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Thermophilic bioleaching of arsenopyrite using *Sulfolobus* and a semi-continuous laboratory procedure

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SUMMARY

A laboratory equipment for pumping slurries is described. The pumping is performed semi-continuously by using a plastic syringe, a set of different valves, and a programmable electronic unit. The reproducibility of the pumping is demonstrated. Bioleaching of a gold-containing arsenopyrite slurry was done with *Sulfolobus* at 70°C using the semi-continuous procedure and with a retention time of 100 h for the mineral. Arsenic was completely released at a rate of 109 mg l^{-1} h^{-1}. The gold recovery is related to the amount of iron and arsenic dissolved and is shown to have a correlation factor of approximately one relative to the release of arsenic.

INTRODUCTION

Industrial, as well as pilot plant processes, are usually run continuously, and it would therefore be desirable to employ continuous procedures even at the laboratory scale in order to study different parameters of a process under steady state conditions.

Continuous processes are well established for bacterial growth using particle free nutrient media. In these processes the continuous flow of liquid is obtained with peristaltic pumps using flexible rubber or silicon tubings. However, when the growth medium contains particles of various sizes, e.g. in bioleaching experiments with ground minerals, there are some practical problems with the pumping. Due to the normally slow flow rates, the minerals have a tendency to settle in the tubings and thereby reduce the flow rate. Also, high wear of the tubings affects the reproducibility of the mineral feeding rates.

Through the periodical addition of mineral suspensions to the reactor these practical problems can

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be avoided. Either a manual procedure or an automatic device may be used. The feeding of the mineral suspension can, e.g. be performed with a peristaltic pump operated at a fast pumping rate for a few seconds each hour and controlled by a timer [5]. The leachate is usually removed from the reactor through overflow into the collecting tank. The overflow technique may also be used between the leaching vessels in multi-stage reactors [2].

Most published work on bioleaching of sulphide ores use the mesophilic bacterium *Thiobacillus ferrooxidans*. However, thermophilic bacteria have received increased attention in recent years, because with these bacteria the bioleaching process could be faster due to the elevated growth temperature. In our leaching experiments with a concentrate of arsenopyrite (FeAsS), we have used *Sulfolobus* at 70°C. We have found (not published) that, in batch culture of *Sulfolobus*, the leaching rate is reduced above 1.5% (w/v) in pulp density of this concentrate, due probably to the toxic level of dissolved arsenic. Therefore, the soluble arsenic concentration must be controlled during the bioleaching of arsenopyrite.

We describe here an automatic semi-continuous procedure, which minimizes these problems. Preliminary results of leaching a gold-containing concentrate of arsenopyrite with this equipment, using *Sulfolobus* at 70°C, are also presented. A short communication of this paper has been published earlier [7].

MATERIAL AND METHODS

Organism and growth conditions

Sulfolobus acidocaldarius 'strain BC' [9] was kindly obtained from Dr. P.R. Norris. Its optimum growth temperature is about 70°C. In all leaching experiments the mineral salts medium 9K (without ferrous iron) of Silverman and Lundgren [10] was used. The *Sulfolobus* cultures used as inoculum was kept on sterilized mineral suspension, 1.5% (w/v) pulp density, at 65°C in 250-ml Erlenmeyer flasks in a Mark X incubator shaker (LH Fermentation Ltd, U.K.). Usually 3–5% (v/v) inoculum of a 4–5 days old culture was used to start the leaching experiments, which normally were performed with unsterilized mineral suspensions.

Mineral

An arsenopyrite flotation concentrate, Olympus, Greece, obtained from Boliden Mineral AB, Boliden, Sweden, with the following main composition was used: Au 27 g/ton, Ag 28 g/ton, Fe 38.7% (w/w), S 40.5% (w/w), As 11.3% (w/w), Zn 0.74% (w/w) and Cu 0.13% (w/w). The main minerals were pyrite (FeS_2) and arsenopyrite (FeAsS). Assuming that all arsenic is in arsenopyrite the estimated composition was 66% (w/w) pyrite and 24.5% (w/w) arsenopyrite. The mineral particles were less than 0.5 mm. The size distribution is shown in Table 1.

Batch leaching for gold recovery analysis

Bioleaching of 800 ml unsterilized mineral suspensions, 1.5% (w/v) pulp density, was performed in 2-l Erlenmeyer flasks, shaken at 150 rpm in the same incubator as above at 65°C. Humidified air enriched with 0.5% (v/v) CO_2 was introduced into each culture via a glass wool filter and a glass tube fitted to the cap of the flasks. The amount of iron and arsenic dissolved during leaching was determined by sacrificial sampling. The flasks were weighed before the daily sampling and distilled water was added to compensate for evaporation. The flasks were harvested periodically, one at a time,

Table 1

Size distribution of the minerals in the slurry

Particle size (PS) (μm)	Weight fraction (%)
PS < 74	34.6
74 ≤ PS < 125	20.9
125 ≤ PS < 250	29.7
250 ≤ PS < 500	14.8
	Sum 100.0

The dry mineral was sieved for 0.5 h and the different fractions were weighed and then carefully mixed again before used in the slurry.

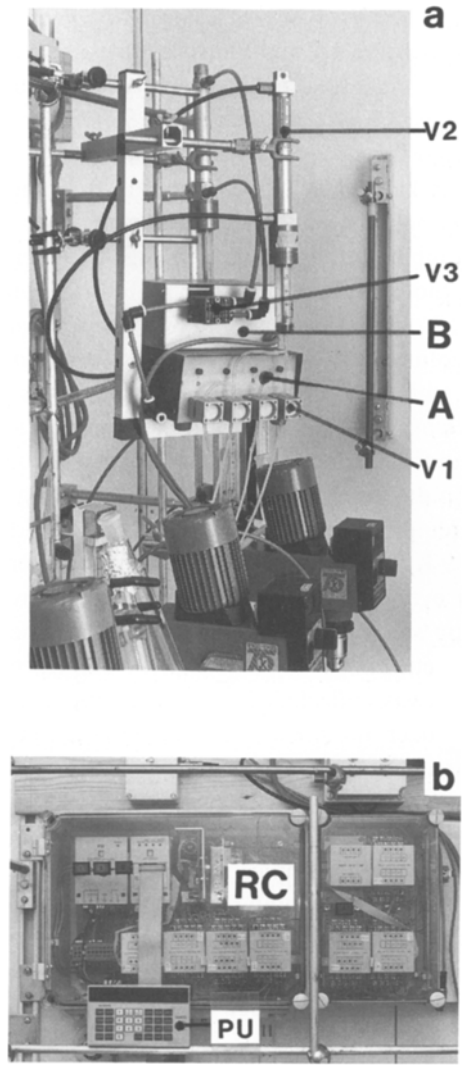

Fig. 1. Equipment for semi-continuous pumping. (a) The pumping unit. Two identical sets, A and B, of valves V1 are mounted on top of each other, facing opposite directions. Valves V1 and V2 belong to system A and valve V3 to system B. (b) Regulatory unit, RC, has 3 × 8 outputs and 16 inputs. Not all were used. PU is the programming unit.

and the leaching residue was collected on a Whatman glass filter GF/A, washed first with 5 M HCl, and then with distilled water. The mineral residue was subsequently treated with cyanide to extract the gold.

Semi-continuous leaching

Semi-continuous pumping was obtained using two valve systems (Figs. 1 and 2A, B). Each system was equipped with four valves V1 (Micro solenoid pinch valves, type T120-C02, 24V DC, from SIRAI, Italy) and a syringe (20 ml from Millipore), which was operated by a pneumatic valve, V2 (Atlas Copco, Sweden, type C1-20-8-100-ES) and a magnetic 4-way valve, V3 (type 420-G-SL 6/4) from Burkert, F.R.G.). The syringe was fitted to valve V2 through a metal block made of brass and the rubber part of

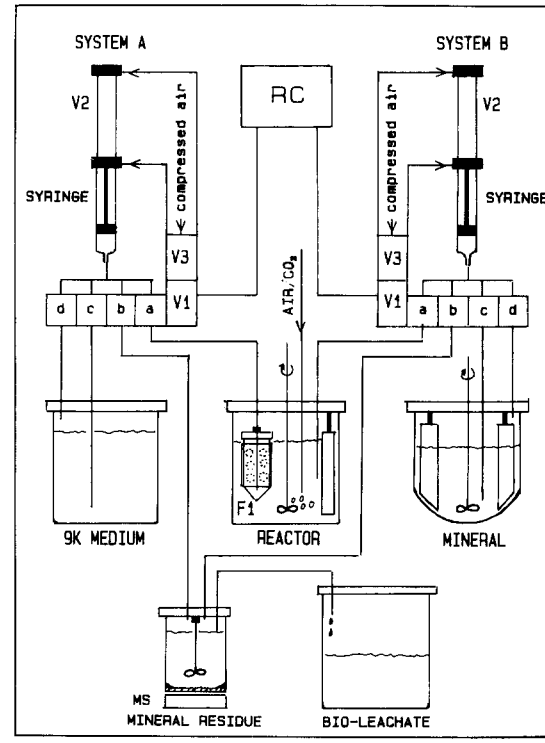

Fig. 2. Diagram of the semi-continuous process. The process was controlled by the regulatory unit, RC. In system A the toxic leachate was removed from the reactor through the filter F1 and replenished with iron-free 9 K medium. In system B the bio-leached mineral was removed from the reactor and collected in the mineral residue tank. Fresh mineral suspension was then transferred to the reactor tank. The two systems A and B operate independently of each other. Both the toxic leachate and most of the precipitates overflowed from the mineral residue tank into the bio-leachate tank. All reservoir vessels were kept at ambient temperature. Abbreviations: MS, magnetic stirrer; V1a-d, four micro-solenoid pinch valves; V2, pneumatic valve; V3, magnetic 4-way valve regulating valve V2 and the syringe.

the plunger of the syringe was fitted to the piston of the valve V2. Silicon tubing, about 10 cm long and with a 4 mm outer diameter and 2 mm inner diameter, was used through these valves to the syringe. The tubings connected to the vessels were of polyethene with an outer diameter of 3.2 mm and an inner diameter of 2.0 mm. The process was controlled by a regulatory unit, RC, (Fig. 1b) built in our electronic workshop, using Satellite Mini V12 Programmable System from Sharp, Japan.

Ordinary two liter polycarbonate anaerobic jars, BBL 60463 (Becton Dickinson and Co, Cockeysville), were used as leaching vessels in the semi-continuous process. The different outlets/inlets are through plastic (polyamide) tubings, 6 mm in outer diameter, and were sealed by O-rings fitted into polycarbonate plastic plugs. The motor driven teflon stirrer was sealed with an oil-seal in the same type of plug. The jar was equipped with two baffles made of polycarbonate to make the stirring more efficient.

Air, enriched with 0.5% (v/v) carbon dioxide was passed through glass wool and introduced at about 300 ml/min. Toxic leach medium was removed through a special filter (F1) which retained most of the mineral residues within the reactor. The polyacrylnitrile textile, DN 0051 of this filter was purchased from Svenska Textilfilter AB, Kinna, Sweden. The estimated pore size is 40 μm. The filter F1 was constructed from an ordinary 50 ml Falcon graduated conical tube. Several holes were drilled around the wall and the textile was wrapped around the tube in a double layer and kept in place with plastic bands. The outlet tubing was fixed to the cap. Evaporation was controlled by tap water cooling of the exhaust air.

The mineral tank used was a 10 liter round bottom glass vessel, equipped with two baffles, containing 10% (w/v) mineral suspension in demineralized water. Stirring was done at 400 rpm using a teflon stirrer.

The heating system consists of an insulated water bath with a heater from HETO, type 01 PF623, (1200 W).

Leaching of the FeAsS-concentrate was started batch-wise in 1.5% (w/v) pulp density in 1.5 liters of mineral medium 9 K [10], using *Sulfolobus* at 70°C. The stirring speed was 100 rpm during the first 3–4 h after inoculation and then increased to 300 rpm. This speed was not harmful to *Sulfolobus* at this pulp density.

After batch leaching for about 70 h, the semi-continuous process was started using system A of the equipment diagrammed in Fig. 2 for removing toxic bioleachate and system B for removing the leached mineral residue and adding fresh mineral. The volume was adjusted to ca. 15 ml in both systems and each cycle, by choosing tubings of the right length. A 10% (w/v) mineral suspension was used in the feeding tank and system B was run every 60 min during the semi-continuous leaching phase. The removal of toxic bioleachate and adding of fresh 9 K medium was programmed in system A to occur every 15 min. The liquid removed from the reactor was first collected in a vessel equipped with a magnetic stirrer. In this vessel most of the mineral residue was collected, but most of the precipitate flowed over into the collection tank, due to the gentle stirring.

Sample treatment and analysis

Usually 2 ml samples were withdrawn from the carefully mixed leaching cultures using a piece of tubing attached to a plastic syringe. After cooling to room temperature, the pH and redox potential were measured. A well blended sample of 300 μl was diluted with 1200 μl 5M HCl and digested at 70°C for 30 min in an Eppendorf centrifuge tube to dissolve any precipitates formed during leaching. After cooling to room temperature and centrifugation in an Eppendorf centrifuge for 3 min, the supernatant was diluted in 1% (w/v) HCl and analyzed for the metal content.

The acid and water washed mineral residues were resuspended in 40 ml distilled water and the pH was adjusted to about 11 by adding 5 M NaOH. Distilled water was then added to a final volume of 50 ml. The suspension was stirred on a magnetic stirrer at 600 rpm and aerated at 300 ml air/min, for 2 h. The pH was checked before KCN was added to 0.1 M. Cyanidation was performed for 48 h at room temperature. Then the cyanide liquid was collected af-

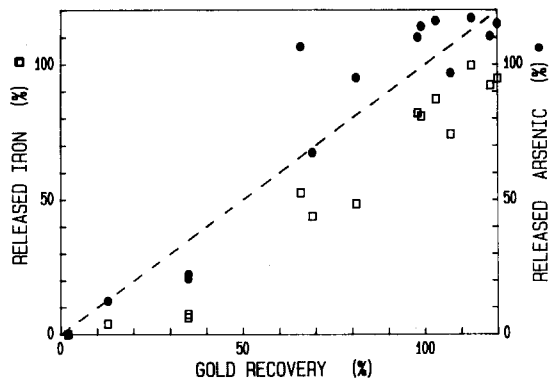

Fig. 3. Gold recovery from bio-leached mineral. Gold was extracted by cyanidation of the mineral residue after bioleaching for different time intervals. The recovery of gold, % (w/w), was plotted against the total amount of released iron, %-w/w (□), and of released arsenic, %-w/w (●). The dashed line represents the 1:1 ratio of gold to iron and arsenic.

Table 2

Pumping of a 10% (w/v) mineral slurry

Sample (no.)	Collected volume (ml)	Mineral (g dry weight)	(% of expected)
1	765	72.3	94.5
2	480	48.6	101.2
3	510	51.7	101.3
4	670	69.9	104.3
5	610	58.9	96.6

The slurry, 10% (w/v) in demineralized water, was stirred at 400 rpm and the slurry was collected with the syringe during various time intervals and transferred to a volumetric flask. The dry weights of the mineral in these samples were compared to the amounts expected from the same volume of a 10% (w/v) slurry.

ter centrifugation at 11 000 × *g* for 15 min and analyzed for the gold content.

Total iron, arsenic and gold were measured by flame atomic spectrophotometry (Varian, AA 875) using acetylene plus air for iron and gold, and acetylene plus nitrous oxide (N_2O) for arsenic.

RESULTS

Gold recovery after batch leaching at 70°C

In order to determine the degree of bioleaching needed for quantitative gold recovery, different extents of batch bioleaching of arsenopyrite were done with *Sulfolobus*. The gold was then extracted from the residue by cyanidation. In Fig. 3 the amount of extracted gold is plotted versus the total amounts of both bioleached arsenic and bioleached iron. As can be seen, the recovery of gold is closely correlated to a 1:1 ratio of solubilized arsenic.

Reproducibility of the pumping system

The reproducibility of pumping a 10% (w/v) mineral slurry with this pumping facility was determined. Suspensions from the mineral tank, run according to Material and Methods, were removed by system B (Fig. 2), collected during different time intervals in a volumetric glass, and the volume was measured. The mineral was then collected on a Whatman glass filter, GF/A, dried, and weighed. The results are shown in Table 2. The resulting amounts of mineral transferred agree very well with the amounts expected for the volumes collected.

The reproducibility of the volume removed per cycle with the syringe was also determined. Fifteen consecutive samples were collected in pre-weighed tubes and the weight of the sample was determined. The results show a mean value of 16 ± 0.18 g.

The semi-continuous process

This semi-continuous procedure has been used here with only one leaching vessel. The results presented in Fig. 4 show that during the batch phase of this experiment the amount of arsenic released reached a plateau value of 10 mM after 24 h of leaching, while the level of released iron continued to increase to 60 mM during this phase. The level of solubilized arsenic stayed rather high and constant at 30 mM, during the semi-continuous phase. The iron concentration, however, gradually increased up to 95 mM during the first 5–6 days of the semi-continuous phase. This is probably due to the formation of jarosite, which can not penetrate through the filter and therefore accumulates in the reactor

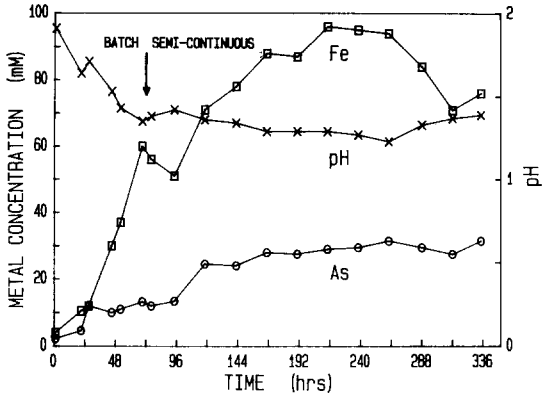

Fig. 4. Solubilized metals in the reactor tank. Samples were withdrawn at the indicated times and treated as described in Materials and Methods. The total amounts of solubilized iron (□), and solubilized arsenic (○), and the pH (×) in the reactor tank during the semi-continuous experiment are plotted against time (hrs). The arrow indicates the end of the batch leaching period and the start of the semi-continuous leaching process at 76 h.

Table 3

Semi-continuous leaching rates of arsenopyrite with *Sulfolobus*

Time (h)	Total leaching rates ($mg\ l^{-1}$) h^{-1}	
	Fe	As
94	76	50
118	272	92
142	254	96
166	278	121
190	240	101
214	275	113
238	253	111
262	265	121
288	249	111
312	204	102
334	238	118
Mean during 94–334 h	253	109

The semi-continuous process was started at 76 h and the bioleachates were collected between each indicated time. The collected volumes were measured and a sample was withdrawn as described in Materials and Methods. The amounts of total released iron and arsenic were estimated for the different time intervals. The feeding rates for iron and arsenic were calculated to be 387 and 113 $mg\ l^{-1}\ h^{-1}$, respectively.

tank. The treatment of the samples involves dissolving any precipitate formed. However, we have not analyzed for jarosite or other precipitates in the leaching vessel.

During the initial batch leaching step the pH decreased from 2.0 to 1.3, but stabilized during the semi-continuous phase at this level. The redox potential was also monitored during leaching (not shown here), using a standard silver chloride electrode. The redox value increased from 475 to about 600 mV during batch leaching and decreased gradually at the beginning of the semi-continuous leaching, stabilizing at 510 mV. This decrease is probably due to a change in the Fe^{3+}/Fe^{2+} ratio due to chemical leaching of the added mineral, which both releases Fe^{2+} and reduces Fe^{3+} to Fe^{2+}.

The leaching rates were calculated from the amount of the bioleachate collected daily. The rates were corrected for changes of the concentrations in the reactor tank and in the mineral-residue collecting tank at the time of sampling. The results are presented in Table 3. The leaching rates for both arsenic and iron were fairly constant during the semi-continuous phase, with the mean arsenic leaching rate being 109 $mg \cdot l^{-1}\ h^{-1}$ from 94 h and onwards. For iron the estimated mean leaching rate was 253 $mg \cdot l^{-1}\ h^{-1}$. This is equivalent to approximate 1.5 mmol arsenopyrite and 3 mmol of pyrite dissolved per liter and hour.

During the semi-continuous phase five consecutive samples were collected from the reactor tank using system B (Fig. 2). The samples were diluted 4-fold with 5 M HCl and digested for 30 min at 70°C. The mineral residue was then collected on a Whatman GF/A glass filter, washed with distilled water, and dried. The mean value of the mineral residue in these samples was 0.85 g ± 0.02, which corresponds to a pulp density of about 5% (w/v) in the reactor tank.

DISCUSSION

Pumping slurries of, for example, minerals with low flow-rates is always problematic. Here, we have

used a semi-continuous procedure to minimize these problems. By using a syringe operated with a pneumatic valve, the filling and emptying each cycle can be adjusted to a flow-rate of the mineral suspension at which the settling of the mineral particles is of little significance. The speed of the piston of valve V2 is regulated by the compressed air flow through valve V3. However, due to fatigue of the rubber plunger, the plastic syringe must be change after approximatly 200 cycles. In the system diagrammed in Fig. 2 we have used 4 valves, VI-a to V1-d. Of course more valves can easily be added to the system if needed.

We have found that the programmable system used here in the RC-unit is very flexible, handy, and reliable. The time schedule for the cycles can easily be changed during the operation. However, the operating system must be turned off, when reprogramming is necessary. But normally, reprogramming takes only a few minutes.

Our preliminary batch bioleaching studies of the arsenopyrite flotation concentrate have shown that the leaching rate decreased above 1.5% (w/v) for this flotation concentrate. We interpreted this to be due to the toxic level of arsenate. During batch-leaching with *Sulfolobus* at 70°C, about 80% of the released arsenic remained in solution as arsenate, which corresponds to about 18 mM arsenate. We have used ion chromatography [6] for the speciation of soluble arsenic in our leachate and only found arsenate towards the end of the leaching process.

In our gold recovery studies using batch cultures with this flotation concentrate of arsenopyrite we have used 1.5% (w/v) pulp density to avoid the toxic level of arsenate. Without any pretreatment of the concentrate with bacteria there is very little recovery; about 2% (w/w) of the gold was obtained from this mineral by the cyanidation process. We have in our cyanidation experiments used 0.1 M KCN, which is equivalent to about 30 mg KCN/g mineral; in other words, well in excess for the amount of mineral or mineral residue usually used.

The 1:1 correlation to the leaching of arsenic and gold suggests that the gold is locked within the arsenopyrite particles. In most of our batch cultures of this mineral we have also seen that arsenic and iron are released at the same rate initially, but that arsenic reaches a maximum level before iron (Fig. 4, batch phase). This suggests that in a mixture with pyrite, the mineral arsenopyrite is preferably leached by the bacteria. This has also been reported for other types of such minerals [1,4,8].

We have used the overall metal content of the mineral (see Material and Methods) in our calculations and it is obvious, since we have recoveries greater than 100%, that the arsenic and gold content varies between different mineral batches.

In the semi-continuous experiment shown here the amount of mineral fed to the leaching reactor was 1500 mg per hour during the semi-continuous leaching phase, which is equivalent to an input as estimated from the given composition of the mineral (see Material and Methods) of 387 mg Fe and 113 mg As per hour per liter of leaching media. Hence, the leaching rates of arsenic shown in Table 3 are very close to the estimated maximal rates. For iron only 65–70% of maximum is reached. This means that asenic leaching rates, and not those for iron, should be used in optimization studies with this type of mineral.

The almost constant and maximal estimated levels of the arsenic leaching rates (Table 3) indicates that the feeding rate of the mineral could be increased further. This, however, may create problems with the soluble arsenate concentration in the leaching reactor, which is kept close to the toxic level by removal of leachate through the filter F1 with the valve system A. The capacity of this filter is therefore critical. During leaching, we have noticed that precipitates form and attach to the filter and render it less permeable to the toxic leachate. However, daily rinsing of this filter by blowing compressed air through the pores of the textile into the leaching reactor have kept the capacity sufficiently high.

In this work we have used only one leaching reactor with the retention time for the mineral of 100 h. With the equipment used here it is very easy to add one or two more reactors in series in order to minimize the short circuiting of the mineral. Recycling of the biomass to the first reactor can also be included, to avoid washing out of the bacteria.

With this programmable system of valves we have been able to reproducibly pump slurries of minerals in a semi-continuous manner at a fairly low overall flow rate suitable for laboratory work. With our semi-continuous process we have at the laboratory level succeeded in leaching gold containing arsenopyrite at 70°C with *Sulfolobus acidocaldarius* 'strain BC' at promising leaching rates and with a procedure approaching a continuous one. We have therefore started comparison leaching studies of mesophilic and thermophilic bacteria with this equipment using two leaching reactors in series and with recycling of the biomass in order to decrease the retention time and to further improve the leaching rates.

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REFERENCES

1 Bruynesteyn, A., R.P. Hackl and F. Wright. 1986. The Biotankleach Process. Gold 100. Proceedings of the International Conference on Gold. Volume 2: Extractive metallurgy of Gold. pp. 353–365, South African Institute of Mining and Metallurgy, Johannesburg.

2 Groudev, S.N. 1986. Continuous bacterial leaching of copper sulphide concentrates. In: Fundamental and Applied Biohydrometallurgy. (Lawrence, R.W., Branion, R.M.R. and Ebner, H.G., eds.), pp. 43–50, Elsevier, Amsterdam.

3 Instruction manual of the Satellite Mini V12 Programmable System, Sharp, Japan.

4 Lazer, M.J., M.J. Southwood and A.J. Southwood. 1986. The release of refractory gold from sulphide minerals during bacterial leaching. Gold 100. Proceedings of the International Conference on Gold. Volume 2: Extractive metallurgy of Gold. pp. 287–297, South African Institute of Mining and Metallurgy, Johannesburg.

5 Le Roux, N.W. and D.S. Wakerley. 1988. Leaching of chalcopyrite ($CuFeS_2$) at 70°C using *Sulfolobus*. in: Biohydrometallurgy. (Norris, P.R. and Kelly, D.P., eds.), pp. 305–317, Science and Technology Letters, Kew Surrey, U.K.

6 Liang, K.T. and J.E. Dutrizac. 1985. Determination of Arsenic(III) and Arsenic(V) in Ferric Chloride-Hydrochloric Acid Leaching Media by Ion Chromatography. Anal. Chem. 57: 2615–2620.

7 Lindström, E.B. and L. Gunneriusson. 1988. Semi-continuous leaching of Arsenopyrite with a *Sulfolobus* strain at 70°C. In: Biohydrometallurgy (Norris, P.R. and Kelly, D.P., eds.), pp. 525–527, Science and Technology Letters, Kew Surrey, U.K.

8 Marchant, P.B. 1986. Commercial Piloting and the Economic Feasibility of Plant Scale Continuous Biological Tank Leaching at Equity Silver Mines Limited. In: Fundamental and Applied Biohydrometallurgy (Lawrence, R.W., Branion, R.M.R. and Ebner, H.G., eds.), pp. 53–76, Elsevier, Amsterdam.

9 Norris, P.R. and L. Parrot. 1986. High temperature, mineral concentrate dissolution with Sulfolobus. In: Fundamental and Applied Biohydrometallurgy (Lawrence, R.W., Branion, R.M.R. and Ebner, H.G., eds.), pp. 355–365, Elsevier, Amsterdam.

10 Silverman, M.P. and D.G. Lundgren. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high yields. J. Bacteriol. 77: 642–647.