table 3.12). It was apparent that iron (Fe II), potassium and probably ammonia were the only nutrients depleted in the course of the leach. These were potentially limiting.

Bacterial leach tests, in which each of the 9K medium components in turn was removed, revealed that only the absence of ferrous iron had a detrimental effect on the leach (Table 3.13). The absence of potassium, or the presence of sodium, seemed beneficial. From the cyanide leaches it was noted that cyanide and lime consumptions dropped when ferrous iron was not present in the bacterial leach. Low cyanide consumptions were also observed in the absence of potassium and phosphate.

Table 3.12: Nutrient concentrations in flotation concentrate bacterial leach solution.

Chemical	Analysis of Leach Solution (mg/l)	
	Before Leach	After Leach
Iron	10 050 (Fe II)	12 331 (Fe III)
Sulphur	6 549	12 950
Calcium	2	351
Potassium	97	<0,5
Phosphorous	18	74
Nitrate	5	1 500
Ammonia	841	700 (from acid neutralization with NH_3)
Magnesium	49	225
Chlorine	48	18

Table 3.13: Nutrient requirements and effects on the leaching of the flotation concentrate. Bacterial leach product values have been corrected for weight changes. "*" denotes the two nutrients that were linked as K_2HPO_4 in 9K medium, each nutrient being proportionally replaced in the form of Na_2HPO_4 or K_2SO_4, respectively, as needed.

	Bacterial Leach					Cyanide Leach			
Absent	Solution		Product			Reagents Consumed		Residue	Gold Dissolution (%)
Nutrient	Total As(g/l)	FeIII(g/l)	Pyritic S (%)	As (%)	Au(g/t)	KCN (Kg/t)	CaO (Kg/t)	Au (g/t)	
Unleached control	-	-	32,90	5,50	148,9	15,93	12,50	80,1	46,2
None	4,17	3,61	24,99	1,17	147,9	35,44	72,04	18,8	87,3
Iron	3,43	2,63	22,03	1,74	147,8	20,67	21,51	25,1	83,0
Ammonia	4,38	3,25	26,92	1,61	148,3	33,62	39,71	19,1	87,1
Nitrate	3,66	4,36	25,72	1,37	141,4	37,64	81,51	17,8	87,4
Potassium*	4,47	5,29	22,48	0,77	144,6	20,00	92,61	12,6	91,3
Magnesium	3,90	4,60	25,58	1,23	143,0	31,86	88,43	15,7	89,0
Phosphate*	3,77	3,82	23,09	1,63	147,3	20,78	88,54	17,1	88,4
All	2,20	3,80	23,03	2,12	144,1	28,76	23,96	25,4	82,4

Similar tests using 2,0 g/l Fe II instead of none yielded an acceptable gold dissolution (approximately 86%) in 10 days.

3.3.3.8 Summary of Minor Analyses and Investigations

Batch tests to determine the effects of inoculum size, various acid neutralizing agents, flotation reagents (because normally the concentrate was dried and allowed to stand, which caused the degradation of these reagents) and aeration were done.

The relationship between inoculum size and bacterial leach retention time was studied using 20% solids (Table 3.14). As the amount of inoculum increased the lag period decreased and the rate of gold dissolution increased.

Table 3.14 : Relationship between inoculum size and retention time for the bacterial leach of the flotation concentrate.

Inoculum Size (%)	Retention Time (days)	Gold Dissolution (%)
5	24,0	90
10	16,0	90
20	12,5	90
30	11,5	90
50	6,0	90

Ammonia, sodium hydroxide and lime were used as acid neutralizing agents during bacterial leaching. There were no marked differences in either the bacterial or cyanide leaches.

The presence of flotation reagents appeared to have a beneficial effect on the bacterial leach.

The optimum air flow into a closed system bacterial leach was found to be approximately 1,43 l/min Kg. However, since this figure varied with several parameters, it can only be taken as a guideline.

In addition, throughout much of this work attention was given to the initial acid consumptions, alkali consumptions, particle size changes, mineralogical changes and chemical composition changes of the concentrate that took place during bacterial leaching. Calculated averages from tests yielding high gold dissolutions showed that the average acid consumption was 62,3 Kg H_2SO_4/t concentrate and of alkali 68,1 Kg CaO/t concentrate. A reduction in particle size was also noted (Table 3.15), with most of the arsenopyrite and some pyrite being destroyed while the gangue remained unaltered (Table 3.16). Chemical analyses (Table 3.17) confirmed the breakdown of sulphidic minerals. Both the mineralogical examinations and the chemical analyses indicated 80-90% arsenopyrite and 36-39% pyrite destruction.

Table 3.15: Tyler screen analysis of the head and tail of the bacterial leach of the flotation concentrate.

Screen Mesh Size (µm)	Percent of Total	
	Head Before Leach	Tail After Leach
+ 295	0,21	0,15
+ 208	1,04	0,85
+ 147	2,27	1,19
+ 104	2,19	1,07
+ 74	2,89	2,36
+ 44	8,51	4,07
- 44	82,89	90,31

Table 3.16: Mineralogical examination of the head and tail of the bacterial leach of the flotation concentrate.

Mineral	Percent of Total	
	Head Before Leach	Tail After Leach
Pyrite	37,08	23,70
Arsenopyrite	8,77	<1,00
Chalcopyrite	0,01	<0,01
Sphene	0,01	-
Rutile	0,04	-
Gold	<0,05	<0,5
Gangue	54,06	75,32

Table 3.17: Chemical analysis of the head and tail of the bacterial leach of the flotation concentrate.

Chemical	Analysis of Sample (%)	
	Head Before Leach	Tail After Leach
Fe	29,87	28,38
S	28,79	26,57
Pyritic S	28,04	16,33
As	5,10	1,06

3.3.4 Batch Tests - Control Leaches

The effect of acid (pH 1,8) and acidic-ferric (pH 1,8, 7 g/l Fe III) leaching on the concentrate for up to 10 days was examined. Neither had any significant effect on gold dissolution (Table 3.18).

Table 3.18: The effect of an acid and acidic-ferric solution on the flotation concentrate.

Type of Leach	Sterilization Method	Gold Dissolution (%)
Unleached control	-	50,9
" "	autoclaved	52,5
Acid	thymol	51,7
Acidic-Ferric	thymol	53,2
" "	autoclaved and thymol	54,8
" "	autoclaved	56,9

3.3.5 Continuous Bacterial Leach Tests

From the batch work it was concluded that :

(i) a 14-15 day residence time was optimum. Since the lag period was approximately 2 days, and this is essentially eliminated in a continuous system, a residence time of 12 days was considered suitable for the continuous bacterial leach.

(ii) decantation of leach solution was advantageous. It appeared that 1-2 decantations would be sufficient. Hence, solution was to be removed after 5,0 and 7,5 days of continuous bacterial leaching. Later it was found that only the 5-day decantation was needed.

(iii) milling of the concentrate to 80% -44 μm was necessary. Hence, milled flotation concentrate was to be fed into the continuous leach system.

(iv) the lower the pulp density the more effective the bacterial leach. However, higher pulp densities require smaller leach vessels and lower water and power consumptions. It was, therefore, decided that a pulp density of 12% solids would be used (see Fig. 3.7).

(v) the bacterial leach pH did not affect gold dissolution much, although the lower pH (pH 1,6) gave the best results. Leach pH did, however, affect lime consumption for acid neutralization as well as cyanide consumption. Both increased with increasing pH. Hence, a pH of 1,6-1,8 would be used initially, but the effect of a higher pH would be reinvestigated.

(vi) 28°C was the optimum temperature, 25°C being the minimum temperature for successful bacterial leaching.

(vii) although no extra nutrients would probably be needed, the absence of ferrous iron was deleterious. Since workers have unanimously considered iron, ammonia and potassium as beneficial (and possible phosphate too) it was decided that ferrous sulphate, ammonium sulphate and di-potassium hydrogen phosphate be added to the continuous bacterial leach. A limited investigation in this regard would also be attempted.

(viii) recycle was not necessary in a batch test, but it would be in a continuous test where bacterial washout was possible. A 10% recycle of solids (with a large number of bacteria attached) would be used.

The continuous bacterial leach of the flotation concentrate lasted 2,5 months. Time was allowed for steady state to be achieved, with each of the three runs being of 2-3 weeks duration. Each run allowed the examination of the effect of a particular parameter on the bacterial leach.

Each set of data was an average of approximately 15 days' readings. Data concerning the conditions pertaining to a particular run were those observed during the test period, but the data relating to the processes' overflow were those recorded 3-7 days later (thus taking into account the residence time per stage). Cyanidations were carried out on 2-3 day composites.

Run 1 was carried out under the conditions predetermined from the batch tests. Run 2 examined the effect of a higher pH (pH 2,1 vs pH 1,8) and run 3 examined the effect of a reduced nutrient feed. The Fe II feed was halved and the other nutrients reduced by 20%. A recheck on the effect of different acid neutralizing agents was made concurrently. Lime was expected to reduce cyanide consumption. These parameter changes are shown in Table 3.19.

Table 3.19: Parameter changes made during the continuous bacterial leach of the flotation concentrate.

Run No	Feed $FeSO_4.7H_2O$(Kg/t)	pH	Type of Alkali
1	346,95	1,81	NH_3
2	408,49	2,07	NaOH
3	160,16	1,73	CaO

The process operated smoothly, although only 89,9% of the feed was recovered in the overflow (Table 3.20). Approximately 91,2% of the solution was recovered after it was taken into account that the filter cake contained approximately 25% w/w solution; 52,0% of the concentrate was recovered. A small loss (or gain, as in run 3) of solution was mainly due to inaccurate replacement of decanted solution between bacterial leach stages. Some loss could be accounted for by evaporation. Residue loss was due to bacterial ore breakdown and loss of fines during decantations. In run 3 this loss of residue was smaller ($\pm$ 33%), because lime was used as a neutralizing agent and formed calcium sulphate, which precipitated into the residue.

Table 3.20: Test conditions during the continuous bacterial leach of the flotation concentrate. Residence time : 12,2 days. Inflow pulp density : 12% solids. Temperature : 29°C. Recycle: 9,5% solids. Air inflow: 6,0 l/min. Values in brackets are the minimum and maximum values observed. N.A. = not applicable.

Condition	Run 1	Run 2	Run 3
Ore feed (Kg/d)	0,90(0,82-0,96)	0,96(0,94-0,96)	0,94(0,92-0,95)
Solution feed(l/d)	6,93(5,3 - 8,9)	6,89(6,2-8,0)	7,20(5,4 - 8,5)
$FeSO_4.7H_2O$ feed(Kg/t)	346,95	408,49	160,16
$(NH_4)_2SO_4$ feed(Kg/t)	10,97	10,80	8,49
K_2HPO_4 feed (Kg/t)	8,11	7,99	6,28
Alkali consumed (Kg/t)	158,3(100,0-221,4)	163,3(79,0-232,3)	120,4(59,8-188,3)
Stage 1 overflow:slurry (l/d)	8,23(6,0-10,2)	7,81(7,0-9,5)	8,72(5,5-13,5)
supernatant (ld)	6,50(5,0-8,5)	5,65(5,2-7,2)	6,84(4,0-10,6)
Stage 2 feed : slurry (l/d)	6,89(5,4-9,7)	7,86(6,2-10,0)	N.A.
% solids	12,8(11,2-14,5)	13,9(11,2-16,7)	N.A.
Stage 2 overflow: slurry (l/d)	6,68(3,4-9,7)	7,96(6,2-10,0)	N.A.
supernatant (l/d)	5,13(3,2-8,5)	6,01(4,5-8,2)	N.A.
Stage 3 feed : slurry (l/d)	6,39(4,4-8,7)	6,54(4,4-8,0)	9,12(5,5-13,5)
% solids	12,6(8,9-16,7)	12,5(11,2-14,5)	13,9(11,2-16,7)
Stage 3 overflow: slurry (l/d)	6,17(3,4-7,9)	6,39(3,3-8,0)	9,79(6,9-12,7)
filtrate (l/d)	5,68(3,4-7,3)	5,37(3,2-7,0)	8,87(5,4-11,8)
Residue (Kg/d)	0,34(0,21-0,43)	0,40(0,23-0,51)	0,60(0,39-0,98)
Recycle: solids(Kg/d)	0,075(0,052-0,095)	0,078(0,044-0,126)	0,100(0,084-0,133)
solution (l/d)	0,25(0,17-0,32)	0,23(0,16-0,37)	0,22(0,15-0,29)

The leach vessel temperatures were found to increase spontaneously from 27°C in the first vessel to 31°C in the last one.

On analysis of the head (concentrate) and tail (residue and filtrate) of the bacterial leach it was found that the total sulphur in the residue dropped to about half, while the pyritic sulphur dropped to about one fifth (Table 3.21). Mineral breakdown was taking place. The difference in analyses reflected the fact that the total sulphur assay also detected basic sulphates that had precipitated out of solution. In run 3 the total sulphur value was higher than in the other two runs, because calcium sulphate also precipitated. Total iron in the residue dropped slightly, indicating mineral solubilization. When less ferrous iron was added to the process this was more noticeable. Arsenic was also solubilized. In run 3 the residue value was lowest due to a decrease in arsenic coprecipitation with iron.

The filtrates had low Fe II and high Fe III and As, indicating a good bacterial leach.

Mineralogical and Tyler screen analyses of the head and tail (Tables 3.22 and 3.23) revealed that approximately 45% of the pyrite and 98% of the arsenopyrite were destroyed, but not the gangue minerals. The overall particle size diminished. A time sequence showed that the smallest particles (<20 μm) diminished first, progressing in sequence to the largest (> 147 μm).

Table 3.21: Analyses of head and tail of the continuous bacterial leach of the flotation concentrate. Values in brackets are the minimum and maximum values observed.

Sample Analyses	Run 1	Run 2	Run 3
Head: Au(g/t)	145,86(144,9-147,4)	149,75(145,1-152,6)	151,50(143,7-159,5)
S (%)	32,52(32,02-33,04)	31,73(31,37-32,47)	32,16(31,47-33,00)
Pyritic S(%)	33,77(33,50-34,35)	32,66(31,00-33,48)	33,15(31,31-34,14)
Fe (%)	31,86(31,43-32,20)	31,25(30,61-31,90)	31,54(30,67-32,24)
As (%)	6,29(5,28-7,94)	6,10(5,34-6,89)	6,33(5,32-7,90)
Residue: Au (g/t)	125,72(97,6-148,3)	153,99(140,4-170,5)	123,00(70,8-157,6)
S (%)	13,15(11,44-13,21)	14,16(13,06-14,86)	18,42(15,80-21,90)
Pyritic S(%)	5,11(3,04-8,48)	7,47(5,70-8,65)	17,53(16,23-20,04)
Fe (%)	22,58(21,67-24,87)	22,62(21,92-23,13)	12,18(9,50-16,00)
As (%)	1,19(0,89-1,38)	1,25(1,01-1,43)	0,53(0,40-0,67)
Filtrate:Fe II(g/l)	0,5 (0,1-0,6)	0,9 (0,3-1,3)	0,9 (0,2-1,4)
Fe III(g/l)	12,8 (8,0-24,0)	18,6 (12,0-25,0)	10,4 (7,1-13,9)
Total As (g/l)	6,54(4,2-9,0)	5,83(4,3-8,2)	4,96(3,5-6,2)

Table 3.22 : Mineralogical examination of the head and tail of the continuous bacterial leach of the flotation concentrate. Tr = Trace.

Mineral	Percent of Total	
	Head Before Leach	Tail After Leach
Pyrite	39,34	21,80
Arsenopyrite	5,78	0,11
Chalcopyrite	Tr.	-
Sphalerite	Tr.	-
Titanium Oxide	< 0,05	-
Hematite	< 0,05	-
Gold	Tr.	<0,1
Gangue	54,78	77,99

Table 3.23: Tyler screen analyses of the head and tail of the continuous bacterial leach of the flotation concentrate.

Screen Mesh Size (µm)	Percent of Total	
	Head Before Leach	Tail After Leach
+295	0,77	0,28
+208	1,73	1,67
+147	2,06	2,27
+104	1,99	2,49
+ 74	2,81	2,90
+ 44	8,64	5,69
- 44	82,00	84,70

During the continuous bacterial leach analyses of the leach vessel contents were carried out. The pH and pulp density fluctuated. The pH was most easily controlled by lime addition. The Fe II concentration dropped along the system, while Fe III was continuously generated although results were staggered due to the decantations. The E_h, therefore, followed the same pattern (Table 3.24).

On two separate occasions analyses of the solids in the leach vessels were made (Fig. 3.9). Arsenic and gold were liberated continuously, whereas iron and pyritic sulphur were dissolved in two phases. This tallied well with what was observed in solution (see Fig 3.5).

However, the major criterion for success remained gold dissolution. All these runs gave very similar and satisfactory gold dissolutions of 97,1-97,5% (Table 3.25). For the bacterial leaches at pH 1,8 and 2,1 (cf runs 1 and 2) no significant differences were seen. This was true of results obtained from the cyanide leaches as well as the bacterial leaches. When the amount of ferrous sulphate feed to the bacterial leach was reduced (run 3), cyanide and lime consumptions dropped, as did the need for pre-aeration prior to the cyanidation. This reduction in consumptions was probably related not only to the lower extraneous iron added, but also to the use of lime as a neutralizing agent. Both reduce jarosite and basic sulphate formation.

Table 3.24: Analyses of leach vessel contents during the continuous bacterial leach of the flotation concentrate. Values in brackets are the minimum and maximum values observed. N.A. = not applicable.

Sample Analyses		Run 1	Run 2	Run 3
pH: vessel	1	2,13 (1,8-2,4)	2,48(2,3-2,7)	1,71(1,7-1,8)
"	2	1,49(1,4-1,7)	1,72(1,6-1,9)	N.A.
"	3	1,83(1,6-2,0)	2,05(1,8-2,4)	1,73(1,7-1,8)
"	4	2,03(1,9-2,2)	2,39(2,0-2,6)	1,74(1,7-1,9)
"	5	1,55(1,4-1,7)	1,70(1,5-1,8)	1,75(1,7-1,9)
E_h(mV): "	1	464(442-489)	462(445-490)	540(522-565)
"	2	517(504-528)	508(472-535)	N.A.
"	3	510(485-537)	490(474-510)	544(521-572)
"	4	543(498-580)	493(478-513)	588(560-611)
"	5	589(565-605)	548(502-599)	604(575-640)
% solids:"	1	17,0(12,3-2,27)	23,5(17,0-28,9)	16,2(13,4-18,7)
"	2	15,7(12,3-18,7)	16,3(15,7-16,7)	N.A.
"	3	12,0(9,0-14,6)	13,3(11,3-15,7)	14,6(12,3-17,0)
"	4	11,8(10,0-14,6)	15,0(12,3-16,7)	13,8(11,2-16,7)
"	5	11,8(11,2-13,4)	16,0(15,7-17,7)	14,0(11,2-17,7)
FeII(g/l):"	1	0,7(0,2-1,3)	0,3(0,1-0,6)	0,4(0,2-0,6)
"	2	0,2(0,1-0,3)	0,1(0,1-0,2)	N.A.
"	3	0,2(0,1-0,3)	0,3(0,1-0,6)	0,3(0,1-0,6)
"	4	0,1(0,1-0,2)	0,2(0,1-0,3)	0,2(0,1-0,3)
"	5	0,1(0,1)	0,2(0,1-0,2)	0,2(0,1-0,2)
FeIII(g/l):"	1	6,2(3,0-10,0)	6,6(4,0-8,0)	11,2(8,0-13,0)
"	2	12,9(6,5-20,0)	17,2(14,0-22,0)	N.A.
"	3	7,9 (3,0-11,0)	12,5(8,0-17,0)	7,6(5,0-10,0)
"	4	6,5 (6,0-10,0)	6,2(4,0-9,0)	9,4(6,0-13,0)
"	5	8,8 (4,0-13,0)	16,3(11,0-22,0)	10,0(6,0-15,0)

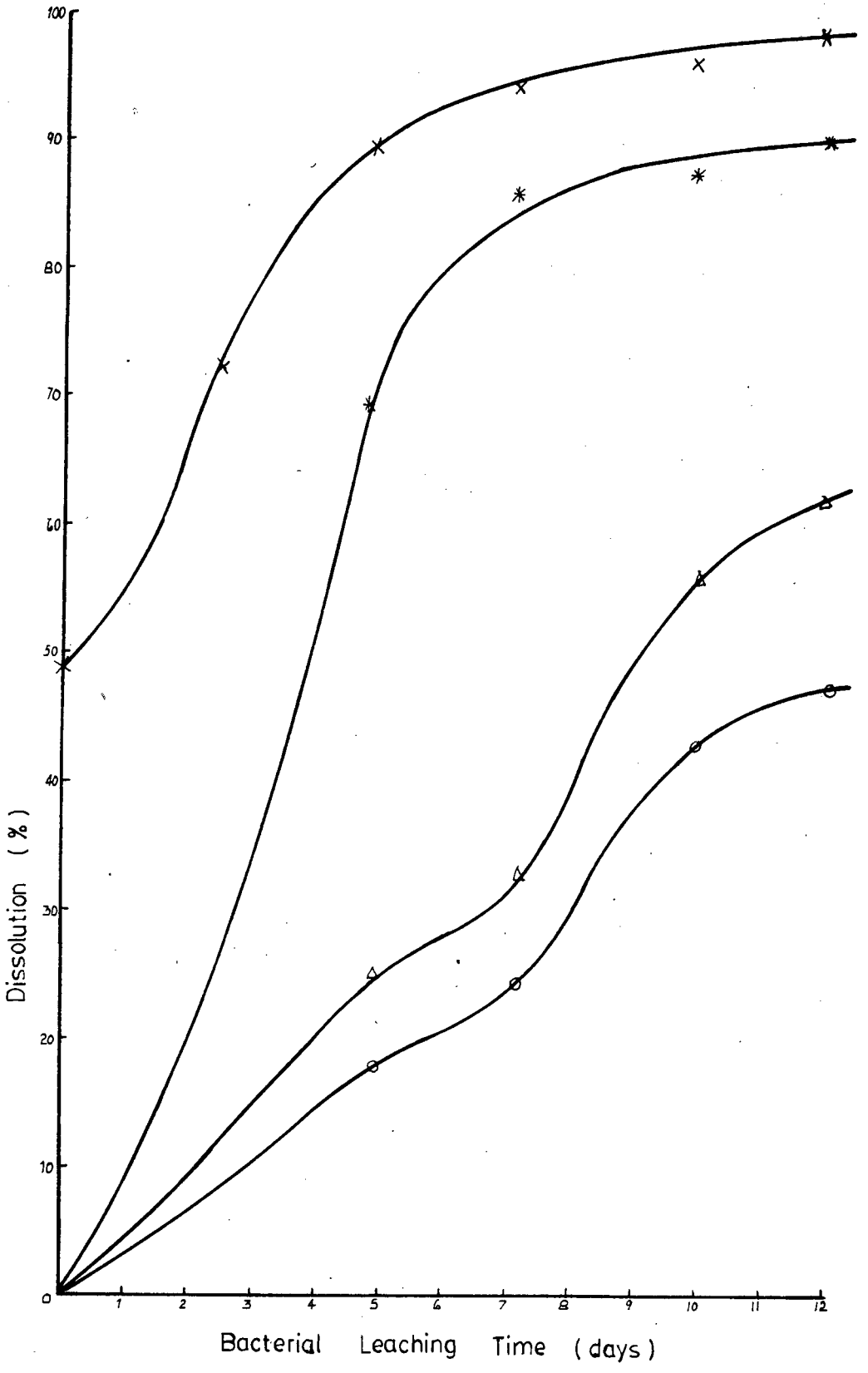


Fig.3.9: Analysis of leach vessel solids during the continuous bacterial leach of the flotation concentrate. Gold (x-x); arsenic (*-*); iron (Δ-Δ); pyritic sulphur (o-o).

Table 3.25: Cyanide leach of the continuously bacterially leached flotation concentrate. Values in brackets are the minimum and maximum values observed.

	Run 1	Run 2	Run 3
Pre-aeration Time (h)	43-45	40-47	21-28
Reagent Consumption:			
KCN (Kg/t)	26,67(23,92-28,38)	28,87(27,58-30,13)	18,03(15,80-21,45)
CaO (Kg/t)	142,45(138,2-151,5)	121,53(117,3-127,2)	49,29(16,2 -95,0)
Head Au (g/t)	125,72(97,6-148,3)	153,99(140,4-170,5)	123,00(70,8-157,6)
Residue Au (g/t)	3,23(2,53-3,59)	3,82(3,26-4,01)	3,51(2,46-3,96)
Gold Dissolution (%)	97,4(96,4-97,8)	97,5(97,1-97,9)	97,1(95,2-98,4)

In addition, on completion of the continuous bacterial leach, an overall material balance was done. It was found that a large weight loss (35-45%) had taken place and that iron and arsenic could be accounted for, but only some of the gold. However, in subsequent work gold accountability was good and concern for gold loss erased.

The process had a few mechanical problems, including no feed, too much or too little alkali addition and no air flow. The effects were minimal.

3.3.6 Microscopic Examination of the Concentrate

Both bacterial and acidic-ferric leaches were carried out for up to 40 days. Most of the work was done with a light microscope, but the scanning electron microscope was used to detect attached bacteria

(thiobacilli and leptospirilli) (Fig. 3.10). It was noted that when the jarositic coating was removed by an HCl wash the bacteria were also removed.

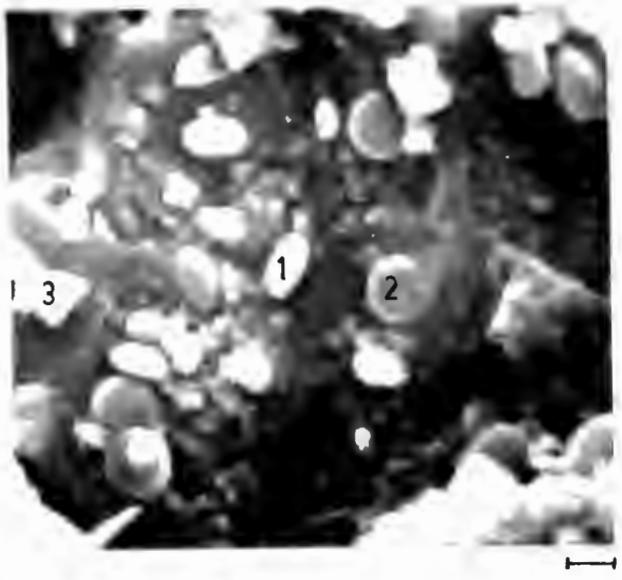


Fig 3.10: Scanning electron micrograph of bacteria attached to the mineral surface of flotation concentrate. Thiobacilli (1) and leptospirilli(2) are present, as well as pieces of the coating (3). The bar represents 1 μm.

Bacterial leaching produced mineral etching, the effects increasing with time. At low magnification (500x) it could be clearly seen that the arsenopyrite was more rapidly and extensively destroyed than the pyrite (Fig. 3.11). In 5-8 days there was no sign of attack on the pyrite, but the arsenopyrite had very fine cracks all over. Within 12-16 days the arsenopyrite surface had been totally destroyed, whereas the pyrite had only just started to show cracks and etches along the edges and surface. Even after 40 days of leaching the pyrite had only limited etching.

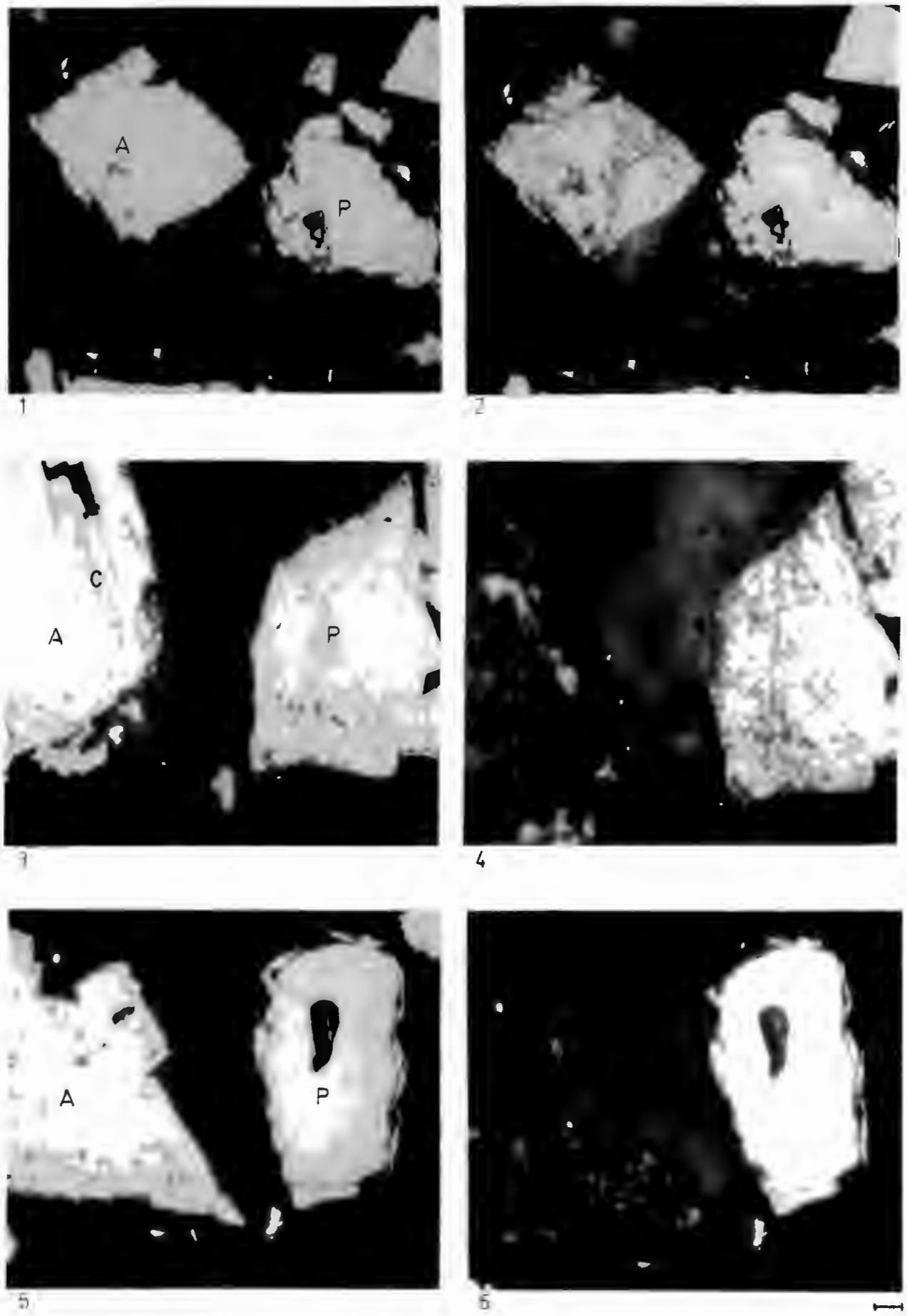


Fig. 3.11: Effect of bacterial leaching on the flotation concentrate mineral as seen with a light microscope. 8-day leach:before(1) and after (2);12-day leach:before(3) and after (4); 40-day leach:before (5) and after(6). Arsenopyrite (A); Pyrite(P);Chalcopyrite(C).The bar represents 10 µm.

At low magnification (500x) acidic-ferric leaching had a similar effect on the flotation concentrate (Fig. 3.12). However, the difference in pyrite and arsenopyrite leachability was not as marked. In 8-9 days both the pyrite and arsenopyrite showed pits and cracks; in 21-23 days (maybe less) the arsenopyrite surface had been totally destroyed, while the pyrite was still recognisable although quite deeply etched. Within 40 days the smaller pyrite particles' surfaces had been destroyed. Pyrite corrosion was greater than with bacterial leaching.

At a higher magnification (2200x) differences in bacterial and chemical leaching were readily visible. During the bacterial leaching of arsenopyrite the attack progressed from very fine cracks (0,2-0,3 µm wide) to depressions about 0,8 µm wide and 1-10 µm long (Fig 3.13 (1)), the approximate size of a bacterium. The pits increased in size to 1 µm x 2-20 µm (Fig 3.13 (2)) and onto larger sizes (as much as 4 µm wide)(Fig 3.13(3)),till they merged and totally destroyed the mineral. The pitting was quite deep (2-5 µm). With the pyrite only the first of these stages was seen (Fig. 3.13(4)) i.e. 0,2-0,3 µm x 2-5 µm pits in less than 20% of the particles.

Acidic-ferric leaching caused a form of pitting illustrated in Fig. 3.14. The pits were 0,5 µm x 1-3 µm, similar on pyrite and arsenopyrite, but very different in appearance from the etches obtained by bacterial leaching.

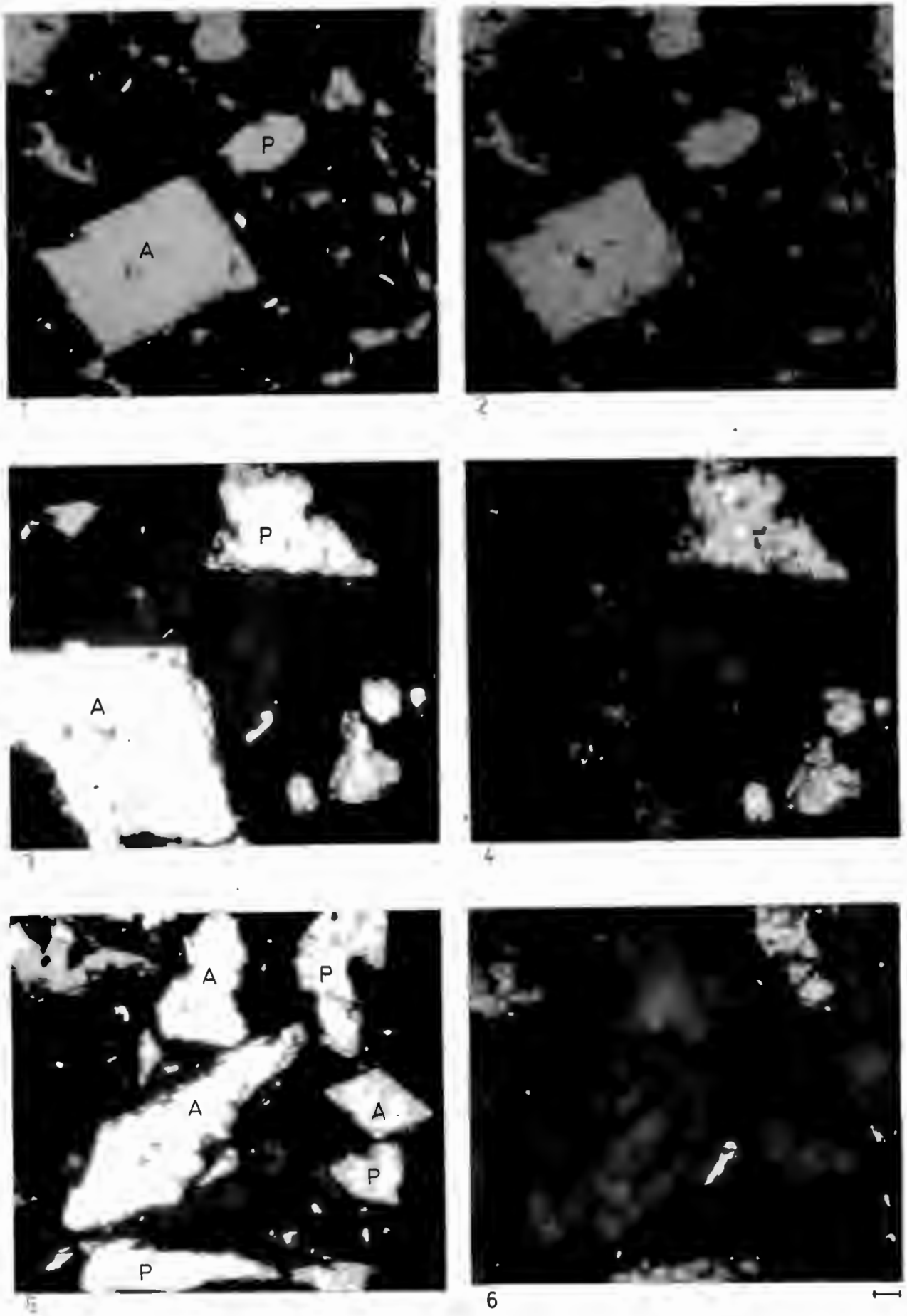


Fig.3.12: Effect of acidic-ferric leaching on the flotation concentrate minerals, as seen with a light microscope.9-day leach:before(1) and after (2); 23-day leach:before (3) and after (4); 40-day leach:before (5) and after (6).Arsenopyrite(A);Pyrite (P). The bar represents 10 µm.

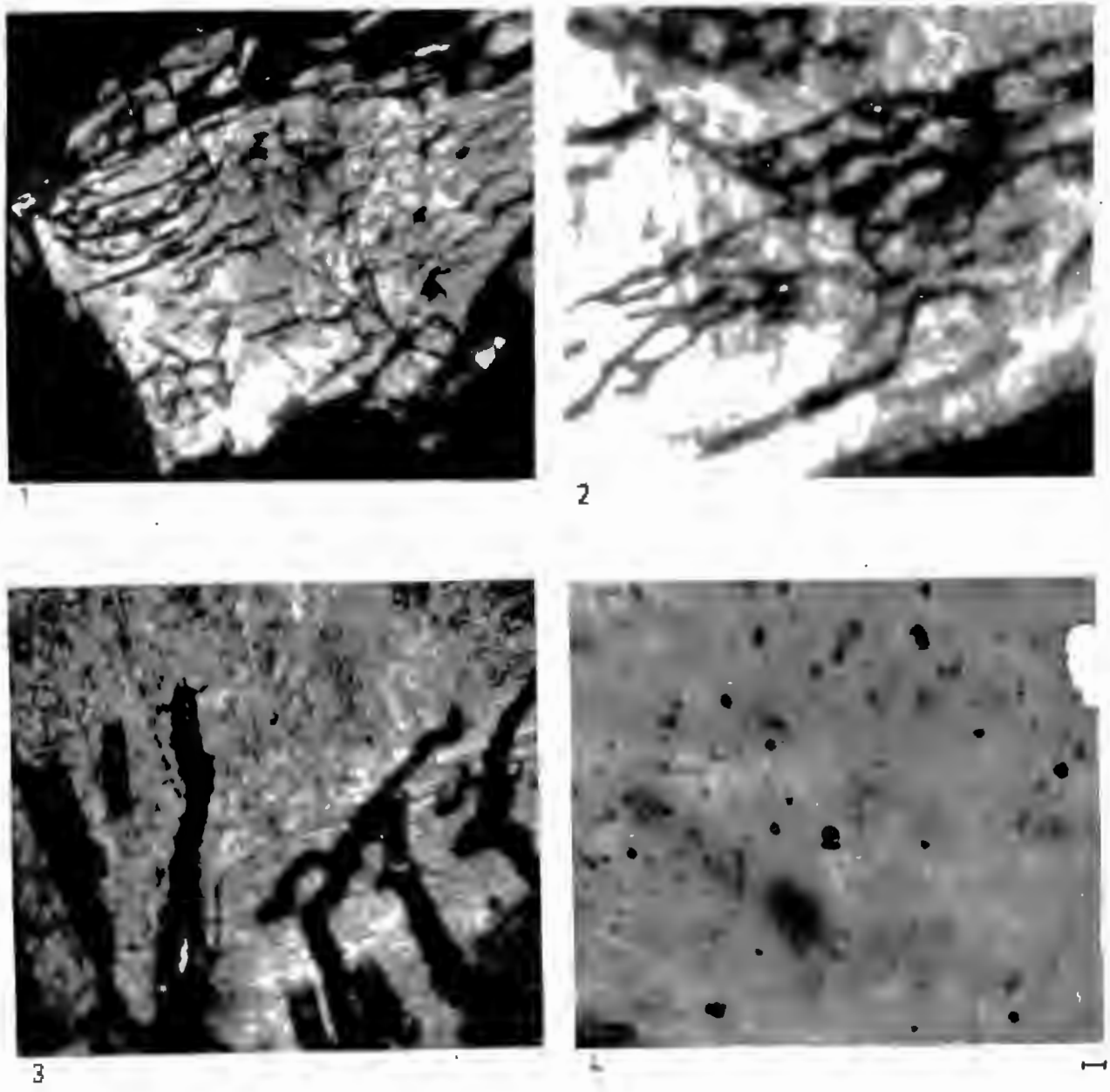


Fig.3.13: Detailed light microscopic view of the effect of bacterial etching on the flotation concentrate minerals. Arsenopyrite etching : Early stages (1); intermediate stages (2); late stages (3). Pyrite etching (4). The bar represents 1 µm.

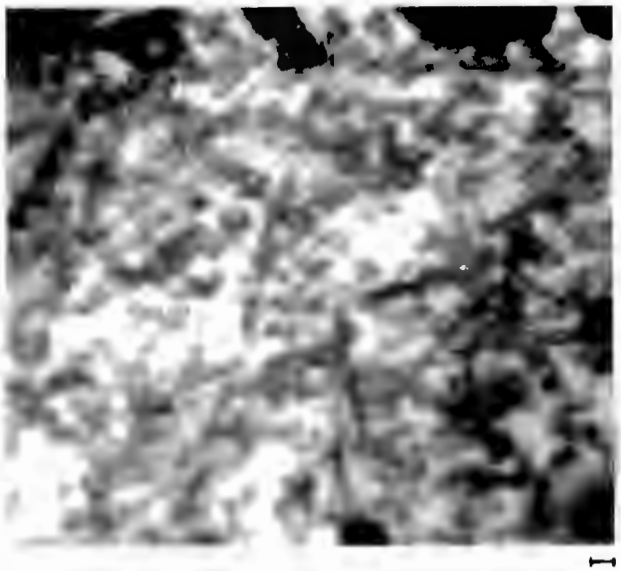


Fig.3.14: Detailed light microscopic view of the effect of acidic-ferric leaching on the flotation concentrate minerals. The bar represents 1 μm.

3.4 DISCUSSION AND CONCLUSIONS

Acid and acidic-ferric leaching can cause metal sulphide dissolution, including pyrite and arsenopyrite, but most workers agree that the effect is limited (Lloyd, 1967; Duncan and Drummond, 1973; Le Roux, North and Wilson, 1973; Bennett and Tributsch, 1978). Groudev (1982b) and Keller and Murr (1982), however, observed clear chemical etching. Since thymol has been thought to be a chemical oxidation inhibitor (Bruynesteyn, 1981) a non-chemical sterility procedure was also used in this work. In this study 0,8% and 2,3% increases in gold dissolution were obtained for the acid and acidic-ferric leaches respectively. When autoclaving (instead of thymol) was used to ensure sterility 4,4% more gold dissolution was obtained, twice as

much as with thymol. It is therefore, quite possible that thymol is a chemical oxidation inhibitor, although this should have been more apparent had a large oxidation effect occurred.

Bacterial leaching, on the other hand, was highly successful. T.ferrooxidans, a sulphur-oxidizing thiobacillus (possibly T.thiooxidans) and L.ferrooxidans were identified. But it is quite possible that other bacteria, such as T.acidophilus, were present. This bacterium has been regularly isolated from T.ferrooxidans cultures grown on iron and pyrite (Arkesteyn and de Bont, 1980; Harrison, Jarvis and Johnson, 1980). L.ferrooxidans prefers pH 3, where it is vibrio - or spirillae-shaped and 0,2-0,4 µm x 0,9-1,1 µm. However, at low pHs (<pH 2) it becomes ring-shaped or coccoid and 0,4-0,8 µm in diameter (Balashova et al, 1974; Pivovarova, Markosyan and Karavaiko, 1981). In this study thiobacilli and coccoid leptospirilli were seen (Fig. 3.10).

The bacteria have been found to be distributed between the leach solution and the mineral surfaces, the proportions varying, but usually most of the organisms are attached. On a zinc sulphide concentrate 65% of the T.ferrooxidans were attached. On pyrite 83%, 60-80% and 98-99% of the T.ferrooxidans have been reported attached, with 5×10^8- 1×10^9 cells/ml solution (Le Roux, North and Wilson, 1973; Gormely and Duncan, 1974; Groudev, 1979; Roy and Mishra, 1981). In this study 98-99% of the bacteria were attached, with $1\text{-}3 \times 10^8$ cells/ml solution.

As with the milled run-of-mine ore leaching sterility was not enforced. Loss of an active culture never occurred, rather the culture adapted progressively more with time. This was particularly true with regard to arsenic tolerance. When the culture was grown in 9K medium plus As_2O_3 only 4 g/l As could be tolerated, but in a concentrate leach as much as 7 g/l As was detected. However, the oxidation state and form of the arsenic was unknown. It probably consisted of a mixture of As_2O_3 and As_2O_5 - which might account for the difference in tolerance levels.

When studying the basic characteristics of the bacterial leach of the milled flotation concentrate a typical growth curve was apparent. A 2-3 day lag phase was followed by a log phase and stationary phase developed after 10-12 days. Analysis of the solution demonstrated Fe II oxidation with concomitant Fe III formation and arsenic solubilization. Ferric iron generation was biphasic. The first phase coincided with the oxidation of the 9K medium ferrous iron and could be attributed, at least in part, to this. The second phase was most likely due to bacterial mineral attack, as was the solubilization of arsenic. In the continuous bacterial leach the decreases and increases in ion concentrations were also observed, but a pattern could not be detected due to the effect of the decantations. As expected, these changes were accompanied by an increasing E_h (from 460 mV to 580 mV). This was slightly higher than the reported 515 mV obtained using T.ferrooxidans for ore oxidation (Sakaguchi, Silver and Torma, 1976; Lundgren and Silver, 1980).

Analysis of the continuous bacterial leach residue showed a similar feature to that obtained from the solution analyses, though from the batch testwork it was not particularly clear. Arsenic dissolution was continuous, whereas iron and pyritic sulphur dissolutions were biphasic (Fig 3.9). If it is considered that in the continuous leach the 2-day lag period was eliminated, Figs 3.9 and 3.5 matched well. The best interpretation for this is that during the first phase arsenopyrite was degraded and then, after a transition period of low bacterial activity, the pyrite was attacked in a second phase. This was later confirmed by the microscopic examination of epoxy resin mounts of the flotation concentrate.

This visualization also confirmed the formation of jarosite, iron arsenate and other precipitates (Fig. 3.10). This was expected, since some leach residue analyses showed increases in iron and sulphur.

The effect of bacterial leaching on gold dissolution was large (the gold dissolution increased from 50% to 98%). For a given bacterial leach time gold dissolution results could vary greatly. This variability decreased with time (see Fig. 3.6). Variations in inoculum size affected the length of the lag phase, but disappeared with time. When no decantations were done there was a greater precipitation of solutes which probably blocked up the minerals and therefore, prevented extensive gold dissolution (always less than 96%, usually 93%).

The cyanidation lime consumption increased with bacterial leaching time, because more acid was generated and had to be neutralized.

The alkali added during the bacterial leach neutralized acidity in solution, but not that associated with the concentrate itself. At 18 Kg/t the cyanide consumption was also high (an average plant consumes less than 1 Kg/t).

Once the feasibility of the bacterial leach had been established the most important parameters likely to affect it were examined. Particle size was the first of these to be investigated. Various workers, using pyrite and arsenopyrite, found that initial particle size was a very important parameter. The rate of mineral oxidation increased with decreasing particle size (Napier, Wood and Chambers, 1967; Pinches, 1975; Polkin et al, 1977). Torma and Bosecker (1982) advocated a particle size of less than 32 µm, but most groups in this field consider 44-37 µm to be ideal and one group used 74-44 µm particle size (Polkin et al, 1975). At B.C.Research -37 µm is considered ideal for chalcopyrite concentrate T.ferrooxidans leaching; for a lead sulphide leach 44-37 µm was considered the optimum; while a concentrate similar to the ore used in this study was leached continuously at an optimum particle size of 40 µm in one case and at 80% -56 µm in another (Polkin et al, 1977; McElroy and Bruynesteyn, 1978; Torma, 1978; Bruynesteyn et al, 1983; Marchant, 1985). The milled flotation concentrate used in this investigation was 83% -44 µm (88% -74 µm). Although very satisfactory results were obtained it had been hoped that the expense of milling could be avoided. However, using unmilled flotation concentrate (68% -74 µm) was unsuccessful, as an extra eight days were needed to achieve the same results.

The choice of initial pulp density was made based on the findings reported for pyrite and arsenopyrite concentrate work. A leach, using T.ferrooxidans and pyrite, used 3-10% solids, maximum oxidation occurring at 3% solids (Roy and Mishra, 1981). Others reported 6% solids as optimum and for vat leaching a pyritic concentrate 16-20% solids could be used (Le Roux, North and Wilson, 1973; Lawrence and Bruynesteyn, 1983; Lawrence and Gunn, 1985). With a pyrite/arsenopyrite concentrate 2,0%, 7,5% and less than 10% solids have been quoted as being ideal (Pinches, 1975; Polkin et al, 1975; Marchant, 1985). Torma and Bosecker (1982) reported that the T.ferrooxidans leaching of a concentrate should be carried out at 10-20% solids. Hence, in these studies 10% solids was used initially. It was found that as the pulp density increased above 5% the gold dissolution dropped. Bacterial activity was totally inhibited between 20% and 30% solids. This was probably due to problems with mass transfer of oxygen, carbon dioxide and nutrients to the microorganisms, microbial inhibition by arsenic and, possibly, erosion of the organisms. Work with other types of concentrates has confirmed the conclusions from this study. With a lead sulphide concentrate 14-20% solids was ideal and with sphalerite 16% was optimum, 13-20% acceptable, but above this zinc extraction dropped dramatically. With a copper-zinc concentrate 25% solids was the limit and with chalcopyrite 10-20% solids and 22% solids were reported as ideal (Torma, Walden and Branion, 1970; Bruynesteyn and Duncan, 1971; Sakaguchi, Silver and Torma, 1976; Polkin et al, 1977; McElroy and Bruynesteyn, 1978; Torma, 1978; Lawrence, Bruynesteyn and Hackl, 1981). In the continuous bacterial leach of this flotation concentrate up to 17% solids was used, with no adverse effects noted.

The effect of pH was also studied. Values in the range of pH 1,3-3,0 have been reported. For a pyrite concentrate pH 2,75-3,0 was considered ideal, but pH 1,8-2,0 has been used, and even pH 1,3. An arsenopyrite concentrate was leached at pH 2,1 in one instance and pH 2,0-2,5 in another. Using a pyrite/arsenopyrite concentrate pH 1,5-2,0 was found to be best in one case, pH 1,25-1,8 in another and pH 2,0-2,3 in yet another (Pinches, 1975; Polkin et al, 1975; Karavaiko, Kuznetsov and Golonizik, 1977; Lawrence and Bruynesteyn, 1983; Lawrence and Gunn, 1985; Marchant, 1985). The effect of pH 1,6-2,2 was examined, as this was thought to be the most suitable range. The batch bacterial leaches indicated that pH did not greatly affect gold dissolution. However, as the pH rose gold dissolution dropped progressively although only by a small amount. In the continuous bacterial leach pH 1,8 was also slightly better than pH 2,1. Although the difference was not marked it could be most clearly seen in the residues, which indicated the lower bacterial activity (Table 3.21). The reason for low pH preference is probably associated with the lack of solute reprecipitation. As the pH increased reprecipitation of solubilized material increased, which probably covered the etched minerals and, hence, blocked gold dissolution. Karavaiko, Kuznetsov and Golonizik (1977) calculated that at pH 1,6-1,8 21-34% of the arsenic precipitated, while at pH 1,8-2,0 this increased to 34-38% and at pH 2,0-2,2 to 38-50%. This clearly shows just how much precipitation can take place. In addition, these compounds are cyanicides and so cyanide consumption increased with increasing pH.

The optimum temperature for the bacterial leaching of the flotation concentrate was found to be 28°C. In the 25-45°C range results were

similar, but at 20°C bacterial activity was markedly reduced. Usually the optimum temperature is accepted as 35°C, as attested by T.ferrooxidans leaches done on chalcopyrite, sphalerite, galena, pyrite and pyrite/arsenopyrite concentrates. The limits are usually 25-40°C, but sometimes up to 45°C (Corrick and Sutton, 1965; Torma, Walden and Branion, 1970; Pinches, 1975; Sakaguchi, Silver and Torma, 1976; Torma, 1978; Lundgren and Silver, 1980; Lawrence and Bruynesteyn, 1983). There are some workers who agree with the results found in this study, that the optimum temperature is 25-30°C. This applied to both pyrite and pyrite/arsenopyrite concentrates (Napier, Wood and Chambers, 1967; Polkin et al, 1975; Karavaiko, Kuznetsov and Golonizik, 1977; Murr and Brierley, 1978).

Another interesting observation was that as the temperature rose above 30°C more of the ferric iron and arsenic precipitated out of solution. At higher temperatures many chemical reactions are accelerated. However, unexpectedly, the cyanide consumptions for gold solubilization did not rise.

Nutrients were another parameter investigated. It is generally accepted that for the bacterial leaching of minerals T.ferrooxidans requires ammonia, sulphate, potassium, phosphate, magnesium and calcium. Most of these are supplied by the ore with the possible exception of ammonia and phosphate (Table 3.1; Lundgren and Silver, 1980). From the batch testwork only ferrous iron appeared to be beneficial to the leach, though not essential. A solution analysis at the end of the leach showed that ferrous iron, potassium and possibly ammonia were depleted, but that enough had apparently been

present to ensure good mineral attack.

Since it has been suggested that ferrous iron, ammonium, phosphate and potassium were beneficial (see previous chapter) ferrous sulphate, ammonium sulphate and di-potassium hydrogen phosphate were added to the continuous bacterial leach in reduced concentrations. These were lower than the 3 g/l $(NH_4)_2SO_4$ and 0,5 g/l K_2HPO_4 recommended by Torma and Bosecker (1982), but more than the 0,05 g/l $(NH_4)_2SO_4$ and no K_2HPO_4 used by Marchant (1985). In the continuous leach 0,5 g/l $(NH_4)_2SO_4$ and 0,37 g/l K_2HPO_4 were added and initially 3,5 g/l Fe II and later 2,0 g/l Fe II. The reduction in nutrient addition had no effect on bacterial activity and gold dissolution. As in the batch tests, a reduction/absence of ferrous iron, potassium and phosphate decreased cyanide and lime consumption during the cyanide leach. This was due to the lessened formation of iron and potassium oxides and jarosite, because of both the absence of these elements and the use of lime as an acid neutralizing agent during the bacterial leach (calcium is divalent and does not generate jarosite).

Tests done in the presence of flotation reagents yielded good gold dissolutions. This was expected, since Polkin et al (1975) and Duncan, Walden and Trussel (1966), working with pyrite/arsenopyrite and chalcopyrite concentrates respectively, found that flotation reagents had no influence on the rate of leaching. Torma (1977), however, reported flotation reagents to be inhibitory to ferrous iron and copper sulphide oxidation. Surfactants e.g. Tween 20,40,60 and 80, on the other hand, gave mixed results (Duncan, Trussel and

Walden, 1964; Burkin, 1967; Duncan, 1967; Torma et al, 1976; Karavaiko, Kuznetsov and Golonizik, 1977; Wakao et al, 1983).

An investigation of the effect of inoculum size was also carried out. Reports on this aspect are varied. Atkins (1978) and Norris and Kelly (1978) worked with pyrite and T.ferrooxidans and found that as the inoculum size increased the lag period dropped. Pinches (1975) confirmed this result, but added that the bacterial growth and leach rates were not affected, while Razzell and Trussel (1963) found that copper dissolution from chalcopyrite increased up to a point with inoculum size, then dropped with further increases. This may account for some workers moving away from "inoculum size" to "bacterial numbers", with 2×10^8 cells/ml at the start considered to be the minimum needed (Groudev, 1982b; Wakao et al, 1982). In this study it was seen that as the amount of inoculum increased so did the rate of gold dissolution, while the lag period was reduced. A minimum of 8×10^8 cells/ml was used at the start, which may account for the success of all the leaches.

A theoretical air consumption had been calculated as 0,18 l/min Kg (assuming total degradation of the sulphides). If a 10-20% aeration efficiency is assumed, then the value obtained (1,43 l/min Kg) was reasonable.

It was also observed that in the initial stages of the bacterial leach the flotation concentrate consumed acid due to its natural pH being 4,30. As the large amounts of sulphides were oxidixed to sulphuric acid alkali (lime) was required to prevent the pH from becoming inhibitory.

A change in grading and compositional analyses of the flotation concentrate was observed as a result of the bacterial leach. As in the previous chapter there was a reduction in particle size which was not due to attrition. Initially the smallest particle size fractions diminished (< 20 μm), progressing to the largest (> 147 μm) in order. This may reflect the fast bacterial oxidation of fines. These findings are in agreement with those of Le Roux, North and Wilson (1973) and Hiltunen et al (1981). They found that the small particles vanished first (20-30 μm) although, in the final analysis, the -20 μm fraction increased due to a general reduction in particle size. Only sulphide minerals were degraded in the leach, with about 50-60% pyrite and arsenopyrite decomposition.

Pyrite and arsenopyrite degradation was investigated via the microscopic examination of epoxy resin mounts of the flotation concentrate. Thiobacilli and leptospirilli were observed through a scanning electron microscope. They were attached, in fairly large numbers, to the sulphide minerals via a coating (Fig 3.10). This has been reported to be quite common, although their concentrations on the minerals (only on energy-yielding ones) was not always high (Berry and Murr, 1978; Lundgren and Silver, 1980; Hiltunen et al, 1981; Keller and Murr, 1982). Coatings of various types have been reported. Pyrite is not always coated but may be covered by a thin, yellow, rough coat. Arsenopyrite can be coated by fine grains or a film, greenish in colour and containing arsenical compounds (Duncan and Drummond, 1973; Pinches, 1975; Polkin et al, 1975; Hiltunen et al, 1981; Derry and Whittemore, 1983).

Bacterial and chemical leaching was selective in that the arsenopyrite was destroyed first, then the pyrite. This can be explained by the galvanic interactions that occur in a mixture such as the one used in this study. The equilibrium electrode potential of arsenopyrite is approximately 490 mV and that of pyrite is approximately 630 mV. Hence, the former is anodic and the latter passivated (Polkin et al, 1975; Karavaiko, Kuznetsov and Golonizik, 1977; Karavaiko and Pivovarova, 1977; Berry, Murr and Hiskey, 1978; Mehta and Murr, 1982). The bacterial leach was more sensitive to this, probably because of the added cathodic effect of the bacteria (Corrans, Harris and Ralph, 1972).

Grooves, pits, holes and jagged edges were all part of the pattern of etching. Mineral defects and weaknesses, crystallography and, to a small extent, crystallographic orientation were influencial on the bacterial etching patterns. Crystallographic orientation preference was observed with the arsenopyrite, the basal section (001) being prefered to the prismatic (110). This was, generally, in agreement with the observations of other workers (Duncan and Drummond, 1973; Tributsch, 1976; Bennett and Tributsch, 1978; Berry and Murr, 1978; Southwood and Southwood, 1985). However, Hiltunen et al (1981) found there was no specific arrangement of etches on pyrite, while Keller and Murr (1982) reported the same and noted that crystallographic orientation also had no effect. Crystallography had the greatest influence on acidic-ferric etching. This is in agreement with the observation of Bennett and Tributsch (1978).

The bacterial pitting strongly suggested direct mineral attack (at

the point of cell contact) via cell-bound enzymes. Alternately, a bacterial metabolite such as Fe III and H_2SO_4 was locally generated and excreted into the area. Bennett and Tributsch (1978) and Lundgren and Silver (1980) were noncommittal, while Polkin et al (1975) and Ehrlich (1978) advocated direct attack and Tributsch (1976) metabolite excretion. Tributsch (1976) and Bennett and Tributsch (1978) worked with galena and pyrite respectively and reported almost exactly what was seen in this investigation.

Southwood and Southwood (1985) worked with auriferous pyrite and showed that there were pores in the mineral, not just superficial etching. Mineral crystallography and weaknesses determined pore direction and size. Since gold is usually particulate inclusions which occupy disruptions in the pyrite crystal, and these are the areas most subject to bacterial corrosion, this could explain why more gold is usually released than sulphide is broken down.

Chemical pitting was very different from that of the bacteria, which quite clearly indicated that the bacteria had a specific role to play in mineral leaching.

From this work it was concluded that (i) the bacterial leach of the milled flotation concentrate was successful; (ii) a retention time of ten days was needed (see Fig. 3.9), with a 9-10% recycle, with one decantation step, yielding a 97-98% gold dissolution upon cyanidation; (iii) milling to 88% -74 μm was necessary; (iv) 12% solids could be used; (v) a pH of 1,7 to 1,8 was suitable and would require a lime consumption of 120 Kg/t concentrate; (vi) 28°C was the optimum

temperature; (vii) probably only 2 g/l Fe II needed to be added, although 0,5 g/l $(NH_4)_2SO_4$ and 0,37 g/l K_2HPO_4 were also added; (viii) the cyanide consumption would be approximately 18 Kg/t concentrate and the lime consumption approximately 50 Kg/t concentrate for gold solubilization.

An economic feasibility study was carried out based on these findings. A bacterial leaching plant for the flotation concentrate was found to be more economic than the conventional flotation-roasting process, with the added advantage of contained environmental pollution (Livesey-Goldblatt, Norman and Livesey-Goldblatt, 1983).

CHAPTER 4

PILOT PLANT BACTERIAL LEACHING OF THE FLOTATION CONCENTRATE

This chapter is a summary of results from the scale-up of the bacterial leach of the pyrite/arsenopyrite concentrate to pilot plant. Operating conditions were based on the continuous bacterial leach laboratory testwork of the flotation concentrate, but differed initially in that there was no decantation step.

From the operation of the pilot plant over a period of about a year the following observations and conclusions were made:

(i) the laboratory testwork was confirmed.

(ii) arsenic tolerance reached approximately 10 g/l As and approximately 39 g/l Fe III could be well tolerated.

(iii) the bacterial numbers increased across the system (6×10^9 - 2×10^{10} cells/ml), with about 9×10^9 cells/ml slurry and about 5×10^8 cells/ml solution. There was, therefore, about 94% attached bacteria. At high temperatures elongated cells (>10 µm) appeared, but were found to not be a new thermophilic species. They were most probably thiobacilli that could no longer divide under the growth conditions.

(iv) the retention time was progressively dropped from 12 to 6 days and could probably have been reduced further, but the heat generated could not be controlled. At a 4-day retention time the agitators had

to be turned off for 30-50% of the time to prevent overheating. Consequently, there was a rise in the residue pyritic sulphur and a drop in gold dissolution.

(v) the need for milling was rechecked. The effect of no further milling (at a 9-day retention) was slightly deleterious, but it was thought that as the retention time decreased this effect would be more pronounced. The plant was therefore operated at approximately 90% -74 μm particle size.

(vi) the pulp density was 11-14% solids. It increased across the tanks due to solution evaporation. At 14-17% solids bacterial leaching was adequate, but not as good.

(vii) on scale-up the process was found to be strongly exothermic. When the temperature was not controlled it rose to approximately 53°C and then plummeted to approximately 28°C, slowly recovered and the cycle repeated itself. The higher the temperature the greater the drop, sometimes causing plant stoppage. Another problem was that above 45°C large amounts of froth formed in the leach tanks. At 30°-40°C bacterial leaching was efficient and stable. Temperatures were maintained in this range by cooling the tanks externally with water sprays. This was necessary on the first few tanks, but the latter tanks remained at 33°-37°C. This drop in heat generating power across the system reflected bacterial activity.

(viii) the need for nutrient addition was also examined. Fe II was not needed (initially about 2 g/l was added), PO_4 was unnecessary

(the process actually improved in its absence), but K was required (initially 0,37 g/l K_2HPO_4 was added as KOH + H_3PO_4). The need for NH_3 was not tested (0,5 g/l $(NH_4)_2SO_4$ was added).

(ix) the pH was maintained at pH 1,6-1,7 . The lime consumption for acid neutralization was 139 Kg/t feed.

(x) the total theoretical air requirement was 440 l/min. Initially a 20% efficiency was assumed (2200 l/min) and more than 450 l/min was added to each of the leach tanks. There was a lot of frothing and the air flow was consequently reduced and found to be perfectly satisfactory. A 38% efficiency was found (1150 l/min), the air requirement decreasing across the system. This, too, reflected the progressive drop in bacterial activity. To the first tanks 300 l air/min was added, dropping to 100 l air/min in the last tank.

(xi) adding 0,03% CO_2 to the air was tested but it had no effect.

(xii) the 10% recycle was found to be unnecessary.

(xiii) the process was found to be stable and, in fact, improved with time.

(xiv) plant assay methods were similar to the laboratory methods, but the Fe III titration was inaccurate at such high values of iron and arsenic. Instead, the Fe III was converted to Fe II and titrated.

(xv) plant methods for handling the overflow solution and fines were

developed. It was limed, allowed to settle and then dumped on a slimes dam. The lime consumption for this was 34 Kg/m^3 of overflow.

(xvi) during cyanidation a lot of frothing occurred. To avoid this froth suppressants were added and 20% solids (instead of 35-50%) used. With about 24 h pre-aeration and a 16 h leach the cyanide and lime consumptions were about 10 Kg/t and 49 Kg/t, respectively. As pre-aeration decreased consumptions rose.

(xvii) gold dissolution was 97-98%.

(xviii) a material balance was done. There was a 3,6% weight gain; 95,5% Fe, 96,8% S, 93,4% As and 92,3% Au could be accounted for.

(xix) an economic feasibility study showed the process as a whole to be practical and viable.

CHAPTER 5

CONCLUSION

The aim of this study was :

(i) to develop an economically viable bacterial leaching process for a pyrite/arsenopyrite ore containing gold.

(ii) to investigate the various parameters which affect a vat leach such as this.

(iii) to investigate the mechanism of bacterial leaching of this ore.

The use of the milled flotation concentrate for bacterial leaching combined high gold dissolutions (97-98%) with economic viability. Initially the milled run-of-mine ore was used. Although bacterial leaching was successful (91-93% gold dissolution was attained) the process was not economically viable.

For the milled run-of-mine ore it was found that particle size and added nutrients had little effect on bacterial leaching. Important leaching parameters were the pulp density, which had to be kept below 30% solids, the pH, at about pH 1,8, and the temperature in the 30-40°C range. A minimum retention time of four days was required. Additional time had little beneficial effect. The arsenic concentration in solution had to be maintained below 1 g/l As.

In contrast, during the bacterial leach of the flotation concentrate

pH and temperature were parameters whose effect was negligible, when used within a reasonable range. Milling was necessary (to -74 μm), the pulp density had to be kept below 20% solids and additional ferrous iron aided bacterial leaching. The minimum retention time needed was ten days. An increased retention time had little beneficial effect. The arsenic concentration in solution had to be maintained below 4 g/l As. Inoculum size affected the rate of gold dissolution as well as the length of the lag period - the larger the inoculum the better.

The pilot plant testwork, however, showed that milling and nutrient addition were not as critical as initially thought. In fact, generally, it demonstrated that the bacterial leaching of the concentrate was more efficient and robust than the laboratory work predicted.

Of interest was the observation that the only three parameters found to be consistently important to the bacterial leaching of this ore were retention time, pulp density and arsenic concentration in solution.

When the run-of-mine ore testwork started the bacterial culture could tolerate only 1 g/l As, but by the time the testwork on the flotation concentrate began 4 g/l As could be tolerated. By the end of this work 6 g/l As could be present in solution and on completion of the pilot plant continuous bacterial leach it was 10 g/l As. This clearly demonstrated the progressive adaptation with time (over four years) of the bacterial culture. This same adaptability accounted for the decrease in minimum residence time.

When the optimum conditions for bacterial leaching were maintained during a continuous vat leach retention time and reagent consumption were low and gold dissolution was high. These three factors were instrumental in achieving economic viability.

Direct and indirect bacterial leaching appeared to occur in this instance. Ferric iron and sulphuric acid etched the pyrite and arsenopyrite considerably but slowly. Bacteria were responsible for another type of etching, which started in the immediate vicinity of the attached organisms. Crystal structure and weaknesses played an important role as sites of crystal attack. Mineral destruction was affected by bacterial enzymes/metabolites and by galvanic interactions, as well as substrate solubility.

APPENDIX A

GENERAL METHODS

DETERMINATION OF AMMONIA IN SOLUTION

The solution was treated with sodium hydroxide, then distilled. The distillate was collected in a standard boric acid solution. The ammonia concentration was determined by back titration of the boric acid solution.

DETERMINATION OF ARSENIC IN SOLIDS

The sample was fused with a Na_2O_2:Na_2CO_3 fusion mix, leached and then acidified with sulphuric acid. It was reduced in the presence of hydrazine sulphate, potassium bromide and hydrochloric acid. The arsenic, as $AsCl_3$, was distilled from the solution matrix. Its concentration was determined volumetrically by titration with standard iodine or potassium bromate solution, using starch as an indicator.

$$As_2O_3 + 2H_2O + 2I_2 \rightarrow As_2O_5 + 4HI$$

DETERMINATION OF ARSENIC IN SOLUTION

1. By Distillation - as above for solids.
2. By ICAP - see under heading "ICAP".
3. By X-ray - the sample was measured against matched standards at

the appropriate wavelengths, using a molybdenum tube.

4. By Liquid Chromatography - the sample was passed through a 40 µm filter and then injected into the system. The arsenic forms (and concentrations) were determined by graphics.

BACTERIAL NITROGEN COUNT

Firstly total nitrogen was determined. Well mixed sample slurry (50 ml) was placed in a Kjeldahl vessel and 40 ml of 50% H_2SO_4 and 1,5 g K_2SO_4 added. Gradually this mixture was heated till strong sulphuric acid fumes were formed. It was rapidly cooled and water added to make it up to approximately 80 ml. It was then neutralized with 50% NaOH until a permanent ferric oxide precipitate was formed ($\pm$ pH 5,5). An excess of NaOH was added and the mixture distilled. The distillate was collected in saturated boric acid solution containing three drops of indicator. The boric acid solution was titrated with 0,1N HCl to purple.

1 ml 0,1N HCl = 0,001401 g N

Secondly free nitrogen was determined. Well mixed sample slurry (100 ml) was placed in a Kjeldahl vessel. An excess of 50% NaOH was added and the mixture steam distilled. The distillate was collected in saturated boric acid solution containing indicator and titrated as above.

Thirdly the total nitrogen content of the ore prior to bacterial leaching was determined, as in the first step.

Finally, the values obtained in steps 2 and 3 were subtracted from the value obtained in step 1:

Total N - free N - ore N ($\pm$0) = non-distillable N (x) mg N/l

$$\frac{x}{0{,}157 \times 10^{-7}} = \text{bacterial numbers (cells/ml)}$$

EPOXY RESIN MOUNT PREPARATION

Sixteen parts of Epofix resin was mixed with two parts of the hardener and stirred for at least two minutes. It was poured into a vaseline-coated container, the sample added and mixed in. After setting for 8 hours at room temperature the mount was removed. It now had to be polished. One end was rubbed for about one minute with P360, P600 and P1200 sandpaper, successively, until the surface looked smooth. Microid diamond compound (9 µm) was placed on a polishing wheel, spread with polishing oil and used to polish the mount for 5-10 minutes. Using different wheels this was repeated using 6 µm and then 1 µm diamond compound. After washing the mount with water it was polished with gamma alumina (0,05 µm), as above. It was then washed, air dried and examined microscopically.

DETERMINATION OF GOLD AND SILVER IN SOLIDS

They were assayed by the standard lead collection fire assay procedure (Dillon, 1955).

DETERMINATION OF GOLD IN SOLUTION

1. In Suspended Solids - The solution was filtered and the residue dried and weighed. It was then fused with a sodium peroxide-sodium carbonate mixture, leached and acidified with 10% aqua regia. The gold was preconcentrated into alamine/solvesso contained in kerosene and its concentration determined by atomic absorption or ICAP.
2. In Solutions - The gold was preconcentrated into alamine/solvesso contained in kerosene and its concentration determined by atomic absorption or ICAP.

ICAP ANALYSIS OF SOLIDS

It can analyse for Al, As, B, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn and Zr.

The sample was dissolved by pressure leaching techniques and read on an Arlab 34000 instrument having sixty spectral channels. Rhodium was the internal standard for viscosity effects. A digital PDP 1103 computer compiled and processed the results.

ICAP ANALYSIS OF SOLUTIONS

The sample was taken and read as above for solids.

DETERMINATION OF TOTAL IRON IN SOLIDS

The sample was fused in a $2Na_2O_2:1Na_2CO_3$ mixture, leached in water and any sodium arsenate removed by filtration. The ferric hydroxide was dissolved in hydrochloric acid and reduced with stannous chloride using heat. It was then titrated with standard potassium dichromate solution as described below ("Determination of Ferrous Iron").

DETERMINATION OF FERRIC IRON IN SOLUTION

1. By Titration - Some sample (5 ml) was pipetted into a flask and 1 g KI added. It was titrated with standard sodium thiosulphate solution to pale yellow. Then 1 ml starch indicator was added and the solution titrated further to colourless. 1 ml titrant = 1 g/l Fe III.
2. By Reduction Followed by Titration - Some sample (5 ml) was pipetted into a flask, mixed ÷ hydrochloric acid added ($\pm$ 20 ml) and the mixture brought to the boil. A 5% stannous chloride solution was added dropwise until the mixture cleared. After cooling 5 ml of saturated mercuric chloride was added to give a fine white cloudiness. It was titrated with standard potassium dichromate solution as described below ("Determination of Ferrous Iron").

DETERMINATION OF FERROUS IRON IN SOLUTION

Some sample (5 ml) was pipetted into a flask, 10 ml H_2SO_4-H_3PO_4-H_2O mixture and a few drops of sodium diphenylamine sulphonate indicator added. It was then titrated with standard potassium dichromate solution to purple. 1 ml titrant = 1 g/l Fe II.

DETERMINATION OF PYRITIC SULPHUR

Bacterial leaching generated various sulphur forms which would be analysed as pyritic sulphur using the normal method. The sample was, hence, firstly treated with a sodium carbonate solution and the residue then analysed for sulphur as described further on (see "Determination of Sulphur").

DETERMINATION OF RESIDUAL CYANIDE

Some sample (130 ml) was pipetted into a flask and a little KI added. This was titrated with standard silver nitrate solution until a permanent, faintly yellow opalescence formed. 1 ml titrant = 0,01% KCN.

DETERMINATION OF RESIDUAL LIME

Some sample (130 ml) was pipetted into a flask and an excess of silver nitrate added (more than that needed for the above titration). A few drops of phenolphthalein indicator were added and the solution titrated with standard oxalic acid solution to colourless (not pink). 1 ml titrant = 0,0022% CaO.

DETERMINATION OF SPECIFIC GRAVITY (S.G.)

It was calculated according to the following formulae:

$$\text{S.G. (slurry)} = \frac{b}{a}$$

with a = weight of a known volume of water

b = weight of same volume of slurry

$$\text{S.G. (solid)} = \frac{c-a}{(b-d) + (c-a)}$$

with a = weight of container

b = weight of container and known volume of water

c = weight of container and sample

d = weight of container and same amount of sample + water to same volume as in "b"

DETERMINATION OF TOTAL SULPHUR IN SOLIDS

1. The sample was digested with aqua regia in the presence of a potassium bromide-bromine mixture. This prevented the formation of acid sulphides during digestion. It was then repeatedly treated with hydrochloric acid and filtered. This dehydrated and removed free silica together with insolubles. The sulphur was precipitated out of the filtrate using barium chloride and ascorbic acid or hydroxylamine hydrochloride (as a reductant, to prevent iron coprecipitation). The resultant barium sulphate was collected by filtration and ignited to constant weight at

850°C. The value of sulphur was determined from the mass of barium sulphate obtained.

2. The sample was fused using a sodium peroxide - sodium carbonate mixture. The alkali leached solution was then made up to a standard volume and the precipitate allowed to settle. An aliquot was filtered and acidified, the silica removed and the sulphur determined as above. This method was used if there were large amounts of lime in the sample.

DETERMINATION OF TOTAL SULPHUR IN SOLUTION

As above for solids.

APPENDIX B

MEDIA AND SOLUTIONS

9K AGAR

Solution A:	$(NH_4)_2SO_4$	3,0 g
	KCl	0,1 g
	K_2HPO_4	0,5 g
	$MgSO_4.7H_2O$	0,5 g
	$Ca(NO_3)_2$	0,01 g
	Agar-agar	20,0 g
	Distilled water	500 ml
Solution B:	$FeSO_4.7H_2O$	50,0 g
	Distilled water	500 ml
	pH adjusted to 1,3 with H_2SO_4	

Solutions A and B were autoclaved separately, then mixed and plates poured.

9K MEDIUM

Solution A : as in 9K agar, but with no agar.

Solution B : as in 9K agar.

Solutions A and B were autoclaved separately, then mixed.

GRAM STAIN

1. Make a water smear of the organism, dry and fix by heat.
2. Stain with crystal violet for one minute, wash with water and drain.
3. Apply iodine for one minute, wash with water and drain.
4. Discolour with fresh acetone alcohol, wash with water and drain.
5. Counterstain with safranin for one minute, wash with water and drain.

GUARGUM 0,05% SOLUTION

Guargum	0,5 g
Water	1 l

MAGNAFLOC 351 0,05% SOLUTION

Magnafloc 351	0,5 g
Water	1 l

NITROGEN COUNT INDICATOR

Methyl red	0,125 g
Methylene blue	0,083 g
Ethanol	100 ml

NUTRIENT AGAR

Nutrient agar	28,0 g
Distilled water	1 l

STANDARD OXALIC ACID SOLUTION (0,1N)

Oxalic acid	6,30 g
Distilled water	1 l

PHENOLPHTHALEIN INDICATOR

Phenolphthalein	5,0 g
Ethanol	500 ml
Distilled water	500 ml

The phenolphthalein was first dissolved in the ethanol, then the water was gradually added while stirring.

STANDARD POTASSIUM DICHROMATE SOLUTION (0,0896N)

$K_2Cr_2O_7$	4,39 g
Distilled water	1 l

STANDARD SILVER NITRATE SOLUTION (0,1N)

$AgNO_3$	16,99 g
Distilled water	1 l

SODIUM DIPHENYLAMINE SULPHONATE INDICATOR

Barium diphenylamine sulphonate	0,32 g
H_2SO_4	1 ml
Distilled water	100 ml

First the water, then the acid was added to the sulphonate. When the barium sulphate had settled the clear reagent was decanted off and used.

STANDARD SODIUM THIOSULPHATE SOLUTION (0,0896N)

$Na_2S_2O_3.5H_2O$	22,24 g
Na_2CO_3	0,05 g
Distilled water	1 l

STARCH INDICATOR

Soluble starch	0,5 g
Distilled water	50 ml

The two ingredients were mixed, boiled, then cooled and used.

H_2SO_4 -H_3PO_4 - H_2O MIXTURE (Mixed Acid)

H_2SO_4(concentrated)	150 ml
H_3PO_4(85%)	150 ml
Distilled water	700 ml

WAKSMANS' AGAR

$Na_2S_2O_3.5H_2O$	5,0 g
NH_4Cl	0,1 g
$CaCl_2.6H_2O$	0,25 g
$MgCl_2.6H_2O$	0,1 g
KH_2PO_4	3,0 g
Agar-agar	20,0 g
Distilled water	1 l

pH adjusted to pH 4-5 with H_2SO_4

WAKSMAN'S MEDIUM

As in Waksman's agar, but with no agar.

APPENDIX C

CHEMICALS

Acetone	UniTEK
Agar Agar	Merck
NH_3	UnivAR
NH_4Cl	Cica (Kanto Chemical Co.)
$(NH_4)_2SO_4$	UnivAR
Antifoam RD emulsion	Dow Corning
As_2O_3	Riedel-de Haën AG
Barium diphenylamine sulphonate	BDH
Boric acid	Protea
$CaCl_2.6H_2O$	Baker
$Ca(NO_3)_2$	UnivAR
CaO	UnivAR
Crystal violet	Cica
Epofix	Struers
Ethanol	UnivAR
$Fe_2(SO_4)_3$	Riedel-de Haën AG
$FeSO_4.7H_2O$	UnivAR
Gamma alumina	Leco Corp.
Guargum	Stein, Hall S.A.(Pty)Ltd.
HCl	Koch-Light
Iodine	UnivAR
Magnafloc 351	Allied Colloids
$MgCl_2.6H_2O$	AnalaR
$MgSO_4.7H_2O$	Cica

Methylene blue	BDH
Methyl red	Riedel-de Haën AG
Microid diamond compound	Leco Corp.
Nutrient agar	Oxoid
Oxalic acid	Protea
pH buffer solutions	UnivAR
Phenolphthalein	UnivAR
H_3PO_4	UnivAR
Polishing oil	Leco Corp.
KCl	UnivAR
KCN	UnivAR
$K_2Cr_2O_7$	PAL Chemicals
KH_2PO_4	Riedel-de Haën AG
K_2HPO_4	UnivAR
KI	UnivAR
K_2SO_4	AnalaR
$K_2S_4O_6$	Fluka
Safranin	Gurr
Sand paper	Norton
$AgNO_3$	UnivAR
Na_2CO_3	Merck
Na_2HPO_4	Merck
NaOH	UnivAR
$Na_2S_2O_3.5H_2O$	AnalaR
Starch (soluble)	Cica
H_2SO_4	UnivAR/UniTEK
Thymol	H & W

LITERATURE CITED

1. Arkesteyn, G.J.M.W. (1979) - "Pyrite oxidation by Thiobacillus ferrooxidans with special reference to the sulphur moiety of the mineral" - Antonie van Leeuwenhoek 45: 423-35.

2. Arkesteyn, G.J.M.W. and de Bont, J.A.M. (1980) - "Thiobacillus acidophilus : a study of its presence in Thiobacillus ferrooxidans cultures" - Can. J. Microbiol. 26: 1057-65.

3. Atkins, A.S. (1978) - "Studies on the oxidation of sulphide minerals (pyrite) in the presence of bacteria" in "Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena" ed. by L.E.Murr, A.E. Torma and J.A.Brierley - Academic Press Inc. (London, New York, San Francisco).

4. Atkinson, B. and Mavituna, F. (1983) - "Biochemical Engineering and Biotechnology Handbook" - MacMillan Publishers Ltd. (Surrey) and The Nature Press (New York).

5. Bailey, J.E. and Ollis, D.F. (1977) - "Biochemical Engineering Fundamentals" - McGraw-Hill Book Co. (New York).

6. Balashova, V.V., Vedenina, I.Ya., Markosyan, G.E. and Zavarzin, G.A. (1974) - "The autotrophic growth of Leptospirillum ferrooxidans" - Mikrobiologiya 43(4): 581-5.

7. Beck, J.V. (1969) - "Thiobacillus ferrooxidans and its relation to the solubilization of ores of copper and iron" in "Fermentation Advances" ed. by D.Perlman - Academic Press Inc. (New York).

8. Bennett, J.C. and Tributsch, H. (1978) - "Bacterial leaching patterns on pyrite crystal surfaces" - J. Bact. 134: 310-7.

9. Berry, V.K. and Murr, L.E. (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" ed. by L.E.Murr, A.E. Torma and J.A.Brierley - Academic Press Inc.(London, New York, San Francisco).

10. Berry, V.K., Murr, L.E. and Hiskey, J.B. (1978) - "Galvanic interaction between chalcopyrite and pyrite during bacterial leaching of low-grade waste" - Hydromet. 3: 309-26.

11. Brierley, C.L. (1978) - "Bacterial leaching" - Crit.Rev. Microbiol. 6: 207-62.

12. Brierley, C.L. (1982) - "Microbiological mining" - Sci. Am. 247(2): 42-51.

13. Brierley, J.A. and Le Roux, N.W. (1977) - "A facultative thermophilic thiobacillus-like bacterium: oxidation of iron and

pyrite" in "Round Table Conference on Bacterial Leaching" ed. by W.Schwartz - Verlag Chemie (New York).

14. Bruynesteyn, A. (1981) - Personal communication.

15. Bruynesteyn, A. and Duncan, D.W. (1971) - "Microbiological leaching of sulphide concentrates" - Can. Met. Quat. 10(1): 57-63.

16. Bruynesteyn, A., Lawrence, R.W., Vizsolyi, A. and Hackl, R. (1983) - "An elemental sulphur producing biohydrometallurgical process for treating sulphide concentrates" in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

17. Bruynesteyn, A. and Viszolyi, A. (1981) - "The effect of pH and E_h on the chemical and biological leaching of a pyritic uranium ore" in "Second SME/SPE International Solution Mining Symposium" (Denver, Colorado).

18. Bruynesteyn, A. et al (1979) - "Bacterially-assisted ferric iron leaching of uranium ores" in "British Columbia Royal Commission of Enquiry into Uranium Mining" (British Columbia).

19. Buchanan, R.E. and Gibbons, N.E.(1974) - "Bergey's Manual of Determinative Bacteriology" 8th edition - The Williams and Wilkins Co. (Baltimore).

20. Burkin, A.R. (1967) - "Review of some recent developments in hydrometallurgy" in "Advances in Extractive Metallurgy" - Metallurgy Proceedings of a Symposium Organized by Institution of Mining (London).

21. Chang, Y.C. and Myerson, A.S. (1982) - "Growth models of the continuous bacterial leaching of iron pyrite by Thiobacillus ferrooxidans" - Biotech. Bioeng. 24: 889-902.

22. Corrans, I.J., Harris, B. and Ralph, B.J. (1972) - "Bacterial leaching: an introduction to its application and theory and a study on its mechanism of operation" - J.S.A. Inst. Min. Met. 72: 221-30.

23. Corrick, J.D. and Sutton, J.A. (1965) - "Copper extraction from a low-grade ore by Ferrobacillus ferrooxidans" - U.S.Dept. of Interior (Bureau of Mines) Report of Investigation 6714.

24. Dart, R.K. and Stretton, R.J. (1980) - "Microbiological Aspects of Pollution Control" - Elsevier Scientific Publishing Co. (Amsterdam, Oxford, New York) - pg 192-201.

25. Dean, A.C.R., Ellwood, D.C., Evans, C.G.T. and Melling, J. (editors) (1976) - "Continuous Culture 6: Applications and New Fields" - Ellis Horwood Ltd., Publisher (Sussex).

26. Derry, R. and Whittemore, R.G. (1983) - "Modelling the in-situ bioleaching of zinc-copper sulphide ore from Avoca, Ireland" in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

27. Dillon, V.S. (1955) - "Assay Practice on the Witwatersrand" - Cape Times Ltd. (Parow).

28. Duncan, D.W. (1967) - "Microbiological leaching of sulfide minerals" - Australian Mining (15 Nov): 21-5.

29. Duncan, D.W. and Drummond, A.D. (1973) - "Microbiological leaching of porphyry copper type mineralization : post-leaching observations" - Can. J. Earth Sc. 10(4): 476-84.

30. Duncan, D.W., Landesman, J. and Walden, C.C. (1967) - "Role of Thiobacillus ferrooxidans in the oxidation of sulfide minerals" - Can. J. Microbiol. 13: 397-403.

31. Duncan, D.W., Trussel, P.C. and Walden, C.C. (1964) - "Leaching of chalcopyrite with Thiobacillus ferrooxidans: effect of surfactants and shaking" - Appl. Microbiol. 12: 122-6.

32. Duncan, D.W., Walden, C.C. and Trussel, P.C. (1966) - "Biological leaching of mill products" - Trans. of the Joint Meeting of the British Columbia Section and Merritt Branch 69: 329-33.

33. Dutrizac, J.E. and MacDonald, R.J.C. (1974) - "Ferric iron as a leaching medium" - Minerals Sci. Eng. 6(2): 59-100.

34. Ehrlich, H.L. (1964) - "Bacterial oxidation of arsenopyrite and enargite" - Econ. Geol. 59: 1306-12.

35. Ehrlich, H.L. (1978) - "Inorganic energy sources for chemolithotrophic and mixotrophic bacteria" - Geomicrobiol. J. 1: 65-83.

36. Gaidarjiev, S., Groudev, S. and Genchev, F. (1975) - "Direct mechanism of bacterial oxidation of sulphide minerals" in "Proceedings of XI International Mineral Processing Congress" (Cagliari, Italy).

37. Golovacheva, R.S. (1979) - "Characteristics of Sulfobacillus thermosulfidooxidans morphogenesis" - Mikrobiologiya 48(5): 863-7.

38. Gormely, L.S. and Duncan, D.W. (1974) - "Estimation of Thiobacillus ferrooxidans concentrations" - Can. J. Microbiol. 20: 1453-63.

39. Groudev, S. (1979) - "Mechanism of bacterial oxidation of pyrite" - Mikrobiologiya 16(1): 75-87.

40. Groudev, S.N. (1980) - "Electrochemical oxidation system during the leaching of chalcopyrite by Thiobacillus ferrooxidans"

- Comptes Rendus de l'Acádemie Bulgare des Sciences 33 (6): 829-31.

41. Groudev, S.N. (1981) - "Oxidation of arsenopyrite by pure and mixed cultures of Thiobacillus ferrooxidans and Thiobacillus thiooxidans" - Comptes Rendus de l'Académie Bulgare des Sciences 34(8): 1139-42.

42. Groudev, S.N. (1982a) - "Microbiological leaching of metals from ores" - USSR/PRP/UNEP/CMEA Project Report.

43. Groudev, S.N. (1982b) - "Differences between strains of Thiobacillus ferrooxidans with respect to their ability to oxidize sulphide minerals" - paper presented at International Seminar "Modern Aspects of Microbiological Hydrometallurgy" (Moscow, June 21-25).

44. Groudev, S.N. (1983) - "Certain modern tendencies of microorganisms - applications in leaching mineral substrates" - Personal communication.

45. Groudev, S. and Genchev, F. (1978) - "Bacterial leaching of copper sulfide minerals with laboratory-bred strains" - Oktobarsko Savetovanja Rudara I Metalurga (Oct.): 344-53.

46. Harrison, A.P. Jr., Jarvis, B.W. and Johnson, J.L. (1980) - "Heterotrophic bacteria from cultures of autotrophic Thiobacillus ferrooxidans: relationships as studied by

means of deoxyribonucleic acid homology" - J. Bact. 143(2): 448-54.

47. Hiltunen, P., Vuorinen, A., Rehtijarvi, P. and Tuovinen, O.H. (1981) - "Bacterial pyrite oxidation : release of iron and scanning electron microscope observations" - Hydromet. 7: 147-57.

48. Ivarson, K.C., Ross, G.J. and Miles, N.M. (1979) - "The microbiological formation of basic ferric sulfates : II.Crystallization in presence of potassium-, ammonium-, and sodium-salts" - Soil Sci. Soc. Am. J. 43: 908-12.

49. Kandemir, H. (1983) - "Fate of sulphide moiety in bacterial oxidation of sulphide minerals : a quantitative approach" in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

50. Karavaiko, G.I., Kuznetsov, S.I. and Golonizik, A.I. (1977) - "The Bacterial Leaching of Metals from Ores" - Technicopy Ltd. (Stonehouse).

51. Karavaiko, G.I. and Pivovarova, T.A. (1977) - "Mechanism of oxidation of reduced sulphur compounds by thiobacilli" in "Round Table Conference on Bacterial Leaching" ed. by W.Schwartz - Verlag Chemie (New York).

52. Keller, L. and Murr, L.E. (1982) - "Acid-bacterial and ferric sulphate leaching of pyrite single crystals" - Biotech. Bioeng. 24: 83-96.

53. Kelly, D.P., Norris, P.R. and Brierley, C.L. (1979) - "Microbiological methods for the extraction and recovery of metals" - SGM 29: 263-308.

54. Khalid, A.M. and Ralph, B.J. (1977) - "The leaching behaviour of various zinc sulphide minerals with three Thiobacillus species" in "Round Table Conference on Bacterial Leaching" ed. by W.Schwartz - Verlag Chemie (New York).

55. Kulebakin, V.G. (1974) - "Bacterial leaching of arsenic from complex tin-containing products"- Akad. Nauk SSR, Sib. Otd. 27: 200-5.

56. Lawrence, R.W. and Bruynesteyn, A. (1983) - "Biological pre-oxidation to enhance gold and silver recovery from refractory pyritic ores and concentrates" - C.M.M. Bulletin 76: 107-10.

57. Lawrence, R.W., Bruynesteyn, A. and Hackl, R.P. (1981) - "Recent developments in the bioleaching of sulfide concentrates" - paper presented at "Twenty Eighth Congress of the International Union of Pure and Applied Chemistry" (Vancouver, Aug.).

58. Lawrence, R.W. and Gunn, J.D. (1985) - "Biological preoxidation of pyritic gold concentrate" - paper presented at "Session on Frontier Technology in Mineral Processing" - The 1985 A.I.M.E. Annual Meeting (New York, Feb. 24-28).

59. Le Roux, N.W., North, A.A. and Wilson, J.C. (1973) - "Bacterial oxidation of pyrite" in "X International Mineral Processing Congress" - Institution of Mining and Metallurgy (London).

60. Livesey-Goldblatt, E., Norman, P. and Livesey-Goldblatt, D.R. (1983) - "Gold recovery from arsenopyrite/pyrite ore by bacterial leaching and cyanidation" in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

61. Lloyd, P.J. (1967) - "The bacterial treatment of sulphide minerals" in "Symposium on the Chemistry of Extraction Metallurgy" - S.A.C.I. and N.I.M.

62. Lundgren, D.G. and Silver, M. (1980) - "Ore leaching by bacteria" - Ann. Rev. Microbiol. 34: 263-83.

63. Mackintosh, M.E. (1978) - "Nitrogen fixation by Thiobacillus ferrooxidans" - J. Gen. Microbiol. 105: 215-8.

64. Marchant, P.B. (1985) - "Continuous vat biooxidation of a refractory arsenical sulphide concentrate" - paper presented at "Seventeenth Canadian Mineral Processors Conference" (Ottawa, Jan. 22-24).

65. Marsh, R.M., Norris, P.R. and Le Roux, N.W. (1983) - "Growth and mineral oxidation studies with Sulfolobus " in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

66. McElroy, R.O. and Bruynesteyn, A. (1978) - "Continuous biological leaching of chalcopyrite concentrates : demonstration and economic analysis" in "Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena" ed. by L.E.Murr, A.E.Torma and J.A.Brierley - Academic Press Inc. (London, New York, San Francisco).

67. McGoran, C.J.M., Duncan, D.W. and Walden, C.C. (1969) - "Growth of Thiobacillus ferrooxidans on various substrates" - Can. J. Microbiol. 15: 135-8.

68. Mehta, A.P. and Murr, L.E. (1982) - "Kinetic study of sulfide leaching by galvanic interaction between chalcopyrite, pyrite and sphalerite in the presence of Thiobacillus ferrooxidans (30°C) and a thermophilic microorganism (55°C)" - Biotech. Bioeng. 24: 919-40.

69. Murr, L.E. and Berry, V.K. (1976) - "Direct observations of selective attachment of bacteria on low-grade sulfide ores and other mineral surfaces" - Hydromet. 2: 11-24.

70. Murr, L.E. and Brierley, J.A. (1978) - "The use of large scale test facilities in studies of the role of microorganisms in commercial leaching operations" in "Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena" ed. by L.E.Murr, A.E.Torma and J.A.Brierley - Academic Press Inc. (London, New York, San Francisco).

71. Myerson, A.S. and Kline, P.C. (1984) - "Continuous bacterial coal desulfurization employing Thiobacillus ferrooxidans" - Biotech. Bioeng. 26: 92-9.

72. Napier, E., Wood, R.G. and Chambers, L.A. (1967) - "Bacterial oxidation of pyrite and production of solutions for ore leaching" in "Advances in Extractive Metallurgy" - Metallurgy Proceedings of a Symposium Organized by Institution of Mining (London).

73. Natarajan, K.A. and Iwasaki, I. (1983) - "Role of galvanic interactions in the bioleaching of Duluth gabbro copper-nickel sulfides" - Sep. Sc. Technol. 18 (12 and 13): 1095-111.

74. Natarajan, K.A., Iwasaki, I. and Reid, K.J. (1983) - "Some aspects of microbe-mineral interactions of interest to Duluth gabbro copper-nickel sulfides" in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

75. Norris, P.R. (1983) - "Iron and mineral oxidation with Leptospirillum-like bacteria" in "Recent Progress in Biohydrometallurgy" ed. by G.Rossi and A.E.Torma - Associazione Mineraria Sarda (Iglesias).

76. Norris, P.R. and Kelly, D.P. (1978) - "Dissolution of pyrite (FeS_2) by pure and mixed cultures of some acidophilic bacteria" - FEMS Microbiol. Lett. 4: 143-6.

77. Pinches, A. (1975) - "Bacterial leaching of an arsenic-bearing sulphide concentrate" in "Leaching and Reduction in Hydrometallurgy" ed. by A.R.Burkin - Institute of Mining and Metallurgy (London).

78. Pivovarova, T.A., Markosyan, G.E. and Karavaiko, G.I. (1981) - "Morphogenesis and fine structure of Leptospirillum ferrooxidans" - Mikrobiologiya 50(3): 482-6.

79. Polkin, S.I., Adamov, E.V., Kazintseva, S.I. and Grishin, S.I. (1977) - "Bacterial leaching of stubborn copper-zinc concentrates" - Sov. Non-Ferr. Met. Res. 5(1): 1-2.

80. Polkin, S.I., Panin, V.V., Adamov, E.V., Karavaiko, G.I. and Chernyak, A.S. (1975) - "Theory and practice of utilizing microorganisms in processing difficult-to-dress ores and concentrates" in "Proceedings of XI International Mineral Processing Congress" (Caglari, Italy).

81. Qiu, R., Wu, Y., Chen, X., Li, Y., Xue, H., Lin, C., Su. S., Tu, W. and Ji, H. (1980) - "Bacterial leaching of cobalt from arsenic-containing sulfide ores" - Acta Microbiol. Sin. 20(3): 303-9.

82. Rawlings, D.E. (1981) - "Nutritional requirements of the microorganisms active in the oxidation of ferrous iron to ferric iron in acid mine leach liquors" - J. Appl. Bact. 51: 267-75.

83. Razzell, W.E. and Trussel, P.C. (1963) - "Isolation and properties of an iron-oxidizing Thiobacillus" - J. Bact. 85: 595-603.

84. Roy, P. and Mishra, A.K. (1981) - "Factors affecting oxidation of pyrite by Thiobacillus ferrooxidans" - Indian J. Exp. Biol. 19: 728-32.

85. Sakaguchi, H., Silver, M. and Torma, A.E. (1976) - "Microbiological leaching of a chalcopyrite concentrate by Thiobacillus ferrooxidans" - Biotech. Bioeng. 18: 1091-101.

86. Schnaitman, C.A., Korcsynski, M.S. and Lundgren, D.G. (1969) - "Kinetic studies of iron oxidation by whole cells of Ferrobacillus ferrooxidans" - J. Bact. 99: 552-7.

87. Silver, M. (1978) - "Metabolic mechanisms of iron-oxidizing thiobacilli" in "Metallurgical Applications of Bacterial

Leaching and Related Microbiological Phenomena" ed. by L.E.Murr, A.E.Torma and J.A.Brierley - Academic Press Inc. (London, New York, San Francisco).

88. Silverman, M.P. (1967) - "Mechanism of bacterial pyrite oxidation" - J. Bact. 94(4): 1046-51.

89. Southwood, M.J. and Southwood, A.J. (1985) - "Mineralogical observations on the bacterial leaching of auriferous pyrite : a new mathematical model and implications for the release of gold" - paper presented at "International Symposium on Biohydrometallurgy" (Vancouver, Aug.)

90. Torma, A.E. (1977) - "The role of Thiobacillus ferrooxidans in hydrometallurgical processes" - Adv. Biochem. Eng. 6: 1-37.

91. Torma, A.E. (1978) - "Complex lead sulfide concentrate leaching by microorganisms" in "Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena" ed. by L.E. Murr, A.E. Torma and J.A. Brierley - Academic Press Inc. (London, New York, San Francisco).

92. Torma, A.E. and Bosecker, K. (1982) - "Bacterial leaching" - Progress in Ind. Microbiol. 16: 77-118.

93. Torma, A.E., Gabra, G.G., Guay, R. and Silver, M. (1976) - "Effects of surface active agents on the oxidation of chalcopyrite by Thiobacillus ferrooxidans" - Hydromet. 1: 301-9.

94. Torma, A.E., Legault, G., Kougioumoutzakis, D. and Ouellet, R. (1974) - "Kinetics of bio-oxidation of metal sulphides" - Can. J. Chem. Eng. 52: 515-7.

95. Torma, A.E. and Sakaguchi, H. (1978) - "Relation between the solubility product and the rate of metal sulfide oxidation by Thiobacillus ferrooxidans" - J. Ferment. Technol. 56(3): 173-8.

96. Torma, A.E., Walden, C.C. and Branion, R.M.R. (1970) - "Microbiological leaching of a zinc sulfide concentrate" - Biotech. Bioeng. 12: 501-17.

97. Tributsch, H. (1976) - "The oxidative desintegration of sulfide crystals by Thiobacillus ferrooxidans"-Naturwissenschaften 63: 88.

98. Tuovinen, O.H. and Kelly, D.P. (1974) - "Use of micro-organisms for the recovery of metals" - Int. Met. Reviews 19: 21-31.

99. Wakao, N., Mishina, M., Sakurai, Y. and Shiota, H. (1982) - "Bacterial pyrite oxidation I. The effect of pure and mixed cultures of Thiobacillus ferrooxidans and Thiobacillus thiooxidans on release of iron" - J. Gen. Appl. Microbiol. 28: 331-43.

100. Wakao, N., Mishina, M., Sakurai, Y. and Shiota, H. (1983) - "Bacterial pyrite oxidation II. The effect of various

organic substances on release of iron from pyrite by Thiobacillus ferrooxidans" - J. Gen. Appl. Microbiol. 29: 177-85.

101. Yudina, I.N., Kostyukova, O.N., Gorenkov, N.L., Panin, V.V. and Kim, D. Kh. (1979) - "Results of the bacterial leaching on a scale-up continuous apparatus (vat process)" - Tsvetn. Met. 8: 109-11.