CHAPTER 1

Bioleaching Applications
A novel bio-leaching process to recover valuable metals from Indian Ocean nodules using a marine isolate

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Abstract
A novel bio-leaching process to recover valuable nonferrous metals from Indian Ocean nodules at near neutral pH and ambient temperature is presented in this paper. Poly-metallic manganese nodules contain a lucrative resource for valuable strategic metals like Cu, Co and Ni. In view of rapid depletion of land-based resources of copper and non-availability of significant resources of Ni and Co in India, these nodules have an enormous potential to effect future metal extraction trends in the country. The significant role of indigenous microorganisms in solubilization of valuable trace metals from the nodules was studied in detail.

A marine organism was isolated from the nodules. The isolate was shown to be a Gram-positive heterotroph of \textit{Bacillus} species. It was grown in artificial seawater medium. The growth studies of the isolate with respect to pH, temperature and salinity of the medium proved that, the isolate grows well at neutral pH, 30°C temperature and 0.25M NaCl concentrations. A growing culture of the isolate as well as cell-free spent liquor containing metabolites was employed to recover transition metals from the nodules. A few chemical leaching experiments in moderate acidic environment were also conducted to generate baseline data.

The bioleaching of the nodules was carried out in two different ways (a) leaching during the growth of the isolate and (b) leaching by spent liquor after removal of fully-grown cells. The leaching efficiency was observed to be more or less the same in both the cases confirming that metabolites produced during growth of the microorganism, played a pivotal role in the leaching process. Around 50% cobalt and 30% of the Cu and Ni came out in the leach liquor at pH of 8.2 in a four-hour interval, indicating that the effect of metabolites was specific to cobalt recovery in comparison to copper and nickel. Kinetic studies revealed that the metal recovery stabilized after four hours. The results for cobalt were quite comparable to those achieved in highly acidic conditions. A strong reducing environment is required to break down the MnO\textsubscript{2} and/or Fe-oxide matrix encapsulating the remaining part of the transition metals. So, the effect of adding a reductant like starch in spent liquor of the isolate was investigated. Starch enhanced the recovery of all the transition metals to about 80%-85%. The effect of pulp density and pH of the leach liquor on the bio-leaching process was investigated. The recovery was observed to be almost independent of size fraction of nodules over a wide range.
1. INTRODUCTION

In view of continuous depletion of land-based resources along with increasing consumption of valuable metals in India, development of environment friendly technologies for tapping alternate sources of metals has gained importance lately. One of them is recovery of strategic metals Cu, Ni and Co from polymetallic Indian Ocean nodules, by biological processing. Fuerstenau and Han (1983) extensively reviewed processing and extraction of valuable metals from manganese nodules discussing both hydro and pyrometallurgical routes. High porosity of the nodules resulting in high moisture content coupled with polluting effluent gases pose major hurdles in pyrometallurgical processing of the nodules. Therefore, hydrometallurgical techniques are potentially viable for extraction of metals from nodules. But often, slow kinetics and poor recovery with dilute acids and corrosiveness of the concentrated ones restrict application of hydrometallurgical extraction. Researchers have studied addition of reducing agents with either mineral acids or ammonia. The reducing environment, so created, enhances leaching, by breaking up the nodule matrix occluding the valuable metals (Niinae et al 1996; Kanungo et al 1988; Jana et al 1999; Trifoni et al 2001; Zhang et al 2001). These processes have varying degrees of success. However, most require high temperature pretreatment and/or costly, corrosive reagents to obtain a sizeable amount of metal recovery with favourable kinetics. As the nodules are low-grade ores of Cu, Co and Ni, use of costly chemical reagents as reducing agents may not be economically viable for large-scale runs.

Ehrlich and his co-workers have studied the biogenesis and microbiology associated with Atlantic Ocean nodules extensively over the last four decades. Ehrlich (1963) isolated and characterized the Mn-reducing organism Bacillus 29 from the Atlantic Ocean nodules. Growing cultures of Bacillus 29 are able to reduce MnO₂ aerobically and anaerobically using glucose as an electron donor. However, microbial ecology of the Indian Ocean nodules has not been studied in detail until now; utilizing microorganisms isolated from the nodules themselves to leach valuable metals from the nodules, still remains an unexplored route.

In the recent past, researchers have been looking into bioprocessing as an alternative route of metal recovery from the nodules. Konishi et al (1997) showed nodule leaching by acidophilic sulphur oxidizing bacteria and thermophilic A. brierleyi. A. Kumari and Natarajan (2001) have been able to extract valuable metals from the nodules by electro-bioleaching using acid producing chemolithotrophs like Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans. But these processes employed a highly acidic environment supplemented with thermal or electrical energy for recovering a sizeable amount of metals.

Major objectives of our present work were to isolate an organism from Indian Ocean nodules, characterize and grow the isolate, and ultimately use the isolate or its growth products for solubilisation of Cu, Co and Ni from the nodules. The recovery of metals through a biological route is compared with that of chemical leaching using dilute acids. The effect of reducing additives in the supernatant, pH of the leaching medium and pulp density were also investigated.
2. MATERIALS AND METHODS

2.1 Materials

Ocean nodule sample was collected from the bed of the Indian Ocean by the National Institute of Oceanography, Goa, India. The sample was ground in a mortar and pestle and sieved to obtain appropriate size fractions. The partial chemical composition of the different size fractions of the as-received sample is presented in Table 1. Phases revealed in the X-ray diffraction pattern of the nodule sample are depicted in Table 2.

<table>
<thead>
<tr>
<th>Table 1. Partial chemical analysis of the ocean nodule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Mn</td>
</tr>
<tr>
<td>Cu</td>
</tr>
<tr>
<td>Ni</td>
</tr>
<tr>
<td>Co</td>
</tr>
<tr>
<td>Fe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. XRD analysis of the ocean nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phases present</td>
</tr>
<tr>
<td>Chemical composition</td>
</tr>
<tr>
<td>Quartz</td>
</tr>
<tr>
<td>Asbolan</td>
</tr>
<tr>
<td>Cobalt manganese oxyhydroxide</td>
</tr>
</tbody>
</table>

2.2 Microorganisms

2.2.1 Isolation of the unidentified marine species

The marine bacteria occurred inherently on the nodule sample as a spore-former. To remove the surface contaminants and germinate the spores, the nodule sample was boiled in water for 30 minutes (Ehrlich, 1963). After boiling, hot water was removed by decantation. The lump of nodule was transferred to an autoclaved porcelain mortar (outer diameter, 150mm) in an inoculating hood, which had been pre-sterilized for at least 30 min by UV rays. The lump was pulverized by pounding in the mortar with a sterilized pestle. One g of this nodule powder was added aseptically to 20ml of sterilized artificial seawater nutrient broth whose composition is given in Table 3. Henceforth the medium will be referred to as ASWNB medium. ASWNB with the nodule powder was incubated at 37°C for 24 hrs. From this enrichment media a loopful of the inoculum was streaked on to artificial seawater nutrient agar plates and incubated at 37°C for 24hrs. The next day round, creamy, smooth surface colonies were found growing on the same medium. A colony was picked from the plate and sub-cultured several times on the same medium to finally obtain the pure strain of the marine isolate. Periodic streaking was done to check for the purity of the isolated strain. Henceforth the bacterium will be referred to as "marine isolate".

<table>
<thead>
<tr>
<th>Table 3. Composition of Artificial Sea water nutrient broth (ASWNB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical components</td>
</tr>
<tr>
<td>Wt. in g. in 100 ml of distilled water</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Potassium chloride</td>
</tr>
<tr>
<td>Calcium chloride dihydrate</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
</tr>
</tbody>
</table>
### Chemical components

<table>
<thead>
<tr>
<th>Chemical components</th>
<th>Wt. in g. in 100 ml of distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulphate heptahydrate</td>
<td>3.5</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>28.13</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.77</td>
</tr>
<tr>
<td>Calcium chloride dihydrate</td>
<td>1.60</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate</td>
<td>4.80</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.11</td>
</tr>
<tr>
<td>Magnesium sulphate heptahydrate</td>
<td>3.5</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.0</td>
</tr>
</tbody>
</table>

### 2.2.2 Growth of the marine isolate

The marine isolate was grown in ASWNB in 250-ml baffled Erlenmeyer flasks at 30°C on a rotary shaker (200 rpm). Ten percent of an active inoculum (from the late exponential phase) containing at least $10^9$ cells/ml was added to the sterilized ASWNB medium. The growth of the microorganism was monitored, by measuring the cell count using a Petroff-Hauser counter employing phase-contrast microscopy. The sodium chloride concentration was kept at 0.25M, which was arrived at by testing at different salt concentrations. Growing cells as well as cell free growth supernatant, containing metabolites produced during growth, were used as bioleaching agents.

### 2.3 Methods

#### 2.3.1 Chemical leaching experiments

Chemical leaching experiments were carried out in 250-ml Erlenmeyer flasks on an incubated rotary shaker at 200 rpm at 30°C. In all the cases, 1 g of properly crushed nodule of 50-75 µm size fraction was used and the solid: liquid ratio was kept at 1:100(W/V). All of these experiments were conducted to generate baseline data to compare with bioleaching results. In order to optimize different parameters, the duration of leaching was fixed at four hours in some cases. Parameters such as choice of mineral acids, time of leaching, requirement of reducing agents, and the effects of organic and inorganic additives were investigated. HCl, HNO₃ and H₂SO₄ solutions of 2.5M concentrations were used as leaching agents. H₂SO₄ solution (pH 2.0) alone or with reducing agents like sodium thiosulfate was employed for leaching also. After leaching, leach liquor was filtered using Whatman 42 filter papers and the collected residue was digested in 1:1 HCl at 60-70°C. The resultant solution, after proper dilutions were made, was analyzed for Cu, Co, Ni, Mn and Fe with an Inductively Coupled Plasma spectrophotometer (ICP). All chemicals used were of reagent grade.

#### 2.3.2 Bioleaching experiments

*Leaching with growing culture:* One g of pre-sterilized, pulverized ocean nodule was placed in 90 ml of sterilized ASWNB media in 250 ml conical flasks; 10% v/v actively growing culture ($10^9$ cells/ml) of the marine isolate was added as inoculum. Growth flasks were removed from the rotary shaker after appropriate time intervals and the solution analyzed for leached metal content.
Leaching with cell-free growth supernatant: To obtain cell free growth supernatant, a fully-grown culture (after 10 hours of growth) was centrifuged at 10,000 rpm for 15 minutes followed by pressure filtration using Millipore ultra-filtration unit. The absence of any cells in the resultant supernatant was assured by observing under phase contrast microscope.

One g of pulverized ocean nodule was added to 100 ml of the growth supernatant and solid: liquid ratio was kept at 1:100. To optimize recovery of metals in leaching, pH of the growth supernatant was varied from an acidic to an alkaline range by adding 10N H₂SO₄ or 0.1N NaOH. The duration of leaching was kept constant at four hours. The size fraction of the crushed nodules was in the range of 50 to 75 microns for all tests.

Leaching with starch added to the growth supernatant:

To observe the effect of starch addition to the growth supernatant, increasing proportions of starch were added to 100 ml of cell free growth supernatant and the solid: liquid ratio was kept constant at 1:100. The duration of leaching was maintained at four hours. The size fraction of the crushed nodule was in the range of 50 to 75 microns. Solution pH in all the cases was within 8-8.5 ranges.

In all the above tests, leach liquor collected after appropriate time intervals was filtered using Whatman 42 filter papers and the residue was digested in 1:1 HCl at 60-70°C. The resultant solution, after proper dilutions were made, was analyzed for Cu, Co, Ni, Mn and Fe by an ICP spectrophotometer.

3. RESULTS AND DISCUSSIONS

3.1 Characterization of the marine isolate

A marine bacterium was isolated from an Indian Ocean nodule sample following the procedure discussed in section 2.2. A preliminary morphological and physiological examination of the marine isolate was carried out. Results are presented in Table 4. The isolated strain was rod shaped, motile and able to reduce Mn (IV) to Mn (II).

Table 4. Characterization of the marine isolate

<table>
<thead>
<tr>
<th>Shape</th>
<th>Bacilli</th>
</tr>
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<tbody>
<tr>
<td>Gram reaction</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
</tr>
<tr>
<td>Manganese reduction</td>
<td>+</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase activity</td>
<td>+</td>
</tr>
<tr>
<td>Aerobic metabolism</td>
<td>+</td>
</tr>
</tbody>
</table>

3.2 Chemical leaching experiments

Baseline leaching tests of the nodules in HCl, HNO₃ and H₂SO₄ of 2.5M concentrations were carried out and the results are summarized in Table 5 below.

We can observe from the table that, though Cu recovery is around 80% in all the cases and Ni recovery is about 60%, Co and Mn recoveries are very low. When leaching with HCl, only 30% Co leaches is leached. Co recovery is negligible for the other two acids. Low Co recovery in all the above tests may be attributed to low Mn recovery. A major part of Co in nodules is supposedly occluded in the MnO₂ matrix, so disintegration of the matrix is an essential prerequisite for Co solubilization. Therefore presence of a reducing
agent, like glucose, under acidic condition with HCl remarkably enhances the recovery of Mn and Co. With glucose in a 2.5M HCl solution 50% Co is extracted while Mn recovery increased to 80%. Again the disparity in Co and Mn recovery proves that Mn and Co recovery do not follow a simple 1:1 correlation. It is possible, however, that all of the cobalt in the nodules might not be directly associated with Mn.

Table 5. Leaching of ocean nodules by mineral acids with and without reducing agents

<table>
<thead>
<tr>
<th>Leaching reagent</th>
<th>% Recovery of metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Co</td>
</tr>
<tr>
<td>2.5 M HCl</td>
<td>30</td>
</tr>
<tr>
<td>2.5M HCl + 20% glucose</td>
<td>50</td>
</tr>
<tr>
<td>2.5M HNO₃</td>
<td>&lt;5</td>
</tr>
<tr>
<td>2.5M H₂SO₄</td>
<td>&lt;5</td>
</tr>
<tr>
<td>pH 2 H₂SO₄</td>
<td>25</td>
</tr>
<tr>
<td>pH 2 H₂SO₄ + 1% thiosulfate</td>
<td>35</td>
</tr>
</tbody>
</table>

Since 2.5 M acid solution is not well suited for industrial applications due to corrosiveness and handling hazards, dilute solutions of acid having a pH of 2.0 were applied. From Table 5 it can be observed that 25% Co, 25% Ni and 40% Cu are recovered with almost 10% Mn and 40% Fe extraction. With 1% sodium thiosulfate in solution with pH 2 H₂SO₄, metal recoveries are observed to increase by 5-10%. In this case it can be observed that adding a reducing agent in the leaching medium enhances the recovery.

3.3 Bioleaching experiments

3.3.1 Leaching by growing cells and cell-free growth supernatant

Leaching tests with uninoculated ASWNB at near neutral pH were carried out to verify whether media constituents could dissolve metal values from nodules. Though 10% Cu came out in solution, recovery of Co, Ni, Fe and Mn was negligible. Cu recovery may be attributed to complexation by media constituents. Then leaching of the nodules was carried out using the growing culture of the marine isolate as well as the cell-free growth supernatant at near neutral pH. Results are shown in Table 6. It was observed from the kinetic studies carried out in the lab that within four hours most metal recoveries stabilized; therefore, leach times were maintained at four hours. Comparing leaching by the growing cells and the supernatant, it can be observed that, although there is a small increase in recovery of Co and Fe in case of leaching by the supernatant, recovery of Cu, Ni, Mn are similar in both the cases. During leaching by the growing culture, attachment of the cells onto the nodule surface might have resulted in a decrease in area available for reaction, thereby decreasing the recovery of Co and Fe.

Another important observation is, in spite of no significant Mn recovery, sufficient Co came into solution in both test cases. Therefore, Co recovery in case of biological leaching may not be directly related to Mn recovery. It has already been shown in section 3.2 that all the Co in nodules may not be directly associated with the MnO₂ matrix. It should be stressed that in both the test cases the pH of the medium was 8.1-8.5. The thermodynamic solubility of all the metals concerned is negligible at that pH range. But the solubility of the transition metals in the complexed state can be markedly different from that in the
uncoordinated state. Therefore complexation at near neutral pH by the metabolites present in the growth supernatant of the marine isolate may be responsible for the unusual solubility of the transition metals.

Comparing the results of leaching by a chemical reagent like 2.5M HCl and bioleaching by the growth supernatant of the marine isolate, some interesting conclusions can be drawn. The recoveries of Co, Mn and Fe are quite comparable in both the cases. So in spite of operating at a near neutral pH, metal recoveries similar to that in highly acidic conditions are achieved. This can have significant industrial application with respect to recovering metals from ocean nodules.

Table 6. Leaching of ocean nodules by the growing cells, growth supernatant of the marine isolate and 2.5M HCl solution

<table>
<thead>
<tr>
<th>Leaching conditions</th>
<th>% Recovery of metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu</td>
</tr>
<tr>
<td>Leaching by the growing cells</td>
<td>20</td>
</tr>
<tr>
<td>Leaching in growth supernatant</td>
<td>25</td>
</tr>
<tr>
<td>2.5 M HCl</td>
<td>80</td>
</tr>
</tbody>
</table>

3.3.2 Leaching by the growth supernatant at different pH values

Leaching experiments using the growth supernatant at different pH values were carried out to observe the effect of pH on metal solubilization by the supernatant. The results are shown in Table 7. Until the pH increases above 10 the leaching recoveries of metals do not change much. But at the highly alkaline pH above pH 12.0 the recoveries of all the metals are considerably enhanced. This indicates a change in chemical nature of the metabolite/metabolites present in the growth supernatant of the marine isolate, intensifying the complexation effect in the leach solution.

A titration of the growth supernatant against 0.1M NaOH showed that the pKₐ value lies between 11.5 and 12.5, confirming deprotonation of the metabolites above that pH.

Table 7. Effect of pH of the medium on leaching of ocean nodules by the growth supernatant of the marine isolate

<table>
<thead>
<tr>
<th>Leaching conditions (pH of the growth supernatant)</th>
<th>% Recovery of metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Co</td>
</tr>
<tr>
<td>2.9</td>
<td>53</td>
</tr>
<tr>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>8.1</td>
<td>45</td>
</tr>
<tr>
<td>10.0</td>
<td>56</td>
</tr>
<tr>
<td>14.0</td>
<td>75</td>
</tr>
</tbody>
</table>
3.3.3 Leaching by the growth supernatant at different pulp densities

From Table 8 it is evident that increasing the in pulp density (g. of solid/100ml of liquid) up to 10% has little effect on recovery of metal values from the nodule sample. Solution pH in all the cases was within a range of 8.0-8.5.

Table 8. Effect of pulp density on leaching of ocean nodules by the growth supernatant of the marine isolate

<table>
<thead>
<tr>
<th>Leaching conditions (pulp density)</th>
<th>% Recovery of metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Co</td>
</tr>
<tr>
<td>1%</td>
<td>45</td>
</tr>
<tr>
<td>5%</td>
<td>40</td>
</tr>
<tr>
<td>10%</td>
<td>45</td>
</tr>
</tbody>
</table>

3.3.4 Leaching by the growth supernatant with addition of starch

Starch was added to the growth- supernatant in increasing proportions: 1%, 3% and 5%. Addition of starch in the leaching media enhanced Cu and Ni extraction significantly while Co, Mn and Fe extraction also improved. Starch provides a reducing environment, which helps to break down the MnO₂ matrix thus liberating Cu, Co and Ni occluded therein. Table 9 shows the enhancement in recovery due to reducing action of starch. In all the cases pulp density was kept at 1% and starting pH was within a range of 8.0-8.5.

Table 9. Leaching of ocean nodules by growth supernatant of the marine isolate with addition of starch

<table>
<thead>
<tr>
<th>Conditions of leaching</th>
<th>% Recovery of metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Co</td>
</tr>
<tr>
<td>Growth - supernatant only (marine isolate)</td>
<td>45</td>
</tr>
<tr>
<td>Supernatant + 0.1% starch</td>
<td>65</td>
</tr>
<tr>
<td>Supernatant + 0.5% starch</td>
<td>85</td>
</tr>
<tr>
<td>Supernatant +1% starch</td>
<td>80</td>
</tr>
<tr>
<td>Supernatant +3% starch</td>
<td>80</td>
</tr>
<tr>
<td>Supernatant +5% starch</td>
<td>83</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

The following major conclusions can be drawn based on the above study:

1) Marine organism isolated from the Indian Ocean nodules grows well in artificial seawater and near neutral pH. It can reduce MnO₂.  
2) The growing cells as well as the cell-free growth supernatant can leach Cu, Ni and Co from the nodules.  
3) Significant dissolution of Co and Fe, comparable to chemical leaching by 2.5M HCl, can be achieved by leaching with the growth supernatant of the marine isolate at near neutral pH.  
4) Metabolites produced during the growth of the isolate solubilize transition metals at neutral pH by complexation.
5) The recovery of metals by the growth supernatant remains unaffected by increasing the pulp density to 10%.

6) Recoveries of Cu, Co and Ni are enhanced in highly alkaline growth supernatant having a pH over 12.0.

7) A considerable enhancement in recovery of Cu, Co and Ni is achieved by introducing a reducing agent like starch into the medium.

ACKNOWLEDGEMENTS

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REFERENCES

A novel biotechnological process for germanium recovery from brown coal

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Abstract

A novel biotechnological process, including two main steps - bioleaching and desorption, to recover Ge from brown coal has been developed. The kinetics of the leaching has also been studied. A mathematical model of the leaching process has been deduced. The reaction order of the process has been calculated as 0.826, and the activation energy of the reaction as 63.641kJ/mol.

Keywords: biotechnology, Ge, brown coal

1. INTRODUCTION

Coal, especially brown coal from certain coal mines such as Kaiyuan Coal Mine in Yunnan province and Shenhua Coal Mine in Inner Mongolia, contains a certain amount of rare metals, such as Ge and Ga. The conventional process to recover Ge from brown coal takes five steps, i.e. 1) burning of the brown coal; 2) recovering of Ge from ashes by sulfuric acid leaching; 3) precipitating of Ge with tannin; 4) roasting of Ge-containing tannin to produce Ge concentrate with the grade of 11%. This process is complex and has a low recovery of 60\%\cite{1}, which is sure to bring about a great waste of resource. In current study, a novel process to recover Ge from brown coal in the presence of microorganism has been developed. A Ge recovery of up to 85.33\% has been achieved, and kinetic study on the leaching process has also been conducted.

2. PRINCIPLE

It is concluded\cite{2} that 97.3\% of Ge in the brown coal exists in the humus, which is the most common component in the brown coal. The humus in brown coal is macromolecule organic substance with a relatively high molecular mass around 300~30,000. According to its solubility in the acid or alkali solution, the humus is classified as three parts: humic acid, fulvous acid, and humin. When humus is treated with an alkali solution, humic acid and fulvous acid can dissolve in the solution, while humin remains insoluble. Neutralizing the solution, humic acid will precipitate while fulvous acid remains in the solution. Research on some brown coal samples shows that 86~89\% of Ge in the brown coal is bound to humin, 10~12\% to humic acid and 1~2\% to fulvous acid, which is why Ge in brown coal can not be recovered by acid leaching or alkali leaching. In order to liberate Ge bound to humus, therefore, Ge-bearing organic complex should be degraded first.
The Ge leaching from brown coal in the presence of microorganism mainly takes place in two stages: 1) biodegradation of brown coal for breaking-down of Ge-containing complex of humus; 2) the recovery of Ge from Ge-containing solution. The flowsheet of this novel process is shown in Fig. 1.

The microbes adopted in the experiments for brown coal degradation were selectively cultured from the brown coal. They are classified into three types: *bacteria*, *actinomycetes*, and *mould*, all of which are Gram-positive. There are two types of *bacteria* known as *spheroplast* and *corynebacterium*. It is observed that at the beginning of the leaching, *actinomycetes’* function in neutral solution remains important, and then *mould* and *bacteria* play an important role in the acid solution.

The natural population of the microbes in natural brown coal is very small, thus, a solution with high microbes population (which is referred to as microbe-rich solution) was prepared and used for the degradation-bioleaching. The brown coal samples were firstly leached in the microbes-rich solution for several days; most Ge was found to be leached into solution. However, some of Ge was found being adsorbed in the pores of coal. Thus the second step, desorption was adopted to extract Ge trapped in the pores from coal.

The average original aperture of brown coal $r_0$ was $1.13 \times 10^{-4}$ cm ($11.3 \mu$m), while the length of microorganism $l_m$ is $1.6 \mu$m. $r_o > 2l_m$. Therefore the leaching process can be considered as a reaction of an aqueous species A with a porous material, the product of the reaction is also an aqueous species. The leaching rate of such process can be presented on the basis of "Pore Model"\[3\] and Petersen Model\[4\]:

$$\frac{dC_n}{dt} = k \frac{\varepsilon_0 (1- \frac{1}{G-1}) C^n_A}{r_0} N \varphi$$

where, $k$ is logic rate constant of the bioleaching reaction; $r_o$-average original aperture of raw material; $\varepsilon_0$ is original porosity of raw material; $C_A$ is concentration of leaching agents; $\varphi$ is reproducing rate of microorganism (=1-$[C(t/\tau)+C''(t/\tau)^2]$, where $C'$ and $C''$ are constants); $N$ is the population of microorganism; $t$ is leaching time; $G$ is generation of organism.

![Figure 1. Flowsheet of the recovery of Ge from brown coal](image_url)

Integration of Equation (1) gives:

$$\alpha = \varepsilon_0 \frac{b k C^n_A}{1-\varepsilon_o} \frac{N \tau}{r_o \rho} \frac{G}{(t+Bt^2+Ct^3)}$$

*Brown coal (Ge: 200g/t)*

\[\text{Crush} \quad \text{Microbes colony} \]

\[\text{Bioleaching} \]

\[\text{Desorption} \]

\[\text{High Ge concentration resolution} \quad ([\text{Ge}]_T>800\text{mg/L})\]
where: $\alpha$ is recovery of Ge; B and C are constants; $\rho$ is the density of raw material; n is reaction order; $N_0$ is the original population of the bacteria; $\tau$ is the time of thorough reaction.

Assuming: 

$$A = \frac{e_o^n b k C^a}{1-e_o} \times \frac{N_0 \tau}{G}$$

(3)

The logarithm of Equation (3) is given by:

$$\log A = \log k + n \log C + \log e_o^n b \frac{N_0 \tau}{1-e_o} \times \frac{G}{\rho}$$

(4)

Thus, from Equation (4), the logic rate constant $k$ and reaction order $n$ of the leaching reaction can be determined.

3. EXPERIMENTAL

The brown coal samples used for the experiments were crushed into three size fractions, say, 0.0998~0.147mm, 2~4mm and 12~14mm. The composition of the brown coal is 56.02%C, 5.21%O, 2.17%H, 1.34%S, 29.33% ash, and 0.0312% Ge. Its combustion value is 16,798 kJ/kg. The porosity of brown coal was 42.25% that was determined by immersing samples into water for 4 hours and measuring the water in the samples. The density of dry coal was 1.12g/cm³.

The leaching experiments were carried out in a static beaker. The leaching solution is microbe-rich solution with one of leaching agents in it, which are industrial pure sodium hydroxide, sulfuric acid, and ammonia sulfate, respectively. The pH varied from 3.5 to 9.0, the operation temperature varied from 22 to 55°. To assure that the concentration of leaching agents remained constant, the ratio of liquid to solid (mL/mS) was controlled as high as 10. During the leaching, the leaching solution was sampled and Ge concentration was analyzed once every day.

4. RESULTS AND DISCUSSION

Following findings and evidences show that microbes has played an essential role in the leaching process:

1. Gas bulbs were observed to be produced on the surface of the coal, and pH of the solution decreased, as shown in Table 1.

2. Oxygen concentration in the coal decreased, as shown in Table 1.

3. In the presence of the microbes, Ge concentration in the solution increased continuously, as shown in Table 1, while in the absence of the microbes, no Ge has been leached;

4. The population of the microbes was observed to increase in the solution.

Table 1. Data indicating the degradation of the Ge-bounded humus complex in the presence of the microbes

<table>
<thead>
<tr>
<th>Leaching time /d</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of the solution</td>
<td>7</td>
<td>6.5</td>
<td>6.5</td>
<td>6.0</td>
<td>5.0</td>
<td>4.5</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Oxygen concentration in the coal /%</td>
<td>5.21</td>
<td>5.02</td>
<td>4.67</td>
<td>4.12</td>
<td>3.68</td>
<td>3.13</td>
<td>2.82</td>
<td>2.63</td>
</tr>
<tr>
<td>Ge concentration in the solution in the presence of microbes /mg/L</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>
4.1 Effect of coal particle size on the leaching rate of Ge

Coal samples of three size samples were leached in microbe-rich solution with 0.001mol/L sulfuric acid in it: 8~12mm, 0.533~2mm, 0.074~0.998mm. The results were shown in Fig. 2. It can be seen from Fig. 2 that the recovery of Ge increases with the decrease of the coal particle size, and the optimal size is 2mm.

![Figure 2. Effect of coal size on Ge recovery (leaching agent as sulfuric acid)](image)

**Figure 2. Effect of coal size on Ge recovery (leaching agent as sulfuric acid)**

1- 0.074~0.998mm  
2- 0.533~2mm  
3- 8~12mm

4.2 Effect of leaching agents on the recovery of Ge

Three agents, sulfuric acid, sodium hydroxide and ammonia sulfate, were used for leaching and all the experiments were conducted in the presence of microorganism. The mass ratio of liquid to solid, e.g. \(m_L/m_S\), remains 10 in all experiments. The pH of the leaching solution varied from 2.5 to 10.0. The experiments lasted for 26d.

The Ge recovery vs. leaching time in the presence of microorganism with different leaching agents is shown in Fig. 3. It is obvious that H\(_2\)SO\(_4\) solution is a preferable leaching agent to recover Ge in the presence of microorganism.

![Figure 3. Effect of leaching agents on Ge recovery](image)

**Figure 3. Effect of leaching agents on Ge recovery**

1- H\(_2\)SO\(_4\), 0.001mol/L  
2- NaOH, 0.001mol/L  
3- (NH\(_4\))\(_2\)SO\(_4\), 0.001mol/L

Figure 4. Effect of sulfuric acid concentration on leaching rate

1- 0.0004 mol/L; 2- 0.0012 mol/L;  
3- 0.002 mol/L; 4- 0.004 mol/L

![Figure 4. Effect of sulfuric acid concentration on leaching rate](image)

**Figure 4. Effect of sulfuric acid concentration on leaching rate**

1- 0.0004 mol/L; 2- 0.0012 mol/L;  
3- 0.002 mol/L; 4- 0.004 mol/L

Figure 5. lgA-lgC diagram in the presence of H\(_2\)SO\(_4\)

![Figure 5. lgA-lgC diagram in the presence of H\(_2\)SO\(_4\)](image)
4.3 Effect of leaching agent concentration

The result of experiments under different acid concentration is shown in Fig. 4. It can be seen, from the Fig. 4, that the higher the concentration of sulfuric acid is, the higher the recovery of Ge is. But the concentration of agent cannot be increased too high because the pH of solution has to be controlled in the range that is favorable for microbes.

The regression of experimental data is summarized as following:

\[
\begin{align*}
\alpha_1 &= 1.2024t - 0.0168t^2 \\
\alpha_2 &= 2.4769t - 0.0569t^2 \\
\alpha_3 &= 4.5083t - 0.1156t^2 \\
\alpha_4 &= 7.8766t - 0.2115t^2
\end{align*}
\] (5)

Supposing \( A \) is the quotieties of the first order of the equation (2) and \( c_{\text{H}_2\text{SO}_4} \) is the concentration of sulfuric acid, the dependence of \( \lg A \) as a function of \( \lg c_{\text{H}_2\text{SO}_4} \) can be described as a linear function as shown in the Fig. 5, from which the reaction rate constant of the leaching \( K \) and the reaction order \( n \) is determined to be 9.113×10^{-4}/d and 0.826, respectively. The reaction order, \( n=0.826 \), indicates that the leaching is a slow process.

4.4 Effect of temperature

The experimental results under different temperatures in the presence of microbes are shown in Fig. 6. The regression of these results are presented in Equation (6):

\[
\begin{align*}
\alpha_1 &= 0.7684t - 0.00956t^2 \\
\alpha_2 &= 1.9517t - 0.0339t^2 \\
\alpha_3 &= 4.486t - 0.11456t^2 \\
\alpha_4 &= 5.7726t - 0.1348t^2
\end{align*}
\] (6)

The Arrhenius plot of \( 1/\lg K - 1/T \) is shown in Fig. 7. The slope of the line is –3323.79. From the slope value, the activation energy of the reaction \( W \) is determined to be 63.641 KJ/mol, which further indicates that the process is controlled by chemical reaction.

A recovery of 72% was achieved for experiments carried out with 0.002mol/L sulfuric acid solution as the leaching agent in 16d at 40–42°. The recovery is not high because, after leaching, the coal residue was porous and some Ge would be trapped in the...
Bioleaching Applications

coa residue. Therefore, the desorption step after bioleaching was adopted for recovering trapped Ge and total Ge recovery of 85.33% was obtained.

5. CONCLUSIONS

1. A novel biotechnological process including two main steps, bioleaching and desorption, has been developed to recover Ge from brown coal of some coal mines.

2. The coal particle size, leaching agents and their concentrations, and temperature are important factors affecting the leaching rate of Ge. The optimal operation condition had been determined as following: leaching with 0.002 mol/L sulfuric acid solution at 40–42° for 16 days; the coal particle size is 2mm. The recovery of Ge could be up to 72%, the desorption after bioleaching can further increase the recovery of Ge up to 85.33%;

3. A mathematical model of the leaching process has been deduced, which gives a good fit to both the calculated and experimental data;

4. The reaction order of the process has been calculated as 0.826, and the activation energy of the reaction as 63,641 kJ/mol.

REFERENCES

Aerobic and anaerobic bacterial leaching of manganese

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Abstract

Heterotrophic bacteria are widely used in the study of bacterial leaching of manganese from manganese dioxide ores and glucose or other organic compounds are used as a source of energy, rendering their commercial utilization uneconomic. In the present research, autotrophic bacteria and a mineral source, such as elemental sulphur as their growth substrate, were used instead in order to develop an economic bacterial leaching process for low-grade manganese ores. Active cultures of bacteria were isolated from mining areas such as Laurium, in Attica (Greece), Kassandra Mines, in Chalkidiki (Greece), Skouriotissa, in Cyprus and Wales (UK). Low-grade manganese dioxide ore samples were obtained from ELBAUMIN S.A., a company which operates a manganese mine in Drama (Greece). Recovery of manganese from these samples by bacterial leaching was studied in stirred tank reactors at both aerobic and anaerobic conditions. A process flow sheet was constructed for the production of electrolytic manganese dioxide from low-grade ores in Greece and a preliminary economic evaluation of this conceptual project was performed.

Keywords: manganese, bioleaching, Thiobacillus, low-grade ores

1. INTRODUCTION

Manganese is a strategic metal. It is essential in the production of iron and steel and no adequate substitute has been found to date. Its non-steelmaking applications, mainly dry-cell batteries, are rapidly growing as well [1]. Manganese ore, however, is mined in large quantities only in a few countries of the world (U.S.S.R., S. Africa), and it is not presently commercially mined in the steel-making countries (U.S.A., Japan, E.U.). This is so because the latter possess low-grade manganese ore reserves, which are not amenable to conventional enrichment and extraction techniques.

The above facts have imposed the need for the development of novel manganese extraction techniques which would eventually solve the supply problems for the industrial countries, which are currently importing enormous quantities of manganese ore or ferromanganese.

The hydrometallurgical treatment of manganese ores has a great potential for future application. Chemical reductive leaching has been tested with a variety of leaching agents with satisfactory results but it creates environmental (SO₂.H₂O leaching) or iron removal (FeSO₄ / H₂SO₄ leaching) problems [2, 3].
On the other hand, the biological aqueous processing of ores has been successfully applied, on an industrial scale, for the extraction of copper, uranium and precious metals from sulphide mineral containing deposits [4, 5]. This large-scale application has proved that bacterial leaching, using autotrophic microorganisms, is a low-cost and environmentally safe process but the sulphide ores are under the course of constant depletion, worldwide. Therefore, the scientific and economic interest for the extraction of metals from non-sulphide resources using microorganisms is ever growing.

Until recently, research efforts on manganese bioleaching have been concentrated on heterotrophic bacteria, which directly reduce the manganese dioxide to the soluble Mn$^{2+}$ form. Marine bacteria and, probably, soil and freshwater species (e.g. *Bacillus*, *Micrococcus*, *Pseudomonas*, *Achromobacter*, *Enterobacter*) biodegrade pyrolusite by enzymatic reduction, using a variety of carbohydrate nutrients (e.g. glucose, molasses) under aerobic and microaerobic growth conditions. Also, certain species of fungi (e.g. *Aspergillus niger*) are known to act on Mn$^{4+}$ by formation of a mixture of extracellular reducing organic compounds, consisting, mainly, of citric, formic and oxalic acids, during fermentative metabolism of sugars. Complete manganese solubilization can be achieved by heterotrophic microorganisms, but the cost of nutrients such as glucose, and other refined sugars, makes their application prohibitively expensive [3, 6-16].

At the Laboratory of Metallurgy of the National Technical University of Athens a research project was undertaken with the aim of investigating the possibility of applying biological leaching to the Greek low-grade manganese ores, which are similar to many pyrolusitic deposits around the world, using autotrophic bacteria [17, 18]. The tasks of this project included:

a) Microorganism strain selection between mixed cultures of autotrophic bacteria (*Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*) of different origins, grown on elemental sulphur.

b) Manganese bioleaching using the selected bacterial strain.

c) Flowsheet design and preliminary economic evaluation of the conceptual project.

Task (b) results have already been presented elsewhere [18]. In the present work tasks (a) and (c) are presented.

2. MATERIALS AND METHODS

2.1 Microorganisms and culture media

The mixed culture of *T. ferrooxidans* like strains was selected among four different strains of autotrophic bacteria:

a) a strain (named Culture C) isolated from a bacterial heap leaching solution, provided by Hellenic Mining Company Ltd. (Cyprus),

b) a strain (named Culture K) isolated from acid mine drainage of the Kassandra mixed sulfide mine (Greece),

c) a strain (named Culture L) isolated from a sulfide waste dump in Lavrion (Greece) and

d) a strain (named Culture W) provided by Professor A. Kontopoulos (sent by Dr. F. D. Pooley, University of Wales, UK).
Initially, the microorganisms were cultivated in an iron-free 0K standard nutrient medium. Later, the nutrient mediums used were ID and MS0b [19], both supplemented with elemental sulphur (0.5 or 1% w/v S).

2.2 Ore and elemental sulphur

The low-grade manganese dioxide ore was provided by ELBAUMIN S.A. mining company (Athens, Greece) and came from a battery-grade ore beneficiation site in Drama, Greece. Its chemical analysis is given in Table 1. The X-Ray diffraction analysis of the ore showed that its mineralogical components were: pyrolusite, quartz and calcite. The elemental sulphur was obtained from an oil refinery in Northern Greece and was ground to –60 and –100 mesh.

Table 1. Chemical analysis of the ore sample M2W (average of two analyses performed on two specimens of the ore sample)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Dry ore composition (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>25.05</td>
</tr>
<tr>
<td>MnO₂</td>
<td>30.65</td>
</tr>
<tr>
<td>MnO</td>
<td>3.30</td>
</tr>
<tr>
<td>MnTOTAL</td>
<td>21.92</td>
</tr>
<tr>
<td>Pb</td>
<td>0.12</td>
</tr>
<tr>
<td>Fe</td>
<td>1.07</td>
</tr>
<tr>
<td>Zn</td>
<td>0.10</td>
</tr>
<tr>
<td>Cu</td>
<td>0.009</td>
</tr>
<tr>
<td>Insolubles (in HCl)</td>
<td>28.27</td>
</tr>
<tr>
<td>CaO (CaTOTAL)</td>
<td>3.80</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>-</td>
</tr>
<tr>
<td>CO₂</td>
<td>-</td>
</tr>
</tbody>
</table>

2.3 Culture optimization, strain selection and adaptation procedures

The adaptation of the mixed culture to use elemental sulphur as its sole energy source was done in shake flasks by serial subculture in a sulphur-containing medium. The adaptation of the bacteria to grow on elemental sulphur was carried out in 500ml shake flasks, incubated in a thermostatic water-bath (30°C) with magnetic stirring. The phase of adaptation of the culture, in the presence of manganese, was carried out in air agitated Pachuca-type reactors.

3. RESULTS AND DISCUSSION

3.1 Factorial Experiment of Strain Selection

A $2^3$ full factorial experiment was conducted using the levels of factors shown in Table 2.
The responses investigated were the sulphur conversion (oxidation) factor, the production of biomass and the production of sulphurous ions. Biomass growth was monitored by optical density measurements of the culture, correlated to actual bacterial mass using the Micro-Kjeldahl technique. The conversion of elemental sulphur to sulfate was monitored by sulfate analysis of the culture medium using a turbidimetric technique after precipitation of barium sulphate. Sulphurous acid concentrations were determined using colorimetry.

Parameters, with constant values throughout the experiments, were the leach solution initial pH (1.5) and the temperature (30°C).

The experimental results are shown in Table 3. Statistical analysis of the data is depicted in Figures 1-6. Due to space limitations, the results for the Kassandra culture are the only given here. Besides, all four cultures exhibited the same behaviour in relation to the factors studied.

Regarding the response "sulphur to sulphate conversion factor", the main effect of factor A as well as the interactions AB and ABC were found statistically significant. The existence of significant interaction, means that the model describing this response is not linear, i.e. the experimental region corresponds to the curvature of the response curve (near a maximum or minimum). More specifically, the sulphur pulp density had a negative effect to the sulphur oxidation, which was attributed to the inhibition of bacterial activity by solid particles, commonly observed in bacterial leaching tests. The interaction AB was significant and negative. This means that the effect of pulp density on the sulphur conversion factor is much greater when a low initial biomass concentration is used, within the present factor limits.

Regarding the response "sulphurous acid production", the main effects of factors A and B as well as the interactions AB, BC and ABC were found statistically significant. The existence of significant interactions, again, means that the model is not linear. Specifically, the sulphur pulp density again had a negative effect to the sulphurous acid production. On the contrary, the initial biomass concentration had a positive effect on this response. The interaction of factors A, B was again significant and negative. This means that the effect of pulp density on the production of sulphurous acid is much greater when a low initial biomass concentration is used, within the present factor limits. The interaction BC was significant and positive. This means that the effect of the initial biomass concentration on the production of sulphurous acid is much greater when the ID culture medium is used than with the MS0b medium.

As far as the response "biomass production" is concerned, only the main effect of factor A and the interaction AB were found statistically significant. Specifically, the sulphur pulp density again had a negative effect to biomass production. The interaction AB was again significant and negative. This means that the effect of pulp density on the
Table 3. Strain Selection. Responses: Sulphur Conversion Factor, Sulphurous Acid and Biomass Production.

<table>
<thead>
<tr>
<th>TREATMENT CODE</th>
<th>Sulphur Conversion Factor</th>
<th>Sulphurous Acid Production (mg/l)</th>
<th>Biomass Production (cells ml$^{-1}$ x10$^8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>Average</td>
</tr>
<tr>
<td>(1)</td>
<td>0.55</td>
<td>0.44</td>
<td>0.49±0.05</td>
</tr>
<tr>
<td>a</td>
<td>0.20</td>
<td>0.29</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td>b</td>
<td>0.41</td>
<td>0.48</td>
<td>0.45±0.05</td>
</tr>
<tr>
<td>ab</td>
<td>0.23</td>
<td>0.19</td>
<td>0.21±0.05</td>
</tr>
<tr>
<td>c</td>
<td>0.39</td>
<td>0.29</td>
<td>0.34±0.05</td>
</tr>
<tr>
<td>ac</td>
<td>0.37</td>
<td>0.45</td>
<td>0.41±0.05</td>
</tr>
<tr>
<td>bc</td>
<td>0.51</td>
<td>0.61</td>
<td>0.56±0.05</td>
</tr>
<tr>
<td>abc</td>
<td>0.20</td>
<td>0.11</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>d</td>
<td>0.57</td>
<td>0.38</td>
<td>0.47±0.05</td>
</tr>
<tr>
<td>ad</td>
<td>0.34</td>
<td>0.14</td>
<td>0.24±0.05</td>
</tr>
<tr>
<td>bd</td>
<td>0.55</td>
<td>0.45</td>
<td>0.50±0.05</td>
</tr>
<tr>
<td>abd</td>
<td>0.24</td>
<td>0.22</td>
<td>0.23±0.05</td>
</tr>
<tr>
<td>cd</td>
<td>0.48</td>
<td>0.34</td>
<td>0.41±0.05</td>
</tr>
<tr>
<td>acd</td>
<td>0.24</td>
<td>0.39</td>
<td>0.31±0.05</td>
</tr>
<tr>
<td>bcd</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84±0.05</td>
</tr>
<tr>
<td>abcd</td>
<td>0.18</td>
<td>0.19</td>
<td>0.18±0.05</td>
</tr>
</tbody>
</table>
biomass production is much greater when a low initial biomass concentration is used, within the present factor limits.

Based on the above results, the suggested models for each of the responses considered are:

\[
Y_{R(\text{sulphur conversion factor})} = 0.38 - 0.13 \times X_1 - 0.066 \times X_1 \times X_2 - 0.063 \times X_1 \times X_2 \times X_3
\]

\[
Y_{R(\text{sulphurous acid production})} \text{ mg/l} = 12.85 - 5.44 \times X_1 + 1.10 \times X_2 - 2.68 \times X_1 \times X_2 + 1.70 \times X_2 \times X_3
\]

\[
-2.53 \times X_1 \times X_2 \times X_3
\]

\[
Y_{R(\text{biomass production})} \times 10^9 \text{ cells/ml} = 4.560 - 1.476 \times X_1 - 0.8360 \times X_1 \times X_2
\]

where:

Y’s are the predicted values of the responses,

X₁, X₂, X₃ are the coded variables corresponding to the natural variables A, B, C. The coded variable is equal to +1 or −1 when the corresponding natural variable is at its high or low level, respectively.

The maximum sulphur conversion factors obtained during these tests by each culture were the following: C: 0.999, K: 0.871, L: 0.970 and W: 0.622. As can be readily seen, strain C, effected a nearly complete sulphur conversion. For this reason, it was selected for further experimental work.

Figure 1. Half-Normal plot of effects on sulphur conversion factor
Figure 2. Half-Normal plot of effects on sulphurous acid production

Figure 3. Half-Normal plot of effects on biomass production
Figure 4. 3-D Response surface of sulphur conversion factor

Figure 5. 3-D Response surface of biomass production
3.3 Bioleaching at Aerobic and Anaerobic Conditions

At aerobic conditions, the bacterial population derives energy from the oxidation of sulphur to \( \text{SO}_4^{2-} \), according to the oxidation pathway below [20]:

\[
\text{APS} \quad S^0 \rightarrow [S] \rightarrow \text{SO}_2^{2-} \rightarrow \text{SO}_4^{2-} \quad \text{(1)}
\]

where, APS: adenosine phosphosulfate, [S]: colloidal sulphur.

It is believed that the main intermediate product \( \text{SO}_3^{2-} \) subsequently reduces \( \text{MnO}_2 \) during leaching, according to the reaction [21-24]:

\[
\text{MnO}_2 + \text{SO}_3^{2-} + 2\text{H}^+ \rightarrow \text{Mn}^{2+} + \text{SO}_4^{2-} + \text{H}_2\text{O} \quad \text{(2)}
\]

At anaerobic conditions, i.e. in the absence of oxygen, according to literature, \( T. \text{ferrooxidans} \) couples the oxidation of inorganic sulphur to the reduction of \( \text{Fe}^{3+} \) to \( \text{Fe}^{2+} \) [25-27]. The product of this anaerobic process is beneficial to manganese leaching, because \( \text{Fe}^{2+} \) ions readily react with \( \text{MnO}_2 \) in the acidic culture medium, according to the equation:

\[
\text{MnO}_2 + 2\text{Fe}^{2+} + 4\text{H}^+ \rightarrow \text{Mn}^{2+} + 2\text{Fe}^{3+} + 2\text{H}_2\text{O} \quad \text{(3)}
\]

Therefore, ferric ions were added to the culture medium at a concentration of 1g/l, thus producing culture medium MS1b.

3.4 Factorial Experiment of Bioleaching

A \( 2^3 \) full factorial experiment was conducted using the levels of factors shown in Table 4.
Table 4. Factorial Design of the Bioleaching Experiment - Minimum and Maximum Levels of Variables

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>VARIABLES</th>
<th>LOW LEVEL</th>
<th>HIGH LEVEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ore pulp density</td>
<td>5% w/v</td>
<td>15% w/v</td>
</tr>
<tr>
<td>B</td>
<td>“Ore / elemental sulphur” (O/S) mass ratio</td>
<td>8.6/1</td>
<td>8.6/2</td>
</tr>
<tr>
<td>C</td>
<td>Fe$^{3+}$ concentration in the leach solution</td>
<td>0g/l Fe$^{3+}$</td>
<td>1g/l Fe$^{3+}$</td>
</tr>
<tr>
<td>D</td>
<td>Gas composition</td>
<td>air + 1% CO$_2$</td>
<td>N$_2$ + 1% CO$_2$</td>
</tr>
</tbody>
</table>

The bioleaching results have been presented elsewhere [18]. Briefly, the main effects A, C and D and also the interactions AD, CD were statistically significant at $\alpha=0.01$. In this range of variables, too, the Fe$^{3+}$ concentration in the leach solution had the largest effect with a wide gap separating it from the remaining contrasts. Regarding the manganese leaching rate, this was found to be dependent on all of the studied factors, particularly on the presence of Fe$^{3+}$ in the solution, which speeded up the reaction. Therefore, the following conclusions were drawn:

- Bacterial leaching of manganese dioxide ores by autotrophic species is possible in stirred tank reactors with dispersion of air plus CO$_2$ or N$_2$ plus CO$_2$.
- Manganese extraction is favored at low pulp densities and in the presence of ferric iron at anaerobic conditions.
- The mass ratio ore / elemental sulphur does not affect the manganese extraction under the experimental conditions used.
- The low values of pH, resulting during leaching, cause the complete dissolution of iron contained in the ore.

4. FLOWSHEET DESIGN AND ECONOMIC EVALUATION

Taking into consideration the existence of an electrolytic manganese dioxide (EMD) plant in Thessaloniki (Northern Greece), a flowsheet was designed which employs ore pretreatment at the mine site (Drama) and bacterial leaching of the washed ore at the electrolysis plant site.

A preliminary economic evaluation of a project based on the above flowsheet was conducted. The assumptions made for this purpose were the following:

i. Head ore grade: 20% Mn
ii. Mining Method: underground
iii. Yearly run of mine ore production: 63,000 tons
iv. Project lifetime: 20 years
v. Overall Mn recovery: 60%
vi. Fe dissolution: 65%
vii. Yearly EMD production: 12,000 tons

The evaluation results are given in Table 5.

In order to economically evaluate the project, the Discounted Cash Flow Method was used (DCF). The performance indicator rate (i) of the investment (ROI), determined by solving the equation: Net Present Value = 0, was found to be equal to 0.2437, which is considered very promising.
Table 5. Commercial Bioleaching Plant Costs (in Euros)

<table>
<thead>
<tr>
<th>OPERATING COST</th>
<th>CAPITAL COST</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>56700 t/y</td>
<td>35000 t/y</td>
<td>56700 t/y</td>
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<tr>
<td>Labour</td>
<td>365,124</td>
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<td>1,684,226</td>
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<tr>
<td>Energy</td>
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<tr>
<td>Various</td>
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<td>373,294</td>
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<tr>
<td>Electrowinning</td>
<td>2,971,701</td>
<td></td>
<td>604,842</td>
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<tr>
<td>Overall Leach-EW</td>
<td>4,694,629</td>
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<td>376,522</td>
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<tr>
<td>Ore Transportation</td>
<td>458,260</td>
<td></td>
<td>9,101,215</td>
</tr>
<tr>
<td>Mining-Beneficiation</td>
<td>1,427,569</td>
<td></td>
<td>8,724,693</td>
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<tr>
<td>TOTAL</td>
<td>6,580,458</td>
<td></td>
<td>1,820,243</td>
</tr>
</tbody>
</table>

5. CONCLUSIONS

The results of the strain selection experimental data led to the establishment of the following optimized conditions for growth of the mixed bacterial culture on the elemental sulphur substrate:
- Nutrient medium: MS0b (iron-free)
- Cell concentration in the inoculum: 10⁹ cells ml⁻¹
- Sulphur pulp density: 0.5%w/v
- Sulphur grain size: -60mesh
- Culture selected for further work: Culture C

The results of the bioleaching experimental data led to the following optimum factor levels for the process of manganese leaching by the mixed bacterial culture:
- Ore pulp density: 5%w/v
- O/S mass ratio: 8.6/1
- Fe³⁺ concentration in the leach solution: 1g l⁻¹ (Nutrient medium: MS1b)
- Dispersed gas composition: N₂ + 1% CO₂

Based on the above parameters, and the flowsheet design, the preliminary economic evaluation for an electrolytic manganese dioxide project in Greece showed favourable results.
Figure 7. Flowsheet of a conceptual manganese beneficiation – bioleaching – electrowinning project in Greece
REFERENCES


Anaerobic iron sulfides oxidation

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Abstract

Several experiments using FeS₂ and FeS have been done to find out if metal sulfides are oxidized under anaerobic conditions at circumneutral pH. In chemical experiments, FeS₂ and FeS were oxidized by MnO₂ but not with NO₃⁻ or amorphous Fe(III) oxide. With MnO₂ as oxidant, elemental sulfur and sulfate were the only products of FeS oxidation, whereas FeS₂ was oxidized to a variety of sulphur compounds, mainly sulfate plus intermediates such as thiosulfate, trithionate, tetrathionate, and pentathionate. Thiosulfate was oxidized by MnO₂ to tetrathionate while other intermediates were oxidized to sulfate. The reaction products indicate that FeS₂ was oxidized via the thiosulfate mechanism and FeS via the polysulfide mechanism under anaerobic conditions which previously had been found for aerobic metal sulfide oxidation.

For anaerobic FeS₂ oxidation with MnO₂ the reaction rates related to the FeS₂ surface area were 1.02 and 1.12 nmol m⁻² s⁻¹ for total dissolved S and total dissolved Fe, respectively. These values are in the same range as previously published rates for the oxidation of FeS₂ by Fe(III). In presence of MnO₂, an Fe(II)/Fe(III) shuttle should transport electrons between the surfaces of the two solid compounds, FeS₂ and MnO₂. At the FeS₂ surface, Fe(III) oxidizes FeS₂ and is thereby reduced to Fe(II) which is reoxidized to Fe(III) by MnO₂.

Bacteria could be enriched from anaerobic marine sediments, which anaerobically oxidize FeS, but not FeS₂, with NO₃⁻ as electron acceptor. Bacteria were not obtained with amorphous Fe(III) oxide as electron acceptor. The result that bacteria do not attack FeS₂ under anaerobic conditions has been confirmed in experiments using ⁵⁵FeS₂ as tracer.

Keywords: pyrite oxidation, metal sulfide oxidation, thiosulfate mechanism, polysulfide mechanism, manganese oxide, iron oxide

1. INTRODUCTION

Biological metal sulfide oxidation at low pH below 4 in presence of oxygen, known as bioleaching, is well documented in the literature [for recent reviews see 1-3]. In the literature about bioleaching it has been regularly stated that bioleaching organisms oxidize metal sulfides by two different ways, "direct" and "indirect". "Direct" means that organisms are attached to the metal sulfide surface, dissolving the metal sulfide without a soluble electron shuttle. "Indirect" means that organisms are not attached to the mineral surface and that the metal sulfide is oxidized via the electron shuttle Fe(II)/Fe(III). So far,
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it has not been shown, how organisms oxidize metal sulfides in a "direct" way. High amounts of Fe bound in a layer of extracellular polymeric substances (EPS) of Acidithiobacillus ferrooxidans and of Leptospirillum ferrooxidans have been detected [4-6]. Recently, Ehrlich [2] suggested that this EPS bound Fe may serve as an electron shuttle, as Fe also does in the "indirect" way. Following this suggestion, Fe(III) is generally the oxidant for biological metal sulfide dissolution, irrespective if cells are attached ("direct") or not attached ("indirect") to the mineral surface. This interpretation is supported by a SEM study of Edwards et al. [7] who detected similar leaching patterns on metal sulphide surfaces in case of bioleaching and of abiotic Fe(III) leaching. As well, Rawlings [3] highlighted the dominant role of EPS bound Fe for bioleaching and stated in his review about the mechanisms of bioleaching, that the mechanism is stricte sensu indirect. A direct contact of a cell to the mineral surface is not essential for bioleaching but increases the rate of bioleaching. He suggests to replace the term "direct leaching" by the term "contact leaching". However, bioleaching of metal sulfides is carried out by acidophilic Fe(II) oxidizing organisms providing Fe(III) to oxidize metal sulfides via the thiosulfate or the polysulfide mechanisms. The intermediary sulfur compounds are either oxidized chemically by Fe(III) or biologically by acidophilic sulfur-/compound oxidizing organisms [5, 6, 8 -10].

Biological metal sulfide oxidation at neutral to alkaline pH in presence of oxygen is less well studied. Bioleaching organisms can not live at this pH and Fe is insoluble. Thus, it is not possible that metal sulfides are biologically oxidized in a similar way as described for low pH. A biological dissolution of the acid soluble metal sulfide FeS at neutral pH has been shown for moderately acidophilic sulfur compound oxidizing organisms like Thiomicrospira frisia [11, 12]. These organisms produce protons by sulfur oxidation which dissolve the acid soluble metal sulfide. According to the polysulfide mechanism, intermediary sulfur compounds like elemental sulfur are formed which are biologically oxidized. In case of the acid insoluble FeS₂, moderately acidophilic sulfur compound oxidizing organisms like Thiomonas intermedia only oxidize intermediary sulfur compounds formed by the chemical FeS₂ oxidation and do not increase the chemical FeS₂ dissolution rate [13, 14]. Growth of microaerophilic, neutrophilic Fe(II) oxidizing organisms with FeS as substrate has been reported [15], but it is not known if these organisms increase the metal sulfide dissolution rate and which sulfur compounds are formed.

In the absence of oxygen, an anaerobic biological metal sulfide oxidation at low pH below 4 has also been demonstrated. In presence of Fe(III) as sole electron acceptor, Acidithiobacillus ferrooxidans enhanced the solubilization of Cu from a CuFeS₂ containing concentrate [16]. At low pH, Fe(III) is soluble and efficiently oxidizes metal sulfides including CuFeS₂. According to the polysulfide mechanism, elemental sulfur accumulates in the course of the chemical CuFeS₂ oxidation. Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Sulfolobus acidocaldarius are able to oxidize elemental sulfur to sulfuric acid by reduction of Fe(III) [17, 18]. The production of sulfuric acid enhances the dissolution of the metal sulfide. However, Fe(II) as a product of the biological elemental sulfur oxidation accumulates because it cannot be oxidized by acidophilic microorganisms in the absence of oxygen. As a consequence, the availability of the oxidant Fe(III) is limited, and a continues supply of Fe(III) is necessary for an anaerobic biological metal sulfide oxidation at low pH. This process seems to relevant in miningimpacted lake sediments [19]. Furthermore, a purely chemical oxidation of FeS₂ with MnO₂ as sole electron acceptor at low pH has been demonstrated, and this process

has been suggested for the dissolution of low grade ores or ocean bed nodules in acid media [20, 21].

In this paper, I report about several experiments being done to find out if and how metal sulfides are oxidized under anaerobic conditions at pH 8 in presence of different electron acceptors. To elucidate the anaerobic metal sulfide oxidation mechanisms, intermediate sulfur compounds were analyzed. Anaerobic metal sulfide oxidation at neutral to alkaline pH seems to be relevant in the environment, e.g. in sulfidic mine tailings or in marine sediments. The experiments have been previously described in detail by Schippers and Jørgensen [22, 23].

2. MATERIALS AND METHODS

2.1 Anaerobic chemical iron sulfides oxidation experiments

FeS was obtained from Aldrich (34,316-1, -100 mesh, 99.9 %, troilite and pyrrhotite were the main minerals). FeS2 and 55FeS2 were prepared from pure chemicals as previously described [22] (pyrite and marcasite in minor amounts were the only detectable minerals. The surface area was 2.9 m2·g−1 measured by the BET-method). As oxidant NaNO3 was used as a pure chemical, amorphic Fe(III) oxide and MnO2 were freshly prepared as described elsewhere [24, 25]. For the experiments, 0.5 g of FeS or FeS2 was weighed into 250 ml flasks. To each assay, 50 ml of a 1 M NaHCO3 solution and 50 ml of either a NaNO3 solution (25 g/l), a suspension of amorphic Fe(III) oxid (1 mol L−1) or a suspension of MnO2 (1 mol L−1) were added. Five parallel assays were prepared for each combination of iron sulphide and oxidizing agent. Additional assays with amorphous Fe(III) oxide in presence of Fe-complexing organic compounds such as salicylic acid, oxalic acid, and citric acid, or in the presence of the electron transporting compound AQDS (2,6-anthraquinone disulfonate) in concentrations of 10 mM, 1 mM, or 0.1 mM each were prepared. Suspensions containing either iron sulfides or oxidizing agents were prepared as controls. The pH remained at 8 (+ 0.5) for all experiments. The flasks were closed with air-tight butyl rubber seals, evacuated, and gassed with a mixture of CO2/N2 (10/90, v/v) three times. All assays were incubated at 20°C in the dark. Chemical analysis was done as previously described [22].

2.2 Enrichment of anaerobic iron sulfides oxidizing bacteria

To enrich anaerobic, neutrophilic FeS and FeS2 oxidizing bacteria using NO3− or amorphous Fe(III) oxide as electron acceptor more than 300 assays were inoculated with material from more than 10 different anoxic marine sediments as previously described [23].

3. RESULTS

3.1 Anaerobic chemical iron sulfides oxidation

FeS and FeS2 were chemically oxidized by MnO2 in a bicarbonate buffered solution at pH 8, whereas NO3− and amorphic Fe(III) oxide did not oxidize these iron sulfides. FeS and FeS2 were also not oxidized by amorphous Fe(III) oxide in presence of Fe-complexing organic compounds such as salicylic acid, oxalic acid, and citric acid, or in the presence of the electron transporting compound AQDS (2,6-anthraquinone disulfonate) in a carbonate buffered solution at pH 8. In the control experiments with iron sulfides and without oxidizing agents, a formation of oxidation products did not take place and amorphic
Fe(III) oxide or MnO$_2$ were not dissolved in the absence of iron sulfides. Furthermore, elemental sulfur was not oxidized by any of the oxidizing agents tested (data not shown).

The products from the chemical oxidation of FeS and of FeS$_2$ by MnO$_2$ are shown in Fig. 1. In the case of FeS, elemental sulfur and sulfate were the only oxidation products detected, whereas the oxidation of FeS$_2$ produced a variety of oxidation products in high amounts. Sulfate was the main product and increased over a month to 30 mM. Sulfate formation was highest during the first 15 days. Tetrathionate accumulated until day 15 to 12 mmol S L$^{-1}$ and then deceased again. Thiosulfate and thionate increased to around 5 mmol S L$^{-1}$, whereas pentathionate occurred only at concentrations below 1 mmol S L$^{-1}$. Longer-chained polythionates or elemental sulfur were not formed. The concentration of Mn(II) increased to 120 mmol L$^{-1}$ due to MnO$_2$ reduction. The concentration of tetrathionate as the main sulfur intermediate decreased after the day 15 and sulphate increased further, indicating an oxidation of tetrathionate to sulfate by MnO$_2$. At the end of the experiment when 2/3 of the total dissolved sulfur had been completely oxidized to sulfate, the dissolved Mn/S molar ratio was 2.5 and the Mn/Fe ratio was 5.

![Figure 1. Products of the chemical oxidation of (A top) FeS and of (B bottom) FeS$_2$ by MnO$_2$ at pH 8. For FeS$_2$ also the formation of Mn(II) due to reduction of MnO$_2$ is shown. The following concentrations were used: 0.5 mol L$^{-1}$ MnO$_2$ and 57 mol L$^{-1}$ FeS (0.5 g) or 42 mol L$^{-1}$ FeS$_2$ (0.5 g) [22]](image-url)
To verify if the sulfur compound intermediate thiosulfate is oxidized by MnO₂ as oxidant as well, a separate experiment has been carried out. Within 5 hours, 2/3 of the initial amount of 10 mM thiosulfate was oxidized by MnO₂, mainly to tetrathionate, some sulfate and a little pentathionate (data not shown).

3.2 Anaerobic biological iron sulfides oxidation

From anaerobic marine sediments bacteria could not be enriched with amorphous Fe(III) oxide as electron acceptor. Instead, bacteria could be enriched, which anaerobically oxidize FeS, but not FeS₂, with nitrate as electron acceptor. The result that bacteria do not attack FeS₂ under anaerobic conditions has been confirmed in an experiment using ⁵⁵FeS₂ as tracer. In this experiment, a FeS oxidizing and nitrate reducing enrichment culture obtained from sediment of the estuary of the Rio Tinto, Spain, was further anaerobically cultivated at 30°C with 2 mM Fe²⁺ and a few mg S⁰ as substrates and 10 mM NO₃⁻ as electron acceptor in the presence of 50 mg "tracer-marked" ⁵⁵FeS₂ to test for co-oxidation of FeS₂. Samples were taken from assays inoculated with bacteria or from control assays without bacteria and analyzed for concentrations of SO₄²⁻, NO₃⁻, Fe²⁺, and ⁵⁵Fe in the medium as previously described [22, 23]. After an incubation period of 2.5 month, SO₄²⁻ was formed and NO₃⁻ and Fe²⁺ were consumed in the assays with bacteria, presumably due to bacterial Fe²⁺ and S⁰ oxidation coupled to NO₃⁻ reduction. Values of ⁵⁵Fe were not higher in the assays with bacteria than in the controls, which means that an anaerobic microbial dissolution of ⁵⁵FeS₂ could not be detected (data not shown).

4. DISCUSSION

4.1 Anaerobic chemical iron sulfides oxidation

The formation and degradation of sulfur compound intermediates in the course of FeS₂ oxidation has been shown previously for the bioleaching of FeS₂ in presence of oxygen by which FeS₂ is degraded via the thiosulfate mechanism [5, 6, 8-10]. According to the molecular-orbital theory Fe(III) hexahydrate ions attack FeS₂, oxidize S₂²⁻ to thiosulfate and consequently cleave the chemical bonding between the Fe(II) and the S₂²⁻ in the FeS₂ lattice. As a consequence, thiosulfate and Fe(II) occur as dissolution products. The Fe(II) is oxidized to regenerate Fe(III) for further attack, while thiosulfate is oxidized via tetrathionate, disulfane-monosulfonic acid and trithionate to mainly sulfate in a cyclic pathway.

The results of the present study are in agreement with the thiosulfate mechanism. Conclusively, the anaerobic FeS₂ oxidation by MnO₂ proceeds via the thiosulfate mechanism as well. Since sulfate is the endproduct of the overall reaction, the oxidation of FeS₂ by MnO₂ proceeds by the following reaction:

\[
\text{FeS}_2 + 7.5 \text{MnO}_2 + 11 \text{H}^+ \rightarrow \text{Fe(OH)}_3 + 2 \text{SO}_4^{2-} + 7.5 \text{Mn}^{2+} + 4 \text{H}_2\text{O} \quad (1)
\]

According to this equation, the stoichiometry of the products is 3.75 for Mn/S and 7.5 for Mn/Fe. At the end of the experiments with FeS₂, 2/3 of the dissolved pyritic sulfur was completely oxidized to sulfate. Stoichiometries of 2.5 for Mn/S and 5 for Mn/Fe were calculated which are exactly 2/3 of the stoichiometries above and, therefore, in agreement with equation (1).

Oxidation rates were calculated per surface area for the FeS₂ oxidation during the first 15 days of the experiment as described by Peiffer and Stubert [26]. For the calculation, the amounts of total dissolved S or Fe after 15 days and the total surface area of the FeS₂ were...
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used. For total dissolved S and total dissolved Fe the rates were 1.02 and 1.12 nmol m$^{-2}$ s$^{-1}$, respectively.

Both FeS$_2$ and MnO$_2$ are insoluble solid compounds and it is not known how electrons are transported from FeS$_2$ to MnO$_2$. Alternatively, a tight contact of the surfaces of the two solid compounds might enable a direct electron transfer, or electrons might be transported via an electron-shuttling compound. It is assumed that Fe(II)/Fe(III) cycling transports electrons based on the following reasons:

A) Fe(III) was detected in our experiments and is a well-known oxidant for FeS$_2$:

$$\text{FeS}_2 + 14 \text{Fe}^{3+} + 8 \text{H}_2\text{O} \rightarrow 15 \text{Fe}^{2+} + 2 \text{SO}_4^{2-} + 16 \text{H}^+$$ (2)

Despite the low solubility of Fe(III) at circumneutral pH, Fe(III) may serve as oxidant if it remains adsorbed onto the surface of FeS$_2$ [26, 27].

B) The surface related reaction rates of 1.02 and 1.12 nmol m$^{-2}$ s$^{-1}$ for FeS$_2$ oxidation by MnO$_2$ are in the low range of the rates described for the oxidation of FeS$_2$ by Fe(III) at circumneutral pH, 1.1-39 nmol m$^{-2}$ s$^{-1}$ [26]. This could imply that in our experiments Fe(III) is the oxidant for FeS$_2$ as well.

C) It was shown that Fe(II) is oxidized by MnO$_2$. Postma and Appelo [28] describe this reaction by the following equation:

$$2 \text{Fe}^{2+} + \text{MnO}_2 + 2 \text{H}_2\text{O} \rightarrow 2 \text{FeOOH} + \text{Mn}^{2+} + 2 \text{H}^+$$ (3)

Fe(III) generated by the reaction of Fe(II) with MnO$_2$ could react with FeS$_2$ before it precipitates as FeOOH.

D) The coupling of the redox pairs FeS$_2$/Fe(III) and Fe(II)/MnO$_2$ has been suggested for the dissolution of low grade ores or ocean bed nodules in acid media [20, 21].

E) Molecular-orbital theory considerations by Luther [29, 30] support Fe(II)/Fe(III) cycling. Fe(II) is a d$^6$ (t$_{2g}^6$) electron configuration and Mn(IV) is a d$^3$ (t$_{2g}^3$) electron configuration. The t$_{2g}$ orbitals are filled in case of Fe(II) and half-filled in case of Mn(IV) which imparts the stability for these metal ions. For both solids, FeS$_2$ and MnO$_2$, to react with each other, a ligand would have to dissociate as both reactants touched, but this does not occur. Soluble Fe(II), which has a t$_{2g}^4$ e$^4_g$ electron configuration, is high spin and labile, thus it can adsorb to and react with MnO$_2$ to form Fe(III). Soluble Fe(III) has d$^5$ (t$_{2g}^3$ e$^2_g$) electron configuration and is therefore a labile cation that can undergo ligand exchange and is therefore able to react with the S$_2^{2-}$ ligand of FeS$_2$.

Summarizing the results, a model of FeS$_2$ oxidation by MnO$_2$ is shown in Fig. 2. It is postulated that electrons are transported via the Fe(II)/Fe(III)-shuttle if FeS$_2$ and MnO$_2$ are in a close contact. Fe(II) and Fe(III) should be adsorbed onto the surface of FeS$_2$ because our experiments with amorphic Fe(III) oxide have shown that precipitated Fe(III) does not oxidize FeS$_2$. However, while amorphic Fe(III) oxides precipitated to the FeS$_2$ surface do not alone oxidize FeS$_2$, they may serve as an electron conduit [31]. Electrons might flow from FeS$_2$ via Fe(III) oxides to adsorbed Fe(III) or via Fe(III) oxides directly to MnO$_2$. In our experiments with MnO$_2$, only precipitated Fe(III) but not Fe(II) was detected by extraction with HCl, indicating that the reaction between Fe(II) and MnO$_2$ is faster than the reaction between Fe(III) and FeS$_2$. All reactions in this model were shown to be purely chemical, however, biological catalysis could be involved in the degradation of sulfur intermediates [9, 32, 33].
Figure 2. Model of anaerobic FeS\textsubscript{2} oxidation by MnO\textsubscript{2} via the Fe(II)/Fe(III)-shuttle [22]

In nature, Fe(III) might be stabilized in solution complexed to organic ligands, thus the Fe(II)/Fe(III)-shuttle might transport electrons even if FeS\textsubscript{2} and MnO\textsubscript{2} are not in close contact. Soluble complexed Fe(III) has been shown to exist in sediment pore waters [34, 35]. Furthermore, the FeS\textsubscript{2} oxidation rate increases in presence of Fe(III)-chelating ligands [26, 30].

Besides MnO\textsubscript{2}, other manganese oxides might oxidize FeS\textsubscript{2} as well because the standard redox potential of the couples MnO\textsubscript{2}/Mn\textsuperscript{2+}, Mn\textsubscript{3}O\textsubscript{4}/Mn\textsuperscript{2+}, and MnOOH/Mn\textsuperscript{2+} are all around 600 mV [36].

In contrast to FeS\textsubscript{2} oxidation, FeS oxidation by MnO\textsubscript{2} only produced elemental sulfur and some sulfate as oxidation products. According to the polysulfide mechanism [5, 6, 10], acid soluble metal sulfides are dissolved by Fe(III) and proton attack. The sulfide is oxidized via radicals and polysulfides mainly to elemental sulfur besides some sulfate, which is in agreement with our results. Therefore, the chemical FeS oxidation is described by the following equation:

\[
\text{FeS} + 1.5 \text{MnO}_2 + 3 \text{H}^+ \rightarrow \text{Fe(OH)}_3 + S^0 + 1.5 \text{Mn}^{2+} \quad (4)
\]

Unlike MnO\textsubscript{2}, amorphous Fe(III) oxide was not an oxidant for FeS\textsubscript{2} or FeS at pH 8 in the experiments of this study, even not in the presence of organic Fe-complexes or of the electron transporting compound AQDS. Luther et al., [30] showed a chemical FeS\textsubscript{2} oxidation by 1 mM ferrihydrite and 10 mM salicylic acid in the pH range of 4 to 6.5. Ferrihydrite and salicylic acid form a Fe(III) salicylate complex which reacts with FeS\textsubscript{2}. Liu and Millero [37] showed that the solubility of Fe(III) in the presence of Fe(III) complexing humic acids is two orders of magnitude higher at pH 4-6 than at pH 8. Presumably, the concentration of complexed Fe(III) in the experiments of this study at pH 8 was too low to enable FeS\textsubscript{2} dissolution.

FeS\textsubscript{2} and FeS were also not chemically oxidized by NO\textsubscript{3}\textsuperscript{-} in the experiments of this study. Ottley et al. [38] have shown, that a chemical oxidation of Fe(II) by NO\textsubscript{3}\textsuperscript{-} can be catalyzed by metals such as copper. Thus, a chemical oxidation of FeS\textsubscript{2} by NO\textsubscript{3}\textsuperscript{-} might exist as well. A FeS\textsubscript{2} oxidation by the reduction of NO\textsubscript{3} has been suggested for aquifers based on geochemical data [39, 40] but clear experimental evidence is lacking. Bacteria may be involved in this process.
4.2 Anaerobic biological iron sulfides oxidation

From anaerobic marine sediments bacteria could be enriched which anaerobically oxidize FeS with nitrate as electron acceptor. This finding is in agreement with results of Garcia-Gil and Golterman [41] who described a FeS-mediated denitrification for a marine sediment. FeS belongs to the acid soluble metal sulfides which are chemically oxidized via polysulfides to mainly elemental sulfur and some sulfate [5, 6, 10]. Due to its acid solubility, protons dissolve FeS according to:

\[
FeS + H^+ \rightarrow Fe^{2+} + HS^- \quad (5)
\]

Both products of this reaction may be oxidized by \( NO_3^- \) reducing bacteria. The \( Fe^{2+} \) can be oxidized according to Straub et al. [42]:

\[
10 \text{FeCO}_3 + 2 \text{NO}_3^- + 24 \text{H}_2\text{O} \rightarrow 10 \text{Fe(OH)}_3 + N_2 + 10 \text{HCO}_3^- + 8 \text{H}^+ \quad (6)
\]

\( HS^- \) may be oxidized by e.g. \textit{Thiobacillus denitrificans} or \textit{Thiomicrospira denitrificans} [11]:

\[
5 \text{HS}^- + 8 \text{NO}_3^- + 3 \text{H}^+ \rightarrow 5 \text{SO}_4^{2-} + 4 \text{N}_2 + 4 \text{H}_2\text{O} \quad (7)
\]

In equation 6, protons are produced which continue to dissolve FeS. Bacteria might be attached to the FeS surface embedded in extracellular polymeric substances (EPS). Bacteria produce EPS to create a microenvironment which favours their metabolisms [5, 6]. In such a microenvironment, the pH might be much lower than 8 enabling FeS dissolution. Consequently, \( Fe^{2+} \) or \( HS^- \) oxidizing and \( NO_3^- \) reducing bacteria can grow with FeS as a substrate, and I was able to enrich these bacteria from different marine sediments. With FeS\(_2\) as a substrate, bacteria did not grow since FeS\(_2\) cannot be dissolved by protons.

Precipitation of Fe(III) hydroxide might explain the absence of \( ^{55}\text{FeS}_2 \) dissolution in a \( S^0 \) and \( Fe^{2+} \) oxidizing and \( NO_3^- \) reducing bacterial culture. The bacteria oxidize Fe(II) to Fe(III) which has to diffuse from the Fe-oxidizing enzyme of the bacteria to the FeS\(_2\) surface to serve as an oxidant for FeS\(_2\). Obviously, Fe(III) precipitates immediately and therefore cannot serve as an oxidant for FeS\(_2\). In aquifers where slightly acidic pH values were detected, a FeS\(_2\) oxidation by the reduction of \( NO_3^- \) has been suggested based on depth profiles of \( NO_3^- \) and \( SO_4^{2-} \) [39, 40]. There, \( Fe^{2+} \) oxidizing and \( NO_3^- \) reducing bacteria and soluble organic Fe(III) complexes could probably catalyze an anoxic FeS\(_2\) oxidation with \( NO_3^- \) as electron acceptor.

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Bacterial growth and propagation in chalcocite heap bioleach scenarios

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Abstract

Heap bioleaching of chalcocite ores is widely practised as a relatively low cost process option, especially for marginal deposits. However, the rates of copper extraction achieved in actual operations are often slower than expected.

A recent study by the authors has shown that chalcocite heap bioleaching is controlled by a two-stage mechanism. The first stage is rapid and ultimately controlled by the supply of acid to the reaction sites. The second stage is intrinsically much slower and controlled by mineral oxidation kinetics. In this work, the results of a column operated with a bacterial consortium grown under idealised laboratory conditions are compared to those of a column run with a native culture and high-TDS (total dissolved solids) raffinate solution from the mine site. The results clearly indicate that while the leach reactions follow the two-stage mechanism in both cases, the overall rate of copper extraction in the high-TDS column is significantly retarded. Bacterial growth in this column is slower and much more limited. It is postulated that in such high-TDS environments the rate of copper leaching is controlled by the rate of bacterial growth and oxidation.

Bacterial growth and propagation trends observed in the experiments have been reproduced with a comprehensive heap simulation tool developed by the authors. The bacterial growth and oxidation model employed is briefly introduced. The simulations confirm that, if the bacterial growth rate is significantly retarded, it can become rate controlling over other factors. In full-scale heaps, however, the principal rate-limiting factor is still the diffusion of acid into large stagnant zones.

Keywords: heap leaching, bio-oxidation, growth kinetics, yield, TDS, growth inhibition

1. INTRODUCTION

Heap bioleaching of chalcocite ores has become widely practised for the economic extraction of copper from low-grade deposits. However, in many operations the rate of copper extraction falls well behind what can be achieved in laboratory columns. Some concerns have been expressed that field conditions (high altitude, low temperatures, high TDS (total dissolved solids) of process waters) are adverse to bacterial growth, and may therefore be the cause for slow extraction rates. Some successes with improving heap performance by adapting cultures to such conditions have been reported [1], but there
appears to be only a limited understanding of the interactions between minerals and bacteria in chalcocite heap leach situations.

The authors have conducted a comprehensive study into the dynamics of chalcocite heap bioleaching, and some results are presented in a previous paper [2]. Based on fundamental electrochemical investigations [3], extensive column studies and simulations with a comprehensive heap leach modelling tool, it has been found that the rate of chalcocite leaching by ferric ions, both in columns and in full scale unconfined heaps, is driven by a two-stage mechanism. In stage 1, approximately 40% of copper from chalcocite is released to form an interim pseudo-covellite compound:

$$\text{Cu}_2\text{S} + 0.8 \text{Fe}_2(\text{SO}_4)_3 \rightarrow 0.8 \text{CuSO}_4 + \text{Cu}_{1.2}\text{S} + 1.6 \text{FeSO}_4$$  (1)

The mineral undergoes significant structural changes during stage 1 leaching, such that the second stage, which releases the remaining copper, must be viewed as a separate reaction:

$$\text{Cu}_{1.2}\text{S} + 1.2 \text{Fe}_2(\text{SO}_4)_3 \rightarrow 1.2 \text{CuSO}_4 + \text{S}^0 + 2.4 \text{FeSO}_4$$  (2)

In bioleaching the necessary ferric is in each case regenerated continuously through bio-oxidation:

$$2\text{FeSO}_4 + 0.5 \text{O}_2 + \text{H}_2\text{SO}_4 \rightarrow \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O}$$  (3)

The first stage of chalcocite oxidation is kinetically very rapid and is controlled primarily by the supply of ferric to the reaction site, and hence by the rate of ferrous to ferric oxidation facilitated by bacteria. As ferric consumption is virtually instantaneous, solution potentials are generally quite low (< 500 mV Ag/AgCl).

In heaps the only acid available for reaction (3) during this stage is that coming in with the feed, since pyrite oxidation is typically limited and no elemental sulfur is being generated for subsequent bio-oxidation to sulfate. This restricts the stage 1 oxidation reaction to a narrow zone, which progresses downwards, commensurate with the rate of acid supply. In unconfined heaps this phenomenon is complicated further by transport effects in stagnant zones. These have been used to explain the large discrepancies in leach rates between full-scale heaps and column experiments [2].

Stage 2 oxidation proceeds in the wake of the stage 1 zone. This step is kinetically much slower at ambient temperatures. During bioleaching, the slow kinetics result in a build-up of ferric near the mineral surface and consequently the reaction proceeds at much higher solution potentials (> 650 mV Ag/AgCl). At the same time elemental sulfur is generated, which can be oxidised to generate sulfuric acid, and pyrite oxidation can also proceed more favourably. As the rate of reaction is relatively slow, it proceeds homogeneously over the entire height of the column (or heap), once stage 1 has passed through.

Bacterial growth and oxidation kinetics in chalcocite heaps must be investigated against this background. It is conceivable that reaction (3) is severely retarded by bacteria growing under adverse conditions, and thus becomes overall rate limiting over other factors. In the present study, column leach experiments using a laboratory culture are compared with those using a native culture growing in a high-TDS raffinate. The different growth rates are quantified and the effect in large columns and heaps is investigated with the aid of a comprehensive modelling tool.
2. EXPERIMENTAL

2.1 Column Studies

The ore used in all experiments originated from a typical copper mining and heap leach operation. Top particle size was ¾ inch and the minus 100 micron fines content was 10%. The sample ore had an average Cu grade of 1.45%, approximately 30% of which was acid-soluble (mostly brochantite) and the rest was chalcocite, with some minor occurrences of chalcopyrite. The pyrite content was approximately 2.5%.

The experimental work was conducted in mini-columns, 400 mm tall and 100 mm in diameter, immersed in a water-bath with the temperature controlled at 25°C. In each experiment a number of these columns were run in series, with solution from one column collected in a small, sealed interim container and pumped from there into the next column by means of a peristaltic pump. The standard experimental irrigation rate was 5 L/m²-hr and all columns were well aerated with air enriched with 1% v/v CO₂. Before charging to the columns, the ore was acid agglomerated in accordance with operating practice at the mine site. All columns were operated on a once-through basis.

Two column experiments, denoted Z5 and Z8, were run in the context of this study. Z5 had 8 columns in series with a total height of 2.5 m. The experiment was inoculated with a laboratory bacterial culture derived from a blend of pure cultures of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. This had been continuously maintained on a minus 40-mesh fines fraction of the original ore sample in standard K9 medium, in an incubator shaking at 150 rpm at 30°C, with weekly transfers. The feed solution consisted of 7.5 g/L H₂SO₄, 0.7 g/L Fe(III) as sulfate and 1.3 g/L Fe(II) as sulfate. This solution reflects the pH, potential and Fe concentrations reported from the mine.

Column experiment Z8 consisted of four columns in series with a total height of 1.2 m. This experiment was inoculated with a native culture obtained from the mine site, which was well adapted to the high TDS raffinate solution, and the original raffinate sampled run-of-production at the mine site. The culture was maintained in the same way as the laboratory culture, but in the original raffinate augmented with K9 culture medium.

The composition of the original raffinate is given in Table 1. Of note are the extremely high concentrations of Al and Mg, which exceed toxicity limits reported in the literature [1, 4, 5], and the elevated chloride levels.

Table 1. Analysed composition of the mine site raffinate (only components >10 mg/L listed)

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration [mg/L]</th>
</tr>
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<tbody>
<tr>
<td>Al</td>
<td>12,200</td>
</tr>
<tr>
<td>Ca</td>
<td>467</td>
</tr>
<tr>
<td>Co</td>
<td>16.2</td>
</tr>
<tr>
<td>Cu</td>
<td>216</td>
</tr>
<tr>
<td>Fe</td>
<td>2,460</td>
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<tr>
<td>Mg</td>
<td>10,100</td>
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<td>221</td>
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<td>29.0</td>
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<td>Na</td>
<td>1,670</td>
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<td>Zn</td>
<td>376</td>
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<tr>
<td>Cl⁻</td>
<td>1,300</td>
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<tr>
<td>F⁻</td>
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<td>105.9</td>
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<tr>
<td>o-PO₄⁻</td>
<td>532</td>
</tr>
<tr>
<td>SO₄⁻</td>
<td>116,880</td>
</tr>
<tr>
<td>pH</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Figure 1 shows the copper extraction vs. time achieved in both tests. Although Z5 was halted to allow early assay of solids, the expected extraction trend (dashed line) - based on
observations from similar experiments - has been added for comparison. In both experiments the first 30% of copper extraction represents the rinsing of acid-soluble copper dissolved upon acid agglomeration. The next 30% of copper extraction corresponds roughly to stage 1 leaching, and it is in this phase where the two experiments differ significantly in terms of extraction rate: In Z5 this reaction is nearly complete within 20 days, whereas Z8 requires almost 40, although being only half as tall. Leaching beyond 60% extraction continues in Z8 at a rate similar to that expected for Z5 (although not measured in this particular experiment).

**Figure 1. Copper extraction in experiments Z5 and Z8. The dashed line indicates trends observed from similar experiments**

Figures 2 shows the effluent pH and the progression of solution potentials along the height of the columns. With regard to Z5 these two figures illustrate that chalcocite stage 1 leaching progresses in a narrow zone down the column, consuming all available acid and ferric, and thus maintaining the solution potential at very low levels.

**Figure 2. pH measured in the effluent and solution potentials measured over the length of the column from experiments Z5 and Z8. The dashed lines indicate trends observed from similar experiments**

In the wake of this zone (stage 2 leaching) the potential is rising rapidly to levels around 700 mV (vs. Ag/AgCl), and once the zone breaks through (not quite achieved in Z5 before shut-down, but observed in similar experiments (dashed line)), the solution pH gradually drops to feed levels. In Z8 the zone is still observed, but it progresses much more slowly, and the transition from stage 1 to stage 2 leaching is much more gradual. Only some of the available acid is consumed, but never depleted, and the pH remains consequently more or less stable with only a minor peak around day 50, when the low potential front breaks through. Thus it is clear that stage 1 leaching proceeds through Z8
within a broad band rather than a narrow zone, and it is not controlled by acid supply as opposed to Z5.

The cause for these discrepancies becomes clear from Figure 3. Plotting the number of bacteria counted in solution as a function of time and depth in the heap shows counts lower by an order of magnitude in Z8 (native bacteria in high-TDS raffinate) as compared to Z5 (laboratory culture in artificial raffinate). Both sets of data show the same progression trends, however, with numbers moving down the columns in a "growing wave". The propagation rate of this wave corresponds to that of the high potential wave in the wake of the chalcocite stage 1 leach front. Therefore, it is postulated that the slower copper extraction rate in Z8 is linked to the slower propagation of bacteria through the column at much smaller numbers. The rate of copper leaching is hence controlled by bacterial growth kinetics rather than acid supply.

![Graph showing bacterial counts over the length of the column in experiments Z5 and Z8](image-url)

**Figure 3. Bacterial counts over the length of the column in experiments Z5 and Z8**

### 2.2 Bacterial Growth Curves

The growth characteristics of the cultures used in the column experiments were investigated in a series of shake-flask experiments. Two sets of seven flasks containing 75 mL of culture medium (standard K9 for the laboratory culture and original raffinate augmented with K9 growth medium for the native culture, each containing 2 g of ore fines) were prepared. At time zero, 25 mL of the respective mature culture (grown for 5 days in the same medium in a shaker incubator at 30°C, 150 rpm) was introduced into each medium and placed in the shaker. Both sets were prepared in duplicate, and a blind test was also prepared.

Flasks were removed after 20 minutes (for the time-zero sample), 1, 2, 3, 5, 7 and 9 days, and left to settle for 5 minutes, after which a 5-mL sample was drawn from the supernatant, and from which the bacterial culture was enumerated.

The resulting growth curves are shown in Figure 4. Clearly the laboratory culture grows much more rapidly and to larger numbers than the native culture. Interesting is the initial "overshoot" in the lab culture, before numbers settle to lower levels. This is thought to correspond to the stage-wise leaching of chalcocite, similar to the trends observed in the column experiments. The growth curves were analysed to extract initial growth rate and final cell yield, as reflected in Table 3. This suggests that the native culture grew about 6 times more slowly than the laboratory culture, and at one fifth of the yield.
3. MODELLING STUDY

The authors have developed a comprehensive heap leach modelling tool (HeapSim) [2, 6], which combines an advection-diffusion model to account for transport of solutes through the heap (or column) to mineral sites in the ore, with a multi-reaction model at the mineral site. Gas adsorption, microbial growth and oxidation, and mineral leaching are represented through appropriate kinetic terms. A comprehensive description of the model is beyond the scope of this paper, but the equations describing bacterial growth and oxidation, as well as some key parameters, are discussed below.

The bacterial growth model essentially follows Monod type kinetics [5]:

\[
\frac{dX}{dt} = X k_g \Pi
\]

where \(X\) denotes the bacterial population per unit volume (i.e. [cells/mL]), \(k_g\) is the growth rate (also commonly referred to as \(k_{\text{max}}, [h^{-1}]\)), and \(\Pi\) denotes the product of a number of terms describing substrate and kinetic limitations (involving \(\text{Fe}^{2+}, \text{Fe}^{3+}, \text{O}_2, \text{acid}, \text{T}, \text{etc.}\)). Some forms of the \(\Pi\) term have been reviewed by Nemati et al. [7]. The rate of ferrous oxidation is related to bacterial growth by a simple yield coefficient, \(Y\) [cells/mol \(\text{Fe}^{2+}\)], thus:

\[
\frac{r_{\text{Fe}^{2+}}}{Y} = \frac{1}{Y} \frac{dX}{dt}
\]

The HeapSim code has been calibrated to model the column data generated in experiment Z5 and Z8 on the basis of as much independent bench and literature data as possible. With respect to bio-oxidation, the bacterial growth rate \(k_g\) was obtained directly from the growth curve experiments described above (values in Table 2). The yield coefficient \(Y\) (which does not correspond to the maximum cell yield given in Table 2) was obtained for Z5 (and a number of other column studies using the laboratory culture, not
detailed here) by trial and error as $2 \times 10^{12}$ cells/mol Fe$^{2+}$. Based on the growth experiments, it was decided to take the yield coefficient for Z8 five times smaller than for Z5, i.e., as $0.4 \times 10^{12}$ cells/mol Fe$^{2+}$.

The simulated copper extraction curves for both Z5 and Z8 and their closeness to the experimental data are shown in Figure 5. It should be stressed that the set of parameters for these simulations was identical except for the values of $k_g$, $Y$, and column height. For Z5 the fit to the experimental data is very close, while the fit of Z8 is reasonable, considering that only the bacterial growth parameters were modified. These results thus lend some credence to the correctness of the modelling approach.

![Figure 5. Modelled extraction curves and experimental data for Z5 and Z8](image)

Figure 5. Modelled extraction curves and experimental data for Z5 and Z8

Figure 6 shows the progression of the bacterial population in solution for both simulations. Both follow the rising wave pattern observed in the experiments, and the peak levels of bacterial counts correspond closely to those observed in the experiments. The simulated and measured peak levels between Z5 and Z8 are at a ratio of approximately 5, confirming that the selection of a yield coefficient for Z8 five times smaller than for Z5 was correct. Different from the measured data, however, the simulated curves do not progress as sharp fronts. In the model, bacterial transport is modelled as advection-diffusion with concomitant Langmuir-type (physical) adsorption. The experimental data suggests, however, that bacteria do not migrate beyond the chalcocite stage 1 zone, but accumulate where there is good substrate (i.e. ferrous and acid) supply. Thus bacterial attachment appears to be part of the growth process rather than merely a physical phenomenon. This is not reflected in the model at present.

![Figure 6. Modelled trends for bacterial propagation in experiments Z5 (left) and Z8](image)

Figure 6. Modelled trends for bacterial propagation in experiments Z5 (left) and Z8

The simulations support the hypothesis that the slower extraction rate in Z8 can be explained by the much lower bacterial growth rate and yield. However, as was stated in the introduction, chalcocite stage 1 leaching in columns is normally limited by acid supply. It is of interest, therefore, to investigate the conditions under which transition from
acid-limited to bacterial growth-limited leaching occurs. A number of simulations of Z8 for various values of $k_g$ (0.015 to 0.17 h$^{-1}$, corresponding to doubling times of 4 to 48 h) and $Y$ (0.4 to $10 \times 10^{12}$ cells/mol Fe$^{2+}$) were run, and the predicted times required to achieve 98% chalcocite stage 1 conversion are plotted against growth rate constant in Figure 7. From this it becomes clear that for growth rates above about 0.07 h$^{-1}$ little improvement in conversion time is achieved, indicating acid-limited conditions. This holds true even for growth rates considerably below 0.07 h$^{-1}$, provided that the cell yield is sufficiently low. The conditions of the native culture in Z8, however, fall clearly outside acid-limited conditions. It must be stressed that this applies strictly only to a short column scenario as given in experiment Z8.

![Figure 7. Time taken for 98% chalcocite stage 1 conversion as a function of various growth rates and yield coefficients, modelled for a Z8-type experiment (1.2 m column). The dashed line indicates the transition from acid-limited to growth-limited conditions](image)

It is now of interest to investigate the effect that growth-limited cultures like native culture used in the present study would have on the rate of extraction in a full-scale heap. Retaining the calibration achieved for Z5, the HeapSim code was run to simulate two 10-m heap scenarios, one with the parameters of the laboratory culture, and one with those of the Zaldívar culture. In addition, two further simulations of 10-m columns, 10 cm in diameter, were also run. Changing from a column simulation to a heap simulation involves changing the model parameter, which describes the length of stagnant pores in the bed, by as much as an order of magnitude [2].

Copper extractions are plotted against time in Figure 8. While the retarded bacteria have quite a noticeable effect on the laboratory columns, the effect is marginal in the heap scenario. As discussed above, the native culture does indeed become overall rate limiting in a narrow-bore column scenario. In unconfined heaps, however, diffusion of acid from the flowing solution into large stagnant zones (through which no solution flow occurs) governs the rate of acid supply to the reaction sites. This process is so slow that even under growth-limiting conditions the rate of bacterial oxidation is still not limiting the overall process.
Figure 8. Copper extraction vs. time simulated for 10 m columns and heaps with and without bacterial rate limiting parameters

4. CONCLUSIONS

Chalcocite bioleaching proceeds in two stages, the first of which requires rapid biooxidation of ferrous to ferric, which is typically limited by the availability of acid at the reaction site rather than bacterial growth. In the present study a native culture growing in a high-TDS raffinate was compared against a laboratory consortium. It was found that in laboratory columns the native culture displayed severely restricted growth behaviour, thus governing the overall rate of leaching. Simulations with the HeapSim modelling tool, calibrated on the basis of bench-scale data, emulated the measured extraction data fairly well and thus confirmed the observed trends in bacterial numbers and propagation. In full-scale heaps, however, acid diffusion through large stagnant zones would still most likely govern the overall rate of copper extraction, despite restricted bacterial growth.

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REFERENCES

Bacterial leaching studies of a Portuguese flotation tailing

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Abstract

Two bioleaching processes - a direct bioleaching and an indirect bioleaching with chemical and biological separate stages - have been applied to the flotation tailings of a chalcopyrite ore. In the present paper the influence of experimental parameters is investigated and the obtained results are described and compared in order to test the efficiency of these processes for the recovery of copper and zinc.

Keywords: bacterial leaching, copper, zinc, tailing

1. INTRODUCTION

Neves Corvo mine – one of the major mines of the Iberian pyrite Belt, located in Southern Portugal – produces high-grade copper (and tin) sulphide ores which are submitted to flotation processes in order to obtain copper and zinc concentrates for sale to international smelters.

The content of copper and zinc in the flotation residue (0.85 and 0.64%, respectively) and the environmental problems caused by its accumulation justify the development of a biohydrometallurgical process, which could be at the same time an economical viable approach for its treatment and for the minimization of its environmental impact.

Bacterial leaching can be a potential treatment for this type of residue. Bacterial leaching is an environmentally safe and flexible alternative.

It is nowadays well established that the most widely used bacterium – \textit{Thiobacillus ferrooxidans} – is able to oxidize Fe\textsuperscript{2+}, S\textsuperscript{0}, as well as other reduced inorganic sulphur compounds. Two mechanisms have been established: the direct mechanism that requires physical contact between bacteria and the particles of the metal sulphide (recently it has been suggested that this mechanism should be renamed to “contact” mechanism [1]) and the indirect mechanism, according to which the bacteria oxidize ferrous ion to the ferric state, thereby regenerating the ferric ion required for chemical oxidation of the sulphide mineral. According to the new integral model for bioleaching, metal sulphides are degraded by a chemical attack of iron (III) ions and/or protons on the crystal lattice [2]. The mechanism of degradation is determined by the mineral structure: pyrite via a thiosulphate mechanism with acid production, and chalcopyrite, sphalerite and galena via a polysulfide mechanism.
The use of bioleaching for copper recovery is usually limited because chalcopyrite is very refractory to oxidation in acid media and so presents problems to bioleaching [3]. Moreover, when bioleaching is carried out in a single reactor several phenomena take place that limit to a great extent the rate of the ferrous biooxidation. Firstly, the mineral particles exert an important abrasive effect on the bacteria with a partial breakdown of them, which has two negative effects on the process kinetics: the active bacterial population decreases and the resulting organic matter would reduce or inhibit the bacterial growth [4]. The slow kinetics results in residence times of several days, even weeks, which can limit its application. In addition, the values of pH, temperature and the use of catalysts are conditioned to those values compatible with bacterial growth. Several approaches, such as the use of thermophilic microorganisms [3] and/or the addition of catalysts [3-6] have been attempted in order to overcome these problems.

Copper concentrates can be effectively bioleached by performing chemical and biological oxidation in separate steps and using silver as a catalyst in the chemical oxidation. For copper-zinc concentrates both metals can be recovered to a great extent by conducting the ferric leaching in two stages, the first one without silver to recover zinc and the second one with silver as a catalyst to extract copper [5]. However, until now this approach has not been applied to flotation tailings in which the content of both copper and zinc are low and they are finely disseminated in the sulphide matrix. The main concern relative to the application of the indirect bioleaching with chemical and biological separate steps to the treatment of flotation tailings is that the kinetics and extent of the leaching reactions and the amount of catalyst required are economically competitive with the direct bioleaching of the residue.

2. EXPERIMENTAL

2.1 Materials

The tailings used in this work were produced by the copper/zinc selective flotation plant of the Neves Corvo mine in Southern Portugal. Its chemical composition is shown in Table 1.

Table 1. Chemical composition of the tailings

<table>
<thead>
<tr>
<th>Element</th>
<th>Content</th>
<th>Element</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (%)</td>
<td>0.85</td>
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<tr>
<td>Zn (%)</td>
<td>0.64</td>
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<td>Pb (%)</td>
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<td></td>
</tr>
<tr>
<td>Sn (%)</td>
<td>0.27</td>
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</table>

The direct bioleaching experiments were performed using a mixed culture of *Thiobacillus ferrooxidans* and other related bacteria isolated from Aljustrel mines drainage waters (Portugal) and routinely maintained at 34ºC on a modified Silverman and Lundgren 9K nutrient medium at pH=2.0.

Chemicals were of reagent grade and all solutions were made up with distilled water.
2.2 Procedure

2.2.1 Direct bioleaching experiments

In general, bioleaching experiments were carried out in 250 cm$^3$ conical flasks with 90 cm$^3$ of 9K nutrient medium (without Fe(II)) at pH=2.0, 5 g of tailings (previously washed with ethanol to remove traces of flotation reagents that may inhibit bacterial growth) and 10 cm$^3$ of inoculum. The incubated flasks were on a thermostatted bath shaker at 34ºC and at a constant speed of 280 min$^{-1}$. The pH was maintained at a constant value by adding 3 M H$_2$SO$_4$. Suspended solids were allowed to settle and liquid samples were drawn daily for copper, zinc and total iron analysis. pH and redox potential were measured by a Pt electrode and an Ag/AgCl electrode as reference. Experiments with an ethanol solution containing 2% (v/v) of thymol instead of inoculum were used as controls.

The effect of variables such as pulp density, air supply and particles size was investigated.

A stirred tank bioleaching experiment was also carried out in a one dm$^3$ glass jacketed vessel. One dm$^3$ of the bioleaching solution was placed in the reactor vessel and heated to the working temperature (34ºC). The experiment was initiated by the addition of 50 g of dried solid. The agitation was obtained with a mechanical stirrer at 500 rpm. Air supply was provided (100 dm$^3$ min$^{-1}$).

2.2.2 Ferric sulfate leaching experiments

Experiments were carried out in 250 cm$^3$ conical flasks with 150 cm$^3$ of ferric sulfate solution. The flasks were continuously agitated at 280 min$^{-1}$ on an orbital shaker supplied with a forced air circulation thermostat. Ferric sulfate solutions were first heated to the desired temperature and the reaction was initiated by adding a dried mineral sample. In all tests, the water losses due to evaporation were taken into account during recovery calculations. At the end of the experiment, the slurry was filtered using 0.45 µm Millipore filters and the residue was washed with distilled water, dried and stored in a desiccator. The leach liquor was analyzed for copper, zinc, total iron and ferrous iron. In catalytic tests, the leaching medium consisted of ferric sulfate solutions with silver. An aliquot of a solution of silver sulfate in aqueous sulfuric acid at pH 1.40 containing 300 ppm of silver was added to the ferric sulfate solution. The amount of catalyst is expressed as milligrams of Ag$^+$/gram of concentrate. Unless otherwise stated, the experimental conditions were: initial pH of solution 1.40, ferric iron concentration 12 g dm$^{-3}$ and duration of the test 8 hours [7]. Because all the experiments were carried out at low ferric iron concentration (12 g dm$^{-3}$) in batch systems, the studied pulp solids concentration have to be low. In a continuous operation the pulp solids concentration of leaching might be higher than the values considered in this study.

The effect of the variables such as ferric iron concentration, temperature and amount of catalyst was investigated. Copper and zinc soluble in acid media were determined by performing a test in sulphuric medium at pH 1.40 without ferric iron.

Copper, zinc and iron in leaching and bioleaching solutions were analysed by flame atomic absorption spectroscopy (AAS). Ferrous iron concentration was determined by standard potassium dichromate solution in an automatic titrator.
3. RESULTS AND DISCUSSION

Pyrite was identified by X-ray diffraction (XRD) as the predominant mineral in the solid material. Considering that the residue is coming from the flotation of chalcopyrite and sphalerite bearing ores, the presence of those minerals, although in much lower contents (and therefore not identified by XRD), should be taken into account. The presence of some oxides should be considered as well.

3.1 Direct bioleaching experiments

Bioleaching tests were performed in order to investigate the possibility of using a biohydrometallurgical treatment for the recovery of copper and zinc from the flotation tailing. Therefore, preliminary studies were carried out taking into account the influence of some relevant experimental parameters on metals extraction.

3.1.1 Influence of pulp solids concentration

Pulp solid concentration is an important parameter that must be considered in bioleaching. Thus, in order to check its influence, bioleaching experiments were carried out in 250-cm$^3$ conical flasks using 5, 10, 15 and 20 g of solid material in 100-cm$^3$ solution.

The evolution of the redox potential in the bioleaching solution with 5% (w/v) pulp solids concentration as a function of time denotes a considerable increase which is consistent with the ferrous ion oxidation to ferric ion and thus, it confirms bacterial growth in the presence of the tailing.

The amounts of copper and zinc recovered as a function of pulp solids concentrations are shown in Figure 1.

![Figure 1. Effect of pulp solids concentration on copper and zinc extraction. Conditions: 34ºC, pH=2.0](image)

This set of experiments shows that copper and zinc recoveries depend on the pulp solids concentration. A fast initial bioleaching rate is observed in all cases, after which it becomes very slow. The best copper and zinc recoveries, 36% and 77%, were achieved with the lower solids concentration. Although the production capacity increases with the use of high solids concentrations, when the solid concentration exceeds a value between 5
to 10%, the rate of metals leaching decreases probably due to important shearing stresses and lower dissolved oxygen concentration. In addition, when high solids concentration are used, the concentration of metals is high which may inhibit bacterial growth. In fact, experiments carried out with solids concentration higher than 5% showed a slight increase in the redox potential probably due to lower bacteria activity, which lead to lower metals recovery.

Iron concentration also increased with time. At the end of the experiment performed with 5% (w/v) about 60% of iron was extracted probably from chalcopyrite and pyrite dissolution. The iron recovered in the rest of conditions varied considerably and was always lower than 30%.

In the control experiments copper and zinc recoveries slightly increased with time and varied from 18% to 22% for copper and from 25% to 31% for zinc. These results show the important role of bacteria in the dissolution of those metals.

In order to improve metals recovery other experiments with air supply and with previous grinding were performed.

### 3.1.2 Influence of air supply

Figure 2 compares copper and zinc extraction in the absence and in the presence of air supply (50 dm$^3$.min$^{-1}$). Air supply does not have considerable influence on metals extraction: a slight increase was observed for copper and a small negative effect was detected for zinc.

![Figure 2:Effect of air supply on copper and zinc extraction. Conditions: solids concentration 5% (w/v), 34°C, pH=2.0](image)

### 3.1.3 Influence of particle size

The tailing was submitted to a granulometric separation after grinding. Five different fractions were separated: < 0.106 mm, 0.106-0.200 mm, 0.200-0.500 mm, 0.500-1.00 mm, 1.00-2.00 mm. The amount of copper, zinc and iron in each fraction was determined and the corresponding values were used for metals recovery calculations (Figure 3). As expected, grinding has a positive influence on zinc recovery. More than 95% of zinc was extracted in all experiments regardless of the particle size. On the other hand, copper recovery does not seem to be affected by grinding. The results also suggest that grinding has a positive effect on the rate of metals recovery, particularly for zinc since more than 95% of metal was dissolved in the first 250 hours from the fractions with particle size lower than 0.5 mm.
3.1.4 Stirred tank bioleaching tests

Considering the usual limitations of the shake flask technique (mainly because of continuously changing conditions which lead to a difficult control of experimental variables) stirred tank bioleaching experiments in similar conditions were carried out. Copper and zinc extractions are presented in Figure 4.

A more efficient aeration and a complete mixing of suspended solids lead to a higher zinc recovery: 92% at 500 hours against only 77% at 623 hours in the shake flask experiment. An improvement of the copper leaching rate was also observed, but only a small increase in its maximum extraction (42% against 35%) was detected.

The high zinc recoveries obtained in the bioleaching experiments were probably due to the presence of chalcopyrite and pyrite. Sphalerite chemical and biological dissolution is reported to be improved in the presence of chalcopyrite and pyrite [9], due to galvanic interactions. Contrarily, the low results of copper dissolution obtained in all tests are probably due to incomplete oxidation of chalcopyrite by bacteria (bioleaching rates of Cu-oxides are reported to be faster [10]). This phenomenon is well known and has been attributed to the formation of elemental sulphur on the mineral surface.
3.2 Ferric sulphate leaching experiments

The leaching of the flotation tailing was initially studied at 70°C at two different pH values, 1.25 and 1.40. Results from these tests are shown in Figure 5 in which copper and zinc soluble in both acid media (without ferric iron) are also shown. Both copper and zinc soluble in acid medium are very high, 26.5% to 28.0% of copper and 45.4% of zinc. The presence of ferric iron does not influence copper extraction, even it decreases slightly, and has a noticeable effect on zinc extraction, that markedly increases. Figure 6 shows the ferrous iron concentration and the final pH of the leaching liquor. In the absence of ferric iron there is a net consumption of acid, as the pH increases, and a small ferrous iron production (0.76 and 0.68 g/dm³ for initial pH values of 1.25 and 1.40 respectively). The presence of 12 g/dm³ of Fe (III) leads to a net production of acid, as the pH decreases, and to a high production of ferrous iron (6.11 and 6.03 g/dm³ for initial pH values of 1.25 and 1.40 respectively). These results, together with those shown Figure 5, indicate that an acid solution with 12 g/dm³ of ferric as ferric sulphate is not able to dissolve the non-acid-soluble copper of the flotation tailing (the majority being as chalcopyrite and other copper sulfides) and it is effective for the dissolution of the sphalerite present. The high ferrous iron production together with the net production of acid in ferric leaching suggests that some reductant component whose dissolution produces acid is being dissolved. This component could be pyrite.

![Figure 5. Effect of the pH on the copper and zinc extraction with and without ferric iron. Conditions: pulp solids concentration 2% (w/v), 70°C, 8 hours](image)

![Figure 6. Ferrous iron production and final pH in tests with and without ferric iron. Conditions: pulp solids concentration 2%(w/v), 70°C, initial pH 1.25 or 1.40, 8 hours](image)
3.2.1 Effect of the ferric iron concentration

The effect of the ferric iron concentration on the copper and zinc extractions was studied over the range 8-12 g/dm$^3$ at 70°C. The leaching results, shown in Figure 7, indicate that an increase of the ferric iron concentration in this range does not affect the copper extraction and has a positive effect on zinc extraction.

![Figure 7. Effect of the initial ferric iron concentration on the copper and zinc extraction. Conditions: pulp solids concentration 2% (w/v), pH 1.40, 70°C, 8 hours](image)

3.2.2 Effect of the pulp solids concentration

The effect of the pulp solids concentration on the copper and zinc extractions was studied over the range 2% to 8% (w/v) at 70°C. Figure 8 shows that as the pulp solids concentration increases both copper and zinc extractions decrease. This effect is not very important in the studied range of pulp density. The increase of the ferrous iron concentration as the pulp solids concentration increases suggests that the decrease in metal extraction observed at high pulp solids concentration could be due to the depletion of ferric iron in those conditions.

![Figure 8. Effect of the pulp solids concentration on the copper and zinc extraction and on the ferrous iron production. Conditions: pH 1.40, 70°C, 12 g/L Fe$^{3+}$, 8 hours](image)
3.2.3 Effect of the amount of catalyst

The effect of the amount of silver on the copper and zinc extraction was studied over the range 0-1 mg silver/g of flotation residue at 70°C and 2% (w/v) of pulp solids concentration. The leaching results, shown in Figure 9, indicate a marked effect of the presence of catalyst on the copper extraction. There is a noticeable increase in copper extraction as silver amount increases from 0.2 to 1 mg/g of flotation residue. Zinc extraction decreases as silver amount increase. These results are in agreement with previous results about the catalytic effect of silver on copper and zinc extraction from a mineral that contains both metals as sulphides [4].

![Figure 9. Effect of the amount of catalyst on the copper and zinc extraction. Conditions: pulp solids concentration 2% (w/v), pH 1.40, 70°C, 12 g/L Fe^{3+}, 8 hours](image)

3.2.4 Effect of temperature

Figure 10 shows copper and zinc extractions in the absence and in the presence of ferric iron from tests carried out at 34°C and 70°C. As it was observed at 70°C, the presence of ferric iron does not have influence on the copper extraction at 34°C and has a positive influence on the zinc extraction. Temperature of 34°C was chosen since it is the temperature of the direct bioleaching experiments and with the aim of comparing the efficiency of chemical and bacterial leaching. The increase of temperature from 34°C to 70°C influence both copper and zinc extraction as it can be observed in Figure 10.

![Figure 10. Effect of the temperature on the copper and zinc extraction with and without ferric iron. Conditions: pulp solids concentration 2% (w/v), pH 1.40, 8 hours](image)
4. CONCLUSIONS

More than 90% of zinc can be recovered by both chemical and biological leaching of the flotation tailing, while only 40% of copper can be recovered by direct bioleaching and 20 to 30% by ferric sulfate leaching. The whole experimental conditions needed to reach those recoveries either by chemical or biological leaching (temperature, pulp solids concentration, leaching time, reagents consumption) should be further evaluated in order to establish the most favourable process. In general, the obtained results are in agreement with previous studies showing that sphalerite is more easily dissolved than chalcopyrite. However, other bioleaching tests could be performed to extend this investigation to the effect of other relevant experimental parameters (i.e. amount of inoculum, initial Fe(II) concentration, pH), which can eventually have a positive influence on copper recovery. Other approaches, such as the use of catalysts, extreme thermophiles or regrinding of the leach residue, should be considered carefully, taking into account the low copper and zinc grade of this type of material.

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Bacterial tank leaching of zinc from flotation tailings


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b Institute of Microbiology, Russian Academy of Sciences, Prospect 60-let Octyabrya, 7/2, Moscow, 117312, Russia

Abstract

The parameters of tank bacterial-chemical leaching of stale flotation tailings containing 5.6% Zn, 12.96% S and 11.6% Fe were investigated. The particle size of the tailings was 65% minus 44 µm. The main minerals were pyrite (25-30%), sphalerite (7-8%), pyrrhotite (7-10%), marcasite, chalcopyrite, galena (5-7%) etc. The gangue rock (55%) was predominantly present in the silicate form. Zinc was leached under continuous conditions at 28-30°C in agitated tanks with a pulp density of 16.7, 28.6 and 40.0% of solids. Acidithiobacillus ferrooxidans strain TFI was isolated and cultivated on zinc flotation tailings and adapted to zinc ions concentrations in the liquid phase of up to 40 g/L. Zinc extraction by leaching at a pulp density of 40% of solids with the return of 10% of the solution from the last tank reached 87.12% while the concentration of zinc ions in the leach solution was in the range of 31.4-32.4 g/L. The solids throughput was increased 8 fold, pulp flow rate 2.4 fold and overall leaching residence time was reduced 2.7 fold as compared to leaching at a pulp density of 16.7% of solids.

Keywords: bioleaching, sphalerite, flotation tailings, acidithiobacillus, pulp density, process flowsheet

1. INTRODUCTION

Huge amounts of technogenic resources including flotation tailings are being accumulated worldwide in the areas adjacent to existent and closed mineral processing plants. The higher grades of ores processed earlier together with imperfection of the technology used are the main reasons why the stale flotation tailings are relatively rich with valuable components. In certain cases they are comparable by tenor with some of the ores mined nowadays and therefore can be reprocessed to extract metals.

Generally the use of expensive pyrometallurgical and pressure leaching technologies to extract valuable components from low-grade technogenic resources is not economically viable. In such cases the technologies with lower operating costs come to the foreground. Primarily these are the cost-effective hydrometallurgical technologies and among them bacterial-chemical leaching should be considered [1-4].

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The aim of this work was to investigate and develop the technological parameters of bacterial-chemical leaching of zinc from the flotation tailings. These parameters include solids content, specific pulp flow rate and flowsheet configuration.

2. MATERIALS AND METHODS

Quantitative chemical analysis showed that the stale zinc-containing flotation tailings with a particle size of 65% minus 44 µm contained 5.6% Zn, 12.96% S and 11.6% Fe. The gangue rock (55%) was predominantly present as silica – 32-38%, mica (biotite, muscovite) – 10-12%, graphite – 5-7% and Fe silicates.

The main minerals were pyrite (25-30%), sphalerite (7-8%), pyrrhotite (7-10%), marcasite, chalcopyrite, galena (5-7%), sulphosalts, Cu sulfate, secondary Fe minerals, magnetite and haematite (partially substituted by Fe hydroxides). More than 70% of valuable minerals were intergrown with other valuable and gangue minerals. Zinc in flotation tailings was present both in the sulfide form (sphalerite) and in the oxide form (sulfates, oxides, silicates and ferrites). The major amount of zinc was found to occur in particle size range of 20-44 µm.

Bacterial-chemical leaching of zinc flotation tailings was performed under continuous conditions at 28-30°C using three agitated tanks connected in a series. The volume of each tank was 1600ml with the mixer speed of 400 rpm. Aeration rate during the experiments was 1L/min per 1L of pulp volume.

Acidithiobacillus ferrooxidans strain TFI was routinely isolated and cultivated on zinc flotation tailings as the energy substrate. Adaptation efforts resulted in obtaining a strain tolerant to 40 g/L of zinc ions in solution.

The cell concentration was evaluated by both Lowry protein measurement [5] and end-point tenfold dilutions method. Strain identification in liquid phase of leaching pulp was performed by analyzing chromosomal DNA structure using pulsed field gel electrophoresis method [6]. Cells activity in leaching pulp and solutions was controlled by manometric method (O2 consumption) and by measuring the rate of ferrous iron oxidation to ferric iron.

Total iron concentration (ferrous plus ferric iron) in solution, as well as zinc concentration in solution and in leach residue (after acid decomposition), were determined by atomic absorption spectrophotometry (Perkin-Elmer mod. 3100). Separately, ferric and ferrous iron concentrations in solution were determined by complexometric titration. Redox potentials were measured using a platinum electrode (combined with a silver/silver chloride reference electrode) and converted to Eh values (relative to a hydrogen reference electrode).

3. RESULTS

It is common practice to leach and oxidize sulfide concentrates in the presence of bacteria at a pulp density of 16-20% of solids. In the present work zinc was bacterially leached at pulp densities of 16.7, 28.6 and 40% of solids.

Experimental results are reported in Table 1. The cells concentration and activity (measured as O2 consumption) together with ferric iron and zinc concentrations in leach solution increased with an increase in the pulp density.

Experimental mass balance of bacterial-chemical leaching of zinc from the stale flotation tailings at different pulp densities is shown in Table 2. The dynamics of zinc leaching at different pulp densities are shown in Fig. 1. High zinc concentrations in leach
solutions were achieved by increasing the pulp density; zinc extraction was still at a high level (87.12%). Zinc content in solid leach residue was reduced from 5.6% to 0.75-0.87%.

Table 1. Technological parameters of bacterial-chemical leaching of zinc from the flotation tailings at different solids content, %

<table>
<thead>
<tr>
<th>Factors</th>
<th>16.7% Values</th>
<th>28.6% Values</th>
<th>40.0% Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leach solution pH value</td>
<td>1.5-2.0</td>
<td>1.4-2.0</td>
<td>1.3-2.0</td>
</tr>
<tr>
<td>Leach solution Eh value (mV)</td>
<td>650-750</td>
<td>700-750</td>
<td>700-750</td>
</tr>
<tr>
<td>Ferric iron concentration (g/L)</td>
<td>5.5-6.0</td>
<td>10.0-14.0</td>
<td>13.6-15.5</td>
</tr>
<tr>
<td>Zinc concentration (g/L)</td>
<td>8.7-10.0</td>
<td>13.2-19.0</td>
<td>31.4-32.4</td>
</tr>
<tr>
<td>Cells respiratory activity (µL O₂/mL-hour)</td>
<td>20.0-44.0</td>
<td>36.0-40.0</td>
<td>40.0-56.0</td>
</tr>
<tr>
<td>Cells concentration (cells/mL)</td>
<td>10⁸-10⁹</td>
<td>10⁹-10¹⁰</td>
<td>10¹⁰-10¹¹</td>
</tr>
<tr>
<td>Flow rate (tank volume/hour)</td>
<td>0.013</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>Pulp throughput (ml/day)</td>
<td>500</td>
<td>1200</td>
<td>1200</td>
</tr>
<tr>
<td>Solids throughput (g/day)</td>
<td>100</td>
<td>480</td>
<td>804</td>
</tr>
<tr>
<td>Single tank residence time (hour)</td>
<td>96</td>
<td>40</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 2. Mass balance of bacterial-chemical leaching of zinc from the flotation tailings at different solids content, %

<table>
<thead>
<tr>
<th>Tank</th>
<th>Leach residue yield (%)</th>
<th>Zinc concentration in solution (g/L)</th>
<th>Zinc content in leach residue (%)</th>
<th>Zinc extraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulp density 16.7% solids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed preparation tank</td>
<td>90.0</td>
<td>8.68</td>
<td>1.40</td>
<td>77.50</td>
</tr>
<tr>
<td>Leaching tank 1</td>
<td>85.0</td>
<td>9.16</td>
<td>1.20</td>
<td>81.80</td>
</tr>
<tr>
<td>Leaching tank 2</td>
<td>83.0</td>
<td>9.96</td>
<td>0.75</td>
<td>88.90</td>
</tr>
<tr>
<td>Leaching tank 3</td>
<td>80.0</td>
<td>10.00</td>
<td>0.75</td>
<td>89.30</td>
</tr>
<tr>
<td>Pulp density 28.6% solids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed preparation tank</td>
<td>95.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaching tank 1</td>
<td>90.0</td>
<td>13.21</td>
<td>2.52</td>
<td>58.96</td>
</tr>
<tr>
<td>Leaching tank 2</td>
<td>86.0</td>
<td>17.40</td>
<td>1.51</td>
<td>76.97</td>
</tr>
<tr>
<td>Leaching tank 3</td>
<td>83.0</td>
<td>19.04</td>
<td>1.01</td>
<td>85.01</td>
</tr>
<tr>
<td>Pulp density 40.0% solids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed preparation tank</td>
<td>95.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaching tank 1</td>
<td>89.8</td>
<td>31.36</td>
<td>1.02</td>
<td>83.64</td>
</tr>
<tr>
<td>Leaching tank 2</td>
<td>85.7</td>
<td>32.39</td>
<td>0.89</td>
<td>86.38</td>
</tr>
<tr>
<td>Leaching tank 3</td>
<td>82.8</td>
<td>32.37</td>
<td>0.87</td>
<td>87.12</td>
</tr>
<tr>
<td>Feed (flotation tailings)</td>
<td>100.0</td>
<td>—</td>
<td>5.6</td>
<td>100.0</td>
</tr>
</tbody>
</table>

High redox potentials and low pH values of the solution were observed during bacterial-chemical oxidation.

At a pulp density of 16.7% of solids zinc extraction in the first tank was significantly higher than at a pulp density of 28.6% due to the extended residence time (96 hours vs. 40 hours). Increasing the pulp density to 28.6% with higher flow rate led to a decrease of zinc extraction in the first two tanks but in third tank extraction reached 85%. At a pulp density
of 40.0% of solids high zinc extraction and high flow rates in all tanks were achieved by returning 10% of the leach solution from the last tank to the first one. Returned liquid phase was characterized by high concentrations of active cells and ferric iron.

It is possible at higher pulp densities to increase the flow rates and hence the overall leaching process throughput while keeping fast enough oxidation. Leaching at a pulp density of 40.0% of solids increased the solids throughput 8 fold, pulp flow rate 2.4 fold and decreased overall leaching residence time 2.7 fold as compared to the pulp density of 16.7% of solids.

Mineralogical analysis of flotation tailings leach residues revealed significant phase transformations and the presence of coarse aggregates of up to 300 µm in size cemented by newly formed substance. Inside these aggregates relic mineral inclusions were found: sphalerite grains (10-15µm), pyrite, graphite and gangue. Only gangue minerals, undestructed pyrite and secondary Fe minerals were observed in "free" form. No "free" sphalerite grains were found in leach residue.

![Figure 1. The dynamics of zinc leaching from flotation tailings at different pulp densities](image)

The substance cementing the mineral aggregates was formed by incompletely destructed gangue rock fragments, whitish-yellow and gray colored matter (elemental sulfur and bacterial metabolites) and unidentified porous material with sub-micron intermetallic inclusions.

Mineralogical data on initial flotation tailings and on bacterial-chemical leach residues correlated the results of chemical analyses and gave evidence that "free" zinc sulfide grains and some other sulfide minerals were leached almost completely.

Sphalerite bacterial-chemical oxidation mechanism can be described according to Fowler and Crundwell [7-9] by the following reaction:

\[
ZnS + Fe_2(SO_4)_3 \rightarrow ZnSO_4 + 2FeSO_4 + S^0
\]  

(1)

Iron oxidizing acidophilic bacteria regenerate ferric iron and oxidize elemental sulfur:

\[
4Fe^{2+} + 4H^+ + O_2 \rightarrow 4Fe^{3+} + 2H_2O
\]  

(2)

\[
S^0 + H_2O + 1.5O_2 \rightarrow H_2SO_4
\]  

(3)
As a result of the reaction according to Eqn. 3, the probability of sphalerite passivation by elemental sulfur layer is reduced greatly; the addition of sulfuric acid is not necessary because the latter is generated as a product of sulfur oxidation process.

Surface-active properties of bacterial metabolites in leaching pulps have a technological significance since they increase the solids settling rate during thickening and dewatering. Since iron is present in bacterial-chemical leach solutions in the oxidized ferric form the lime consumption for Fe hydroxide precipitation prior to zinc extraction from the solution will be lower.

4. CONCLUSIONS

It has been shown in this work that unlike the common practice of leaching and oxidizing sulfide concentrates in the presence of bacteria at pulp densities of 16-20% of solids it is possible to bacterially leach zinc at a pulp density of 40% of solids with high technological results.

Raising the pulp density in tank bacterial-chemical zinc leaching up to 40% of solids with the return of 10% solution from the last tank increased solids throughput 8 fold, pulp flow rate 2.4 fold and allows to reduce 2.7 fold the overall leaching residence time as compared to the leaching at a pulp density of 16.7% of solids.

ACKNOWLEDGEMENTS

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Behaviour of elemental sulphur in the biohydrometallurgical processing of refractory gold-sulfide concentrates of various mineral types

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Abstract

The kinetics of oxidation of gold-sulfide concentrates of various mineral types with the use of Acidithiobacillus ferrooxidans monoculture and mixed culture of mesophilic bacteria has been studied. It is shown that the oxidation of the main sulfide minerals - arsenopyrite, pyrite and pyrrhotite is more efficient when applying mixed cultures. The oxidation of sulfide concentrates is accompanied by release of elemental sulfur. Most amount of elemental sulfur is derived in the course of bacterial oxidation of pyrrhotite-pyrite-arsenopyrite concentrates. With actually complete oxidation of pyrrhotite, arsenopyrite and some pyrite, the process of elemental sulfur biooxidation is not terminated. Even additional bacterial leaching of pyrrhotite concentrate within 7 days does not completely oxidize elemental sulfur. As elemental sulfur has a negative effect on the process of gold recovery by cyanidation of biooxidation residues and leads to high consumption of sodium cyanide, some ways of additional oxidation of elemental sulfur by aeration in lime environment or electrolytic treatment were investigated. The research outcomes were taken into account in design and construction of the first commercial plant in Russia at the Olimpiada gold deposit.

Keywords: gold-sulfide concentrates, biohydrometallurgy, elemental sulfur

1. INTRODUCTION

Since 1986, the bacterial leaching of refractory gold ore and concentrates has been employed for gold recovery at a commercial scale. At present, more than 10 commercial plants are operating in the world: in Australia, Ghana, South Africa, Brazil, Peru, China etc. In Russia, the first plant was commissioned in 1997 at the Olimpiada gold deposit, the Krasnoyarsk territory, its daily capacity being 1 t concentrate with further increasing up to 400 t/day in 2001. The bacterial leaching is carried out around the year under severe climatic conditions, at winter temperatures of -25 to -45°C.

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The known BIOX® technology developed by Gencor, first tested at Fairview and later on applied at the other plants uses a mixed culture of mesophilic bacteria and biooxidation plants are operating within the range of 40° to 45°C.

The other technology - Bac Tech process employs moderately thermophilic bacteria at about 50° C at the Youanmi commercial plant in Australia [1].

Taking into account cold climate and refractory ore reserves in Russia which are to be developed, this paper considers the process of biooxidation of refractory gold-sulfide concentrates of various mineral types with the use of mesophilic bacteria at 28-32°C.

While elaborating technology of treating the pyrrhotite - pyrite - arsenopyrite concentrates from the Olimpiada deposit, we faced some problems caused by release of a great amount of elemental sulfur as a result of biochemical oxidation of sulfides and first of all of pyrrhotite. It is known that \( S^0 \) [2] and \( S^2, S_2O_3^{2-}, SO_3^{2-} \) sulfur-bearing anions [3-6] produce (CSN) and cause high cyanide consumption in the course of cyanidation of biooxidation residues. At the plants using biooxidation technology for the treatment of refractory gold concentrate the cyanide consumption attains 30 kg/t of concentrate [3]. Therefore, several methods of reducing cyanide consumption are applied, such as: increase of pulp density [4, 6], pre-leaching lime aeration [7, 8], pre-treatment in sodium hydroxide solution [9], electrolytic treatment [10].

This paper describes the results of study of the elemental sulfur behavior in the process of bacterial oxidation of various mineral types of concentrates and recommends pre-aeration in the lime environment and electrolytic treatment of pulp prior to cyanidation in order to reduce cyanide consumption and to increase gold recovery from the biooxidation residues.

2. MATERIALS AND METHODS

2.1 Sulfide concentrates

Concentrate samples were produced by mineral processing of refractory gold ore of Russian deposits. Four samples of refractory gold - sulfide concentrates were studied under laboratory conditions, such as: gravity concentrate (1) from the Nezhdaninka deposit, Republic of Sakha -Yakutia, gravity - flotation concentrate (2) from the Albazin deposit, the Khabarovsk region, and two flotation concentrates (3) and (4), accordingly, from Nezhdaninka, Republic of Sakha – Yakutia, and Olimpiada, the Krasnoyarsk region.

The main gold-bearing sulfide minerals in concentrates are arsenopyrite and pyrite. The concentrate (4) contains also pyrrhotite. The quantitative predominance of one or another mineral in decreasing order allows to distinguish three main mineral types of refractory gold-sulfide concentrates: arsenopyrite - pyrite concentrates (1) and (2); pyrite - arsenopyrite concentrate (3); pyrrhotite - pyrite - arsenopyrite concentrate (4).

Gold grade in concentrates is 21.6 - 150 g/t, silver 2.3 - 160 g/t. Total sulfur actually occurs in the sulfide form amounting to 5.68-28.8%; the content of elemental sulfur in pyrrhotite-free concentrates (1 -3) is insignificant (0.11-0.15%) while its grade in pyrrhotite-bearing concentrate (4) is higher by an order – 1.3%. Arsenic occurs, mainly, in the form of sulfide arsenic amounting to 4.5-18.9%. The concentrates also contain non-sulfide constituents, such as (%): 9.15 – 51.6 SiO₂, 0 – 8.16 CO₂, 0.54 – 4.5 C organic.

The mineral composition of concentrates (Table 1) is represented by various contents of the main gold-bearing sulfide minerals - arsenopyrite, pyrite and pyrrhotite as well as sulfides grading 17 to 74.8%. Pyrrhotite is abundant (39%) only in the concentrate (4);
antimonite (1.7%) is also contained, mainly, in the concentrate (4). The grain size of concentrate samples is within the range of 80-90% of the class – 0.044 mm.

### Table 1. Mineral composition of concentrates

<table>
<thead>
<tr>
<th>Sulfides</th>
<th>Arsenopyrite-pyrite</th>
<th>Pyrite-arsenopyrite</th>
<th>Pyrrhotite-pyrite-arsenopyrite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td>FeS₂</td>
<td>33.7</td>
<td>7.2</td>
<td>24.3</td>
</tr>
<tr>
<td>FeAsS</td>
<td>41.0</td>
<td>9.8</td>
<td>10.4</td>
</tr>
<tr>
<td>FeS</td>
<td>single grains</td>
<td>not found</td>
<td>not found</td>
</tr>
<tr>
<td>Sb₂S₃</td>
<td>0.1</td>
<td>not found</td>
<td>single grains</td>
</tr>
<tr>
<td>Total sulfide content</td>
<td>74.8</td>
<td>17.0</td>
<td>34.8</td>
</tr>
</tbody>
</table>

### 2.2 Microorganisms

The research on bacterial oxidation of the concentrate (1) employed mesophilic bacteria: the Acidithiobacillus (A.) ferrooxidans monoculture with concentration of 1.0 x 10⁷cells/ml and mixed culture of A. ferrooxidans and A. thiooxidans with corresponding content of 2.5 x 10⁷ and 2.5 x 10⁴ cells/ml. (Table 2).

### Table 2. Characterization of the applied culture*

<table>
<thead>
<tr>
<th>Mineral type of concentrates</th>
<th>Bacteria species, quantity of cells per milliliter of solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenopyrite-pyrite:</td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>Monoculture – A.ferrooxidans 1.0 x 10⁷</td>
</tr>
<tr>
<td>(1)</td>
<td>Mixed culture: A.ferrooxidans – 2.5 x 10⁷, A.thiooxidans – 2.5 x 10⁴</td>
</tr>
<tr>
<td>(2)</td>
<td>Mixed culture: A.ferrooxidans – 1.6 x 10⁸, A.thiooxidans – 2.5 x 10⁴</td>
</tr>
<tr>
<td>Pyrite-arsenopyrite:</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>Mixed culture: A.ferrooxidans – 3.5 x 10⁹, A.thiooxidans – 5 x 10³</td>
</tr>
<tr>
<td>Pyrrhotite-pyrite-arsenopyrite</td>
<td>Mixed culture: A.ferrooxidans – 4.5 x 10⁹, A.thiooxidans – 4.5 x 10⁴</td>
</tr>
</tbody>
</table>

* The microorganism populations were studied by G.I.Karavaiko, T.F.Kondrateva and T.A.Pivovarova, specialists from the Institute of Microbiology, RAS.

The oxidation of concentrates (2, 3) was performed with the use of the mixed culture of A.ferrooxidans and A. thiooxidans bacteria with cell concentrations of 7.05x10⁸ – 1.0x10⁹ and 2.5x10⁴ cells/ml correspondingly. The pyrrhotite-bearing concentrate (4) was tested with the use of the mixed culture of A. ferrooxidans – 4.4 x10⁹, A. thiooxidans – 4.5 x10⁸, Leptospirillum ferrooxidans – 2.2 x10⁴ and moderately thermophilic Sulfobacillus thermosulfidooxidans bacteria – 2.2 x10⁶ cells/ml.

Bacterial culture available in the TsNIGRI’s biotechnological laboratory which were previously adapted to pyrite-arsenopyrite substratum were used in researches. Adaptation and growth of the biomass was implemented for each particular concentrate (1-4) by transferring bacteria to the 9K environment with portioned addition of concentrates up to the ratio of S(solid):L(liquid) = 1:50-1:20. The biomass was further re-disseminated and
the content of solids increased up to S:L=1:5. The process of bacterial adaptation continued with checking pH = 1.5-2.2, Eh = 500-680 mV, concentration of iron oxide and protoxide, biomass activity measured by oxygen consumption on the Warburg device and the rate of iron protoxide oxidation.

2.3 Biooxidation tests

The process of bacterial oxidation of concentrates was studied on the pilot-laboratory unit under the conditions of continuous bacteria cultivating. The unit includes 5 reactors of capacity 2 litres each, furnished by devices for mechanical mixing, dispersion and additional 1000 cm³/l per min air supply. Bacterial oxidation was performed at S:L = 1:5, pH = 1.5-2.2 and temperature of 28 - 32º C. The pH value was maintained by addition of sulfuric acid or lime slurry depending on the material composition of the concentrate. The required temperature of the process was provided with the help of thermoelectric heaters placed directly in the reactors. The main parameters were constantly monitored, such as: pH, °C, the biomass oxidizing activity; bacterial solutions were analyzed for Fe³⁺, Fe²⁺ and As. Once a day, 45 ml pulp samples were taken to make analyses for solid phase – sulfide As, sulfide and elemental S and sulfide Fe.

3. TEST RESULTS

3.1 Comparison of the efficiency of FeAsS - FeS₂ concentrate bioleaching with the use of A. ferrooxidans and a mixed culture of A. ferrooxidans and A. thiooxidans

In order to select the most effective microorganisms for biooxidation of refractory concentrates containing arsenopyrite and pyrite at 28-32ºC, the comparative tests were made on bacterial leaching of arsenopyrite - pyrite concentrate (1) with the use of A. ferrooxidans monoculture and a mixed culture of A. ferrooxidans and A. thiooxidans. Performance of biooxidation was estimated from the analyses of the contents of sulfide and elemental S and sulfide As in the residues of biooxidation of the concentrate (1) within 5 days (Fig. 1A), and also indexes of the completeness of arsenopyrite and pyrite oxidation (Fig. 1B).

The kinetic curves show higher rate of sulfide components biooxidation in case of the mixed A. ferrooxidans and A. thiooxidans culture as contrasted to the single A. ferrooxidans (curves 1 and 1°). By the end of the 5-th day, residual content of sulfide S was, accordingly, 8.12 and 10.2%, sulfide As 0.1 and 1.0% and elemental S - 0.42 and 1.41%.

When using the mixed bacterial culture, more complete oxidation of arsenopyrite – 99.4% and pyrite 71.4% is attained as contrasted to the monoculture (correspondingly, 94.7% and 62%). Joint use of A. ferrooxidans and A. thiooxidans was recommended as most effective tool for further investigation of bacterial oxidation of other mineral types of sulfide concentrates containing arsenopyrite and pyrite.

3.2 Kinetics of biooxidation

Fig. 1 shows kinetic curves corresponding to biooxidation of sulfide components of three mineral types of concentrates at 28-32ºC with the use of mixed bacteria, the performance of the latter being shown in Table 2.

The kinetic curves of bacterial oxidation of the concentrates (1-3) containing only two main minerals - pyrite and arsenopyrite differ from kinetic curves of biooxidation of the concentrate (4) which additionally contains pyrrhotite.
Bioleaching Applications

1-4 residues of biooxidation of the concentrates (1 – 4) with mixed culture of bacteria.  
1° residue of biooxidation of the concentrate (1) with A.ferrooxidans.

Figure 1. Biooxidation of sulfur, sulfide arsenic, elemental sulfur, pyrite and arsenopyrite during bioleaching of gold sulfide concentrates

The sulfide minerals are known to have different crystalline structures and different stability in sulphate solutions. With pH= 2.5 a row of sulfide stability is as follows: pyrrhotite (0.45B), arsenopyrite (0.50B), pyrite (0.55 – 0.6B).

Pyrrhotite, as most electrochemically sensitive mineral, is oxidized, first of all, chemically and microbiologically according to the reactions:

\[
\begin{align*}
\text{FeS} + \text{Fe}_2(\text{SO}_4)_3 &= 3 \text{FeSO}_4 + \text{S}^0 \quad (1) \\
\text{FeS} + 0.5 \text{O}_2 + \text{H}_2\text{SO}_4 &= \text{FeSO}_4 + \text{S}^0 + \text{H}_2\text{O} \quad (2) \\
\text{bacteria} \\
2\text{FeS} + 4.5 \text{O}_2 + \text{H}_2\text{SO}_4 &= \text{Fe}_2(\text{SO}_4)_3 + 2 \text{H}_3\text{AsO}_4 \quad (3)
\end{align*}
\]

In the course of biochemical oxidation of pyrrhotite Fe\textsuperscript{2+}, Fe\textsuperscript{3+} sulfates and S\textsuperscript{0} are created, which are further oxidized by bacteria: Fe\textsuperscript{2+} up to Fe\textsuperscript{3+}, S\textsuperscript{0} up to SO\textsubscript{4}. 

In the first two days of concentrate biooxidation a considerable growth of S\textsuperscript{0} grade in the residues of biooxidation of pyrrhotite-bearing concentrate (4) is observed - from 1.3 up to 6.85% while in the course of oxidation of the pyrrhotite-free concentrates (1-3) less amount of S\textsuperscript{0} is produced – 0.1-1.3%. This testifies about predominant oxidation of pyrrhotite (90-95%) and lower oxidation rate of arsenopyrite (19-29%) and, especially, pyrite when leaching the FeS - FeS\textsubscript{2} - FeAsS concentrate (4). In all other pyrrhotite-free concentrates, the arsenopyrite oxidation rate in the first two days is 2.5 - 3 times higher and it is oxidized for 78-91%.

The lower rate of arsenopyrite oxidation in pyrrhotite-bearing concentrate as compared to pyrrhotite-free concentrates is, probably, explained by variations in electrochemical characteristics of the environment: increase of pH from 1.8 up to 2.2 and decrease of Eh value from 700 up to 500 mV due to Fe\textsuperscript{2+} accumulation and reduced
soluble sulfur compounds resulted from chemical oxidation of pyrrhotite and, partially, arsenopyrite that is evidenced by diminishing concentration of *A. ferrooxidans* bacteria from 2.5 x 10⁷ up to 2.5 x 10⁴ cells / ml in the first two days of oxidation of the pyrrhotite-bearing concentrate (4). Then S⁰ content decreases from 6.8 up to 3.9% due to microbiological oxidation by *A. Thiooxidans* bacteria the amount of which increases from 2.5 x 10⁷ to 2.5 x 10⁹ cells / ml.

The *Sulfacillus thermosulfidooxidans* bacteria living inside the system also facilitate S⁰ oxidation. At the same time, the rate of arsenopyrite oxidation increases that is evidenced by diminishing concentration of sulfide As from 4.4 up to 0.2-0.4%, accordingly, after 2 and 5 days of bioleaching, and by growing quantity of *A. ferrooxidans* bacteria from 2.5 x 10⁷ up to 2 x 10⁹ cells / ml. For 5 days of the Fe S - FeS₂ - FeAsS concentrate (4) bioleaching, arsenopyrite is oxidized for 98%, pyrrhotite - 99%, pyrite - 35%. The residual content of sulfide S accounts for 6.1% as compared to the initial 28.8% value.

The completeness of sulfide oxidation in the FeAsS - FeS₂ and FeS₂ - FeAsS concentrates is governed by a total grade of sulfides and their quantitative ratio. All kinetic curves of oxidation of pyrite, arsenopyrite, sulfide S and As, and also elemental S in the concentrates (1-3) are ranked as 1, 3, 2. The content of sulfides in concentrates decreases in the same order, %: (1) – 74.8, (3) – 34.8, (2) – 17.0. Hence, the less is the content of sulfides, the more completely they are oxidized. This dependence is most pronounced for pyrite as an example. For 5 days, the highest degree of pyrite biooxidation (94.8%) is attained in the concentrate (2) which contains the least amount of sulfides - 17% (7.2 FeS₂). The worst oxidation of pyrite (71.1%) occurs in the concentrate (1) that contains the greatest quantity of sulfides - 74.8% (33.7% FeS₂).

The duration of bacterial leaching of refractory concentrates is governed by their composition and, first of all, by sulfide content. The concentrate (2) containing the least quantity of sulfides (17%) is oxidized almost completely within 3-4 days. The oxidation of the concentrates (1,3,4) containing 34.8-74.8% sulfides requires 5-6 days of bioleaching with pH = 1.5-2.2, S:L = 1:5, t = 28-32°C with the use of mixed culture of mesophilic bacteria.

The analysis of kinetic curves of S⁰ biooxidation demonstrates that the process of S⁰ oxidation is not terminated after 5 days of concentrate leaching, except for the concentrate (2) with the lowest sulfide grade (17%). A great amount of S⁰ (3.9%) is retained in the pyrrhotite-bearing concentrate (4) while 0.42 and 0.24% S⁰ remains in the concentrates (1) and (3). For microbiologic oxidation of S⁰ the leaching was prolonged from 5 to 12 days (Fig. 1). This allowed to decrease the content of elemental sulfur from 3.9 only to 1.5% while the content of sulfide sulfur dropped from 6.1 up to 5%.

### 3.3 Oxidation of S⁰ by hydrometallurgy before cyanidation

As a rule, the biooxidation residues have a complex composition and contain constituents which consume oxygen and cyanide, and deteriorate the process of gold cyanidation. It is shown in publication [8] that the residue of biooxidation of pyrite – arsenopyrite ore contain S²⁻, Fe (OH) compounds, residual FeAsS, jarosite and S⁰ that are the consumers of oxygen and sodium cyanide. In order to diminish their action, the pulp was treated, prior to cyanidation, with lime at pH=10.5 within 24-40 hours, then sodium cyanide was added to perform leaching within 24 hours.

We studied an effect of several methods of treating the biooxidation residues before cyanidation on the decrease of the content of elemental sulfur, such as: 3-4 repeated water
washing, neutralization, aeration in limy environment, electrochemical treatment. The hydraulic washing of biooxidation residues does not affect the $S^\circ$ content. The neutralization just insignificantly decreases the $S^\circ$ content - from 3.9 up to 3.75%. The aeration of the pulp in lime environment causes a partial oxidation of $S^\circ$ - from 3.75 up to 2.71%. Most effective is the electrolytic treatment of pulp within 1-2 hours at pH=11-12 (Fig. 2A).

![Figure 2. Effect of treatment of biooxidation residues before cyanidation on content of sulfur (A), gold extraction and cyanide consumption (B)](image)

In the course of electrolysis of aqueous solutions the gaseous oxygen is released; its solubility in solution increases up to 20-30 mg/l. The gas saturation of pulp with finely dispersed bubbles increases 100-1000 times as compared to aeration. The process of $S^\circ$ oxidation is enhanced and its content decreases from 3.75 up to 2.17% for 1-2 hours of treating.

A mechanism of pre-aeration in lime environment or electrolytic processing can be represented by two stages:

1. Oxidation and precipitation of oxygen and cyanide absorbers prior to cyanidation:
   
   \[
   \begin{align*}
   \text{Fe}^{2+} & \rightarrow \text{Fe}^{3+} \rightarrow \text{Fe(OH)}_3, \\
   \text{S}^2- & \rightarrow \text{S}^\circ \rightarrow \text{S}_2\text{O}_3^{2-} \rightarrow \text{SO}_4^{2-} \rightarrow \text{CaO} \rightarrow \text{CaSO}_4
   \end{align*}
   \]

2. Lime passivation of sorption-active surface of newly precipitated iron hydroxides and decrease of a sorption capacity of products of bacterial oxidation in relation to cyanide complexes of precious metals.

The applying of aeration and electrolytic processing of pulp allows to stabilize the pulp ionic composition prior to cyanidation. As the duration of preparation increases, the concentration of oxygen and cyanide absorbants decreases, mg/l: from 52.5 up to 10.2 S$^2$; from 35 up to 0 S$\_2$O$\_3$; from 6.4 up to 0 Fe$^{3+}$. A chemical value of oxygen absorption decreases from 480 up to 320 mg/l and remains constant during the whole cyanidation process. The stabilizing of ion composition of pulp allows to reduce 3.3 times the CNS-ion concentration and 1.7 times the SO$\_4$ ions.

The neutralization of harmful constituents allows to improve gold dissolution and to reduce cyanide consumption. The preliminary aeration within 24 hours allows to increase gold recovery from the residue of biooxidation of the concentrate (4) from 90 up to 96%
and to reduce the cyanide consumption from 16.0 up to 7.2 kg /t. The electrolytic processing of pulp before cyanidation increases gold recovery up to 97% and reduces the cyanide consumption from 16 up to 7 kg /t (Fig. 2B).

Table 3 shows that biohydrometallurgical processing of refractory gold-sulfide concentrates is an effective technology of sulfide oxidation and recovery of finely disseminated precious metals. Gold recovery from the studied mineral types of concentrates with the use of biohydrometallurgical technology attains 92-98% as compared to low-effective parameters of gold recovery by cyanidation of source concentrates – 12.9-63.4%.

**Table 3. Effect of biooxidation on gold recovery from concentrates**

<table>
<thead>
<tr>
<th>Processing method</th>
<th>Mineral type of concentrate</th>
<th>Arsenopyrite-pyrite</th>
<th>Pyrite-arsenopyrite</th>
<th>Pyrrhotite-pyrite-arsenopyrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold recovery by cyanidation, %</td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
<tr>
<td>Gold recovery by biohydrometallurgy, %</td>
<td>63.4</td>
<td>12.9</td>
<td>37.5</td>
<td>51.2</td>
</tr>
<tr>
<td>Duration of biooxidation, days</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

4. **CONCLUSION**

$S^\circ$ is formed during biooxidation of all mineral types of concentrates containing arsenopyrite, pyrite and pyrrhotite. The presence of pyrrhotite enables creating of the most amount of $S^\circ$. The process of its biooxidation is slower than sulfides oxidation. $S^\circ$ is saved in biooxidation residues and has a negative effect on the process of cyanidation, leads to high consumption of cyanide because of (CNS) creating. Using of aeration in lime environment or electrochemical treatment enables to decrease cyanide consumption as much as 2.3 times and increase extraction of gold from 90 to 96-97%.

**REFERENCES**

Beneficiation of phosphatic ores from Hirapur, India

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Abstract
The availability of phosphorus in tropical soils is generally low due to immediate precipitation and fixation of applied phosphorus to soil. In India, 98% of the soil contains insufficient amounts of available phosphorus to support maximum plant growth and hence, application of phosphorus is very essential. High grade rock phosphate is in short supply. Therefore, it becomes necessary to import di-ammonium phosphate from abroad.

Hence, it was thought to beneficiate the low grade rock phosphate available at Hirapur in India. Unfortunately, it contains high amounts of iron and silica (3-5%). Hence, microbial beneficiation was attempted using *Thiobacillus ferrooxidans* to remove extra iron by ferrous iron oxidation and by removal of both iron and silica by sieving and cleaning the ore using various physical treatments. This resulted in a cleaner ore, with nearly 70% iron and 25% silica impurity removed. The factors for such a beneficiation process were standardized at laboratory level (optimum pH = 3, temperature = 30°C, 1% pyrite added as nutrient). These factors are used for scaling up the process at field site at one t.p.d. level. This is an important first step in production of indigenous phosphate source to eliminate import - dependence in agriculture.

*Keywords:* phosphatic ores, *Thiobacillus ferrooxidans*, beneficiate

1. INTRODUCTION
The availability of phosphorus in tropical soils is generally low due to immediate precipitation and fixation of the applied phosphorus. In India, 98% of the soils contain insufficient amounts of available phosphorus to support maximum plant growth. Application of phosphatic fertilizers, therefore, becomes very essential (1, 2). According to several workers (3, 4), about 85 to 90% of the added phosphorus in the fertilizer gets fixed in the soil within 24 to 48 hours of its application and becomes unavailable to the plants. Thus, efficiency of utilization of phosphatic fertilizers in soils ranges from 10 to 15% only. The problem of phosphorus fixation was found to be very acute in neutral to alkaline soils in India, where the phosphorus is precipitated as calcium phosphate upon application of superphosphate. This results in non-availability of applied phosphorus in soils, which is a considerable loss from the point of view of productivity and economy to the nation.

Manufacturing of phosphatic fertilizers in India is faced with serious problems, as it requires the use of non-renewable resources, such as high grade rock-phosphate and sulphur, which are in short supply and are being depleted progressively; thereby, becoming costly.
The magnetic or igneous rocks are the principle sources of phosphorus on our planet and the igneous rocks rich in phosphorus are industrially exploited. In India, the Mussourie rock phosphate is currently exploited, as it has 8.6% phosphorus or 20% $P_2O_5$. At Hirapur, M.P. India, in addition to phosphorus it contains high amounts of iron along with silica. Therefore, it becomes difficult to remove iron and silica by conventional chemical and physical means.

Hence, the aim of the present study was to screen and select microorganisms, giving maximum phosphate solubilizing activity to remove the phosphorus part or removing the impurities of iron and silica from the phosphatic ore of Hirapur, India.

2. MATERIALS AND METHODS

2.1 Screening and selection of microorganisms

2.1.1 Phosphate solubilizing activity

Our previous studies had indicated 10 species of bacteria, two of yeasts and four mycelial fungi to have a good phosphate dissolving activity. (5). These microorganisms were isolated from nine different ecosystems containing phosphates or having come into contact with phosphates. A chemical analysis of the rock phosphate from Hirapur is shown in Table 1.

When these microorganisms were screened qualitatively by using Pikovskaya's agar medium or quantitatively in Pikovskaya's liquid medium, (6) it was found that the culture of *Arthrobacter* species gave maximum solubilization of 27.1% in 14 days (Table 2). This culture was used to find out the amount of phosphorus released from rock phosphate of Hirapur.

2.1.2 Removal of ferrous iron and silica from phosphate ore

Such a beneficiation was attempted using *Thiobacillus* species on the phosphate ore crushed to -30 mesh size. From the different thiobacilli tried, *Thiobacillus ferrooxidans* strain B-101 from MACS Culture Collection MCM (7) was able to remove 86.5% ferrous iron from the ore along with silica. The phosphorus and silica content was analyzed by standard techniques (8) before and after the treatment and the analysis is shown in Table 3.

2.1.3 Optimization of the beneficiation technique

Since it was observed that instead of only solubilisation of phosphate, the beneficiation achieved by *Thiobacillus* species yielded good results, the optimisation was attempted only with *Thiobacillus* species, which removed the impurities of both silica and iron from the ore.

The growth of the culture was attempted under various conditions of pH, temperature and nutrients to optimize the parameters and the results are reported in Table 4.

3. RESULTS

It can be seen from Table 1 that the ore contains, a high amount of iron and silica. Normal beneficiation operations, such as sieving and cleaning of ore using different techniques have very little effect in removal of these impurities.
Table 1. Chemical analysis of samples of phosphatic rocks from Hirapur, India

<table>
<thead>
<tr>
<th>Element</th>
<th>Average Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaO</td>
<td>65 - 63%</td>
</tr>
<tr>
<td>P(_2)O(_5)</td>
<td>25 - 20%</td>
</tr>
<tr>
<td>SiO(_2)</td>
<td>5 - 3%</td>
</tr>
<tr>
<td>MgO</td>
<td>2 - 0.5%</td>
</tr>
<tr>
<td>Al(_2)O(_3)</td>
<td>1 - 0.5%</td>
</tr>
<tr>
<td>Fe(_2)O(_3) and FeO</td>
<td>5-3 %</td>
</tr>
</tbody>
</table>

It is also seen from Table 2 that even though the *Arthrobacter* species is able to solubilize phosphorus to some extent, the net effect is that most of the phosphorus in the ore remains insoluble, probably due to impurities associated with the ore. Therefore, the second aspect of the studies, i.e. beneficiating phosphatic rocks by removing silica and iron was considered promising and was attempted.

Table 2. Phosphatic solubilizing activity of *Azotobacter* sp., *Arthrobacter* sp. and *Candida* sp. in Pikovskaya's broth

<table>
<thead>
<tr>
<th>Cultures used</th>
<th>% phosphatic solubilization after 7 days</th>
<th>after 14 days</th>
<th>after 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Azotobacter</em> sp.</td>
<td>5.75</td>
<td>10.50</td>
<td>7.04</td>
</tr>
<tr>
<td><em>Arthrobacter</em> sp.</td>
<td>13.82</td>
<td>27.10</td>
<td>17.82</td>
</tr>
<tr>
<td><strong>Yeast:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> sp.</td>
<td>6.74</td>
<td>14.05</td>
<td>8.71</td>
</tr>
</tbody>
</table>

When the *Thiobacillus* strain B-101 was tried, it was found that on an average 86.5% of iron and 30.4% silica impurities were removed at laboratory level (Table 3). Therefore, it was thought that this method could be used for beneficiating the phosphatic rocks from Hirapur after optimization of various parameters for heap leaching on a large scale.

Table 3. Beneficiation of phosphatic ore from Hirapur, India using a culture of *Thiobacillus ferrooxidans* (MCM B-101)

<table>
<thead>
<tr>
<th>Ore analysis</th>
<th>Fe</th>
<th>SiO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>4.5</td>
<td>2.3</td>
</tr>
<tr>
<td>After treatment</td>
<td>0.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Upon optimization, it was found that the *Thiobacillus* culture can optimally grow at pH 3 and at 35° C. It becomes necessary to add a source of sulfur, such as pyrite as part of the nutrient supplement to allow the cultures to grow optimally.

When the experiments are scaled up from the laboratory level to the heap leaching level, it was found that the efficiency of the process decreased slightly, when it was observed that 70% of the iron and 25% silica impurities were removed at that level. However, the process takes place in a continuous manner and therefore is advisable to use. The process parameters for treatment of 300 kg heap of ore are listed in Table 5.

Based on the encouraging results obtained, it is proposed that the beneficiation of rock phosphate first by using microbiological means followed by physical and chemical treatment would be an important first step for producing clean phosphatic rocks for use as fertilizer in India.
Table 4. Optimization of various parameters for growth of *Thiobacillus ferrooxidans* B-101

<table>
<thead>
<tr>
<th>Parameter tested</th>
<th>Growth (cell density in 48 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. pH</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>$4 \times 10^7$</td>
</tr>
<tr>
<td>3.0</td>
<td>$1.3 \times 10^8$</td>
</tr>
<tr>
<td>4.0</td>
<td>$2 \times 10^7$</td>
</tr>
<tr>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>II. Temperature</td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>$0.5 \times 10^7$</td>
</tr>
<tr>
<td>30°C</td>
<td>$6.6 \times 10^7$</td>
</tr>
<tr>
<td>35°C</td>
<td>$1.3 \times 10^7$</td>
</tr>
<tr>
<td>40°C</td>
<td>-</td>
</tr>
<tr>
<td>III. Energy source</td>
<td></td>
</tr>
<tr>
<td>Sulfur (1%)</td>
<td>$4 \times 10^8$</td>
</tr>
<tr>
<td>Ferrous sulfate (47%)</td>
<td>$1.6 \times 10^8$</td>
</tr>
<tr>
<td>Pyrite (1%)</td>
<td>$3 \times 10^7$</td>
</tr>
</tbody>
</table>

Table 5. Beneficiation of phosphatic ore by heap leaching (Heap size = 300 kg)

<table>
<thead>
<tr>
<th>Optimum pH</th>
<th>pH 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Suitable Nutrient (Energy Source)</td>
<td>1% Pyrite</td>
</tr>
<tr>
<td>Process Efficiency Removal of</td>
<td>Fe   SiO₂</td>
</tr>
<tr>
<td></td>
<td>70% 25%</td>
</tr>
</tbody>
</table>

4. DISCUSSION

It was observed earlier in the microbial beneficition of manganese ores, which was tried to remove phosphorus from the ore, that a combination of physical and chemical treatments, such as passing the crushed material after sieving through immersion magnetic separator (physical), followed by chemical precipitation in flotation cells, etc. had very little beneficial effect (9). Even though, some amount of iron and silica content was reduced, due to such treatments, it was not found effective in reducing the phosphorus content of the ore. However, a culture of *Arthrobacter* was observed to leach out 70% to 85% phosphorus from manganese ore (10).

In the present study, it was observed that time 70% iron and 25% silica were removed from the phosphatic rocks in six days using a *Thiobacillus* culture. If physical and chemical beneficiation techniques are tried later on these 'cleaned' ores, it would provide an economically feasible operation to beneficiate phosphatic rocks, to be used as fertilizer. This is being tried at site on 1 t.p.d. plant now.

REFERENCES

Biohydrometallurgy of antimony gold-bearing ores and concentrates

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Abstract
Experimental data on bacterial leaching of antimony-bearing ores of the Sarylakhsk and Sentochansk deposits (Yakutia) and ores of Tajikistan deposits. Bacterial accumulated culture Desulfovibrio desulfuricans have been employed as leaching and chemical agents. Encouraging results have been obtained in isolation antimony into the solution (recovery 96-98%). Thiobacillus ferrooxidans used for flotation selection of Hg minerals from antimonite and for transformation of antimonite into commercial product of antimony trioxide.

1. INTRODUCTION
Antimony gold-bearing ores (Sb₂S₃-Au) are widespread in Russia, People's Republic of China, Republic of South Africa and Bolivia. In the Russian Federation, the main sources of antimony production are sarylakhsk flotation to produce antimony gold-bearing concentrates, obtained by enriching ores of the Sarylakhsk and Sentochansk deposits, Republic Sacha (Yakutia) [1].

The current processing of Au-Sb concentrates is accomplished using pyrometallurgy. The gold is concentrated at the bottom of Au-containing antimony alloy ingots; the Au content ranges from 2 to 4% [2]. Only commercial trioxide of antimony is a salable product.

When a hydrometallurgical alkaline leach method of production is applied, antimony is leached with the extraction of gold in cake. Soluble antimony is then subjected to cathodic electrodeposition to recover antimony [3, 4].

The existing methods for processing gold-antimony concentrates do not solve the problem of their complex use, since these processes do not make it possible to concentrate gold to products suitable for refining. Not all of Russia’s antimony needs are currently met. In this regard there are needs for geological prospecting of new deposits in Yakutia, the Khabarovsk region, the Baikal region, in the Arctic and in Central Ural and new efficient technology developments for processing.

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A widely available cyanidation process is not applicable for gold extraction from Sb$_2$S$_3$-Au ores and concentrates, because of large cyanide consumption due to Sb reaction with NaCN and the locking of Au in the sulfide matrix. Use of thiourea allows to extract gold from gold-bearing stibnite, however, this development is still under laboratory investigation [5]. The problems of antimony leaching can be solved by applying an oxidizing acid leach. Other investigators have used ferric chloride to leach antimonite (stibnite) obtaining antimony trioxide [6-9].

Several microbial processes, including oxidizing and reducing type reactions, which can alter the physio-chemical, sorptive and flotation properties have been evaluated on various antimony minerals [10-12]. Some of these microbial processes are promising. When the surfaces of mercury and antimony sulfides are biomodified, selective separation is possible. It may also be possible to develop new microbial-produced solvents for Sb$_2$S$_3$ that would yield soluble antimony chlorides. Also, conventional bacterial leaching is promising.

Kenzhalov et al. [17] from the Institute of Metallurgy and Mineral Processing, Republics Kazakhstan, have selected a heterothroph, *Pseudomonos aureofaciens*, from a Kazakhstan mineral deposit. They have studied in detail the influence of this heterothroph on antimonite, defining of the kinetics of the reaction, the order of reaction, the velocity constant, and the maximum velocity of product formation—(M$_{max}$ and the Michael’s constant, K$_m$). Rapid dissolution of antimony from antimonite was obtained in the presence of the bacteria (0.11x10$^{-4}$); without the bacteria the rate was 0.29x10$^{-5}$. Bacterial affinity to antimony was quite high (K$_m$ = 0.07-0.37). Dissolution of sulphur surpassed that of antimony.

Sulphate-reducing microorganisms (SRB) are wide-spread in nature. They utilize sulphate-sulphur as an electron acceptor. The most representative organism of the SRBs is *Desulfovibrio desulfuricans*. Intact cells of SRB rapidly reduce sulphate, while simple organic compounds or molecular hydrogen as follows:

\[
\begin{align*}
C_{org} + SO_4^{2-} &= S^{2-} + 2CO_2 \\
4H_2 + SO_4^{2-} &= S^{2-} + 4H_2O \\
SO_4^{2-} + H^+ + 4H_2 &= HS^- + 4H_2O \quad \Delta F = -46410 \text{ kal}
\end{align*}
\]

A number of enzymes participate in the above reactions. Many studies have been conducted using SRBs in flotation of oxidized antimony and lead ores, as a desorbing and depressor in flotation of concentrates, for selection of lead from zinc minerals, and for the separation of molybdenite from chalcopyrite [10]. However researchers pay little attention to utilizing SRBs to produce H$_2$S reagents for ore leaching and especially for leaching antimony-bearing materials. Lyubavina and coworkers (personal communication) have perfected a nutrient medium for the large-scale production of SRBs; this medium is based on the use of linter dust, a waste product of cotton-seed processing, as a carbon source for the organisms.

Only alkaline leaching of antimony sulfides has achieved industrial application. This hydrometallurgical process results in high selectivity for the noble-metal groups, which remains in a leaching cake. Mel’nikov and coworkers found that dissolving antimony sulphide and antimony oxide in sodium sulphide and caustic soda results in sodium thioantimonite and thioantimonate formation according to the following reactions [2]:

\[
\begin{align*}
H_2S + 2NaOH &= Na_2S + 2H_2O \\
Sb_2S_3 + 3H_2S + 6NaOH &= 2Na_3SbS_3 \\
Sb_2S_3 + 4NaOH &= Na_3SbS_4 + NaSbO_2 + 2H_2O
\end{align*}
\]
The objectives of this study were to:

1. Evaluate the use of sulphate-reducing bacteria (SRBs) as agents to produce H₂S for the leaching of gold-bearing antimony sulfide minerals, and
2. Assess antimonite transformation into Sb₂O₃ using *Thiobacillus ferrooxidans*.

2. EXPERIMENTAL

2.1 Materials and methods

Antimonite (an antimony-sulphide mineral) ore and concentrates were investigated. A flotation sulphide concentrate, containing 39.55% antimony, was studied using 250-ml conical flasks at 28°C. An apparatus, specially designed for bacterial leaching, was also employed. Ores and concentrates for study were sterilized by boiling. In some experiments the solutions were filtered from insoluble residue. pH, the number of bacterial cells and antimony content were measured. Some antimony compounds were identified using spectral analyses with a quantometer VRA-2.

For research purposes a SRBs were isolated from the Tyrny-Auz molybdenum wolfram deposit. Postgate medium [12] was used to cultivate the SRBs under laboratory conditions. Using a direct observation method a maximum of 220x10⁶ cells ml⁻¹ of SRBs were found out to grow on the fourth day of culturing. During this period of time 400 mg l⁻¹ of soluble H₂S was obtained; that was 10 times less than the maximum solubility of H₂S in water. SRBs a ready-to-employ reagent solution of H₂S for the leaching process after four days of growth.

2.2 The ores

Ore from the Joint-Stock Co. Gold of Sacha (Yacutiya) was used to produce a flotation concentrate of antimony. This concentrate, produced by the Indigir-Gold plant, contained, %: 55 Sb; 22 S; 0.4 As; 30 g t⁻¹ Au and 3 g t⁻¹ Ag. An antimonite concentrate procured from the Adichanskiy operation at the Sentochanskaya Au-Sb deposit contained, %: 37 Sb; 14 S; 0.16 As; 60 g t⁻¹ Au, and 25 g t⁻¹ Ag. *Thiobacillus ferrooxidans* were cultured from the Bakyrchik and Olimpiadinsk deposits. The experimental testwork was performed in 250-ml Erlenmeyer flasks using 9K medium.

3. RESULTS AND DISCUSSION

3.1 Sulfate-reducing bacteria as antimonite solvents

Recovery of gold from refractory ores requires a pretreatment to liberate the gold particles from the host mineral. The antimony forms stable compound with NaCN during the cyanidation process. Pretreatment is usually an oxidation step. As an alternative, chemical or bioleaching can be applied to liberate the gold particles from the sulfur matrix.

Emphases deserves an operation of sulfide-alkaline leaching as a way of selective separation of stibium from gold-bearing concentrates. Known developped by Irgiredmet (Irkutsk) technology of metallurgical conversion of rich gravity concentrates of Sarylachsk dressing plant (Au – 1050 g t⁻¹; Sb - 65 %) [18]. The scheme includes sulfide-alkaline leaching with the following electrolytic extraction of stibium from solutions. Three-phase recleaning of stibium leaching tailings from concentration tables, melting of secondary gravity concentrates (contents Au – 48 kg t⁻¹) on the dore metal and cyanidation
of tailing from final gravity concentration, received total extraction in corresponding commodity products Au - 98 %, Sb - 96.5%.

The authors demonstrated the technical feasibility recovery of Sb by Na₂S and NaOH leaching, the successive gold solubilisation by conventional cyanidation process and the recovery of Sb and Au from the respective leach solutions by electrowinning [4].

Chemical basic leaching of pure stibnite by Na₂S and NaOH under different experimental conditions at 40°C has been studied in order to optimise the reagents concentrations for the antimony dissolution process. Response surface methodology has been used to find the best experimental concentrations to maximize the Sb extraction yield. 98-100% of antimony recovery was obtained by using 1g Na₂S and 1g NaOH per gram of pure stibnite.

Treatment of a gold-bearing stibnite ore with cyanide yielded only 4% Au extraction; however, after seven days of bioleaching 85.5% Au recovery was attained (Table 1) [13]. Tests were conducted at laboratory scale utilising a refractory stibnite ore [14]. The gold content of the sample was 32 g·t⁻¹. Bacterial cultures utilised in the biological test consisted predominantly of *Thiobacillus*.

### Table 1. Gold recovery by cyanidation, with and without biooxidation [14]

<table>
<thead>
<tr>
<th>Time, hours</th>
<th>Recovery Au, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No bioleaching</td>
</tr>
<tr>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>24</td>
<td>4.0</td>
</tr>
</tbody>
</table>

At laboratory scale was investigated the best conditions for alkaline leaching of a refractory gold-bearing Sb₂S₃ (13.25 Sb₂S₃; 30 g·t⁻¹ Au) coming from South America [19]. The solution was constituted by sodium sulfide and sodium hydroxide. Main parameters studied were: Na₂S concentration, NaOH concentration, pulp density and temperature.

It was reasonable to check a possibility of leaching stibium by bacteria.

The selective flotation and separation of cinnabar from antimonite minerals using *T. ferrooxidans* [15] is illustrated in Figure 1. Bacterial conditioning of 5 h did not affect cinnabar flotation (recovery 89.6%), while the antimonite recovery by flotation decreased from 89 to 6.2%; this led to almost complete selection of the minerals. The results presented in Figure 1 indicate the superiority of biological separation compared to chemical separation.

![Figure 1. Changes in flotation recovery (%) of Sb₂S₃ and HgS minerals in processing by *T. ferrooxidans* from time, h](image)
As a result of bacterial oxidation, antimonite is converted to antimony trioxide, the mineral senarmontite. *T. ferrooxidans* oxidizes the surface of antimonite crystals while cinnabar remains intact. As a result, HgS is floated and extracted into the concentrate, while Sb₂S₃ is coated by a fine film of oxides (Sb₂O₃) and removed in the tailings. Partly regenerated culture can be recycled in this process. When the bacterially-oxidized products of the antimonite (stibnite) concentrate were analyzed by x-ray diffraction, no Sb₂S₃ was observed. However, XRD analysis of the original concentrate revealed intensive lines, belonging to antimonite [16]. Biooxidation also reduced the content of other elements in the stibnite concentrate.

Antimony leaching was done by somewhat different technique. Antimony-containing portion was mixed up with SRB of different hydrogen sulphide concentration and with caustic soda solution. The pulp was heated up to 90°C and it was stirred with S:L = 1:16 ratio. Antimony solubility is most effective at 120 g l⁻¹ caustic soda concentration and maximum hydrogen sulphide concentration in SRB. Special experiments established that the time necessary for leaching of antimony is 1 or 1.5 h. To increase antimony transition into the solution, it is necessary to increase contact time with SRB during leaching. However, when the time of contact was increased, it was necessary to control antimony ions in the solution whose optimal concentration slowed down the leaching process. Therefore, antimony leaching was done in two stages with gradual addition of reagents. The first stage lasted 1 h, after that the solution was decanted, then once again necessary reagents were added and contacted for 0.5 h. Effective antimony leaching was observed under maintained optimal conditions. Isolation of antimony into the solution under those conditions was about 96.5-98.0%.

Results obtained in antimony leaching from antimony-bearing raw materials are given in Table 2. These data show the extent of effective application of bacteria as compared to sodium sulphide in antimony leaching. Furthermore, the suggested technology of antimony leaching has a number of advantages. The presence of intact alkali in electrolyte leads to considerable increase of electric conductivity in the solution and promotes better results of the subsequent electrolysis.

Table 2. Effect of sulphate-reducing bacteria (SRB) and Na₂S on leaching Sb from antimony-bearing materials*

<table>
<thead>
<tr>
<th></th>
<th>Stage I</th>
<th>Stage II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antimonite</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRB, mg l⁻¹</td>
<td>8.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Sb recovery, %</td>
<td>80.5</td>
<td>83.2</td>
</tr>
<tr>
<td><strong>Antimony concentrate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRB, mg l⁻¹</td>
<td>25.4</td>
<td>70.9</td>
</tr>
<tr>
<td>Sb recovery, %</td>
<td>41.3</td>
<td>46.7</td>
</tr>
<tr>
<td><strong>Antimonite</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂S, mg l⁻¹</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Sb recovery, %</td>
<td>51.4</td>
<td>78.8</td>
</tr>
</tbody>
</table>

*NaOH 120 g l⁻¹, leaching time 1 h, temperature 90°C
Thermodynamic calculations show that hydrogen sulphide oxidation occurs at a lesser rate, than that of sodium sulphide. The concentration of $S^{2-}$ and SH$^-$ ions in the pulp containing SRB is weaker than that in the pulp, containing sodium sulphide. Traditional technology of antimony leaching requires higher concentrations of sodium sulphide, than in the case of SRB leaching, the latter reducing yield on the electric current. Given cake is processed by usual methods:
- cyanidation, since contents of stibium does not render influences upon the leaching of gold;
- presence in cake sulfur (more than 14%) must be sodium neutralized;
- by gravity concentration methods in centrifugal devices of Knelson type with following separation of concentrate in ferro-magnetic liquid to receive rich Au-containing concentrate, ready for affinage.

3.2 Transformation of antimonite into antimony trioxide by *Th. ferrooxidans*

Information on antimonite biooxidation is of certain interest [14]. According to the results of investigations carried out by Irgiredmet, Sb$_2$S$_3$ oxidation with *Th. ferrooxidans* was described in [20, 21].

Antimonite biooxidation realized in relatively pliant regime to improve the technological characteristics of cyanided material due to the release of gold associated with Sb$_2$S$_3$ and transformation of antimony to the less active chemical form [16]. It was established that bacterial oxidation of gold-arsenic concentrates by *Th. ferrooxidans* occurred within 100-120 h, the high degree of sulfide oxidation achieved (%) 96-98 of arsenopyrite, 97-98 of pyrrhotite, 92-95 of antimonite, and 65-84 of pyrite [17].

Under biochemical leaching was observed greater amount of the oxidized forms of stibium and also its oxides of high valences regardless of initial contents of stibium minerals. After bacterial influence an intensity of lines D 5.05 D 5.66 A, belonging to Sb$_2$S$_3$.

Thionic bacteria oxidized animonite sulfur and formed antimony trioxide of cubic syngony of senarmontite type (the heat of formation of $\Delta H_{278} = 165.5$ kJ) according to the following reactions:

$$2\text{Sb}_2\text{S}_3 + 12\text{O}_2 + 6\text{H}_2\text{O} = \text{Sb}_4\text{O}_6 + 6\text{H}_2\text{SO}_4 \quad (8)$$

$$3\text{Sb}_2\text{S}_3 + 3\text{Fe}_2(\text{SO}_4)_3 + 6\text{H}_2\text{O} = \text{Sb}_4\text{O}_6 + 12\text{FeSO}_4 + 3\text{S} \quad (9)$$

$$\text{S} + \text{H}_2\text{O} + 3/2\text{O}_2 = \text{H}_2\text{SO}_4 \quad (10)$$

$$2\text{FeSO}_4 + 1/2\text{O}_2 + \text{H}_2\text{SO}_4 = \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O} \quad (11)$$

$$\text{Sb}_4\text{O}_6 + \text{H}_2\text{SO}_4 = 2\text{Sb}_2(\text{SO}_4)_2 + 6\text{H}_2\text{O} \quad (12)$$

The rhombic form of Sb$_2$O$_3$ is obtained in hydrolysis of antimony-chloride solutions. Table 3 presents the data of influence exerted by bacterial processing on the change in phase content of minerals under different conditions of the experiments. The action of diluted H$_2$SO$_4$ on antimony sulfate results in hydrolysis with formation of antimonyl sulfate:

$$\text{Sb}_2(\text{SO}_4)_3 + 2\text{H}_2\text{O} = (\text{SbO})_2\text{SO}_4 + 2\text{H}_2\text{SO}_4 \quad (13)$$

Senarmontite processed into antimony trioxide by the reactions:

$$\text{Sb}_2\text{O}_6 + 12\text{HCl} = 4\text{SbCl}_3 \quad (14)$$

$$2\text{Sb}_2(\text{SO}_4)_2 + 6\text{HCl} = 2\text{SbCl}_3 + \text{H}_2\text{SO}_4 \quad (15)$$

$$\text{SbCl}_3 + \text{H}_2\text{O} = \text{SbOCl} + 2\text{HCl} \quad (16)$$
SbOCl + 2NH₄OH = Sb₂O₃ + 2NH₄Cl + H₂O \quad (17)

**Table 3. Transformation minerals by *Th. ferrooxidans***

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Initial content, %</th>
<th>Contents after treatment, %</th>
<th>Samples numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1</td>
<td>No 1'</td>
<td>No 2</td>
</tr>
<tr>
<td>Senarmontite</td>
<td>0</td>
<td>94.6</td>
<td>93.9</td>
</tr>
<tr>
<td>Antimonite</td>
<td>71.7-81.5</td>
<td>26.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Pyrite</td>
<td>13.3-6.8</td>
<td>0.1</td>
<td>—</td>
</tr>
<tr>
<td>Quartz</td>
<td>15.0-11.7</td>
<td>2.7</td>
<td>46.0</td>
</tr>
</tbody>
</table>

![Flow sheet of processing Au-Sb concentrates](image)

**Figure 2. Recommended flow sheet of processing Au-Sb concentrates**

Selected stibium trioxide, the contents not below 99.5% Sb₂O₃ and recovery of stibium in the product - 90.5%. On the contents of allowing admixtures stibium trioxide corresponds to analytical grade (Tech.Cond. 6-09-3267-84) and exceeds requirements for the lavsan production (Tech.Cond. 6-09-2897-77). Offered technological conversion scheme for Sb-Au concentrates settles problems of their complex using and allows to get high-quality stibium trioxide and gold-containing product.

One of the ways of oxidation of stibium minerals is a hydrogen peroxide oxidation, prodused by heterothrofic bacteria [17]. In the reactionary ambience, oppressing development of bacteria, occurs improvement of respiratory activity that brings about hypersynthesis of H₂O₂. Senarmontite disolves in the alkaline water-glycerin medium containing 250-300 g l⁻¹ glycerin, 50-60 g l⁻¹ NaOH, and 100-20 g l⁻¹ Sb with the complex formation:
Sb$_2$O$_3$ + 2NaOH + 2C$_3$H$_8$O$_3$ = 2NaOSbO$_2$C$_3$H$_5$OH + 3H$_2$O \hspace{1cm} (18)

Leaching was carried out for 30-60 min without electrolyte heating and with mechanical air mixing. During electrolysis of water-glycerin solutions, antimony was separated from the complex anion on the cathode:

\[
\text{SbO}_3 \text{C}_3\text{H}_5\text{OH}^- + \text{H}_2\text{O} + 3e^- = \text{Sb} + 2\text{OH}^- + \text{C}_3\text{H}_5\text{O}_2\text{OH}^2^- \hspace{1cm} (19)
\]

Thus, the technological scheme is proposed for producing antimony trioxide or cathode antimony (Fig. 2). Realization of the technology will make it possible to begin development of the Sentachansk deposit (Yakutia).

The bacterial transformation of Sb$_2$S$_3$ into Sb$_2$O$_3$ from gold-antimony concentrate favours production of the material suitable for cyanidation. In this case, several advantages can be expected:

1. An increase in market cost of antimony in concentrate due to its production in the form of trioxide (up to 83.53%), decrease in volumes of ore-mass transportation, and reduction in arsenic content in bacterial processing.
2. Reduction in time for gold and silver return to metallurgical conversion.
3. Removal of arsenic from concentrates, which will favour improvement of their quality and release of gold-bearing minerals.
4. Creation of small-scale production of antimony trioxide and diluted sulfuric acid suitable for ore processing and other purposes directly in the deposit.

The bacterial transformation of antimony sulfide in trioxide from gold-antimony concentrates produced material applicable for the cyanidation. Herewith possible expect a number of essential advantages.

4. CONCLUSIONS

1. The most advanced results in bioleaching of minerals were achieved with thiobacteria on sulphides of iron and some non-ferrous metals. These data showed the extent of effective application of bacterial strains as compared to sodium sulphide in antimony leaching. Furthermore, the suggested technology of antimony leaching has a number of advantages. Antimony recovery, as well as transformation of sulfates into Sb$_2$O$_3$, discussed here, are of great interest.
2. Increased stibium market value in the concentrate as Sb$_2$O$_3$ (up to 83.53%) in contrast with Sb$_2$S$_3$ (71.69%) and reduced volumes of transportation, reduction of arsenic contents in the process of biological conversion.
3. Increased return of gold and silver to metallurgical processing.
4. Removal of arsenic from concentrates promotes both: a raise of concentrate quality and opening gold-containing minerals.

REFERENCES

Bioleaching of Argentinean sulfide ores using pure and mixed cultures

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Abstract

The objective of this work was to evaluate the efficiency of the bioleaching of three different Argentinean sulfide ores using pure and mixed cultures of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The samples used were obtained from La Silvita, La Resbalosa and Mallín Quemado ores. Their main constituents are quartz, galena, sphalerite, and chalcopyrite and their chemical composition includes 8.98% Zn, 0.046% Cu, 5.86% Pb and 0.55% Mn (La Silvita sample), 17% Zn, 0.064% Cu, 2.37% Pb and 0.56% Mn (La Resbalosa sample) and 7.95% Zn, 0.022% Cu, 64.7% Pb and 1.5% Mn (Mallín Quemado sample). Bioleaching experiments were carried out in glass columns with the percolation of the medium through the minerals. Solubilized metals (zinc, copper, manganese and total soluble iron) were determined using an atomic absorption spectrophotometer while iron(II) was measured by titration. Solid residues recovered by filtration were analyzed by means of X-ray diffraction. Metal recoveries from La Silvita and La Resbalosa were significantly enhanced by inoculation. The highest extraction was reached using *Acidithiobacillus ferrooxidans*.

Keywords: bioleaching, sulfide ores, zinc, *acidithiobacillus*

1. INTRODUCTION

Bioleaching is applied to ores which cannot be treated economically by conventional processes like flotation and roasting [1]. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* bacteria are capable of acting directly or indirectly on metallic sulfides, oxidizing the sulfides to sulfate and thus releasing the metals in those cases in which the respective sulfates are soluble. For this reason, these microorganisms were traditionally used in bioleaching processes of low-grade ore on a commercial scale [2].

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Although there are several minerals amenable to bioleaching or biooxidation on a commercial scale, only two metals, copper and gold, are currently recovered using this technology. Nowadays, important efforts are being done to adapt heap biooxidation technology to treat sphalerite concentrates [3]. On the other hand, in western Patagonia (Argentina) there are some reservoirs containing important amounts of base metal sulfides, which could be suitable to biohydrometallurgy commercial application. Therefore, it is interesting to study the application of this technique at these ores thoroughly. The objective of this work was to evaluate the efficiency of the bioleaching process of three different complex Zn-Mn-Fe-Pb sulfide ores from Neuquén (Patagonia-Argentina) using pure and mixed cultures of *A. ferrooxidans* and *A. thiooxidans*.

2. MATERIALS AND METHODS

2.1 Microorganisms and media

Pure and mixed cultures of *A. ferrooxidans* (DSM 11477) and *A. thiooxidans* (DSM 11478) were used throughout this study. The first strain was cultivated routinely in 9K medium of initial pH 1.80 [4]. The cells were harvested when the culture had consumed 90% of the iron (II) available. The culture was filtered through blue ribbon filter paper to retain the jarosite deposits and then through a filter Millipore of 0.22 microns in order to retain the cells. Cells were washed at least twice with iron-free 9K medium and finally resuspended in the same medium pH 1.8. The second strain was cultivated routinely in iron-free 9K medium with sulfur as energy source. The cells were collected when the pH descended below 1.0. The procedure for the preparation of the inoculum was similar to the one applied for the other strain. These suspensions (with bacterial populations of approximately 2x10^8 cells/ml) were used as inoculum at the 10%v/v.

2.2 Mineral

The samples used were obtained from La Silvita, La Resbalosa and Mallín Quemado ores (Province of Neuquén, Patagonia Argentina). Chemical Analysis and Mineral Composition of the samples are given in Table 1.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Fe (%)</th>
<th>Zn (%)</th>
<th>Pb (%)</th>
<th>Mn (%)</th>
<th>Cu (%)</th>
<th>Mineral Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>La Silvita</td>
<td>15.03</td>
<td>8.98</td>
<td>5.86</td>
<td>0.54</td>
<td>0.046</td>
<td>ZnS 5.5%, PbS 3%, FeS2 12%, CuFeS2 0.5%</td>
</tr>
<tr>
<td>La Resbalosa</td>
<td>9.34</td>
<td>17.01</td>
<td>2.37</td>
<td>0.56</td>
<td>0.064</td>
<td>ZnS 12%, PbS 2%, FeS2 8%, CuFeS2 1%</td>
</tr>
<tr>
<td>Mallin Quemado</td>
<td>2.94</td>
<td>0.795</td>
<td>64.75</td>
<td>1.55</td>
<td>0.022</td>
<td>PbS 96%</td>
</tr>
</tbody>
</table>

2.3 Detection of indigenous bacteria

In order to detect sulfur or iron oxidizing bacteria two tests in 250 ml shake flasks on a rotatory shaker at 180 rpm and 30±0.5 °C were carried out. Each flask, containing the ore (pulp density of 10%), was respectively filled with 100 ml of the appropriate liquid medium (9K iron free pH=3.0 supplied with 1% w/v of sulfur powder or 9K medium pH=1.8).
2.4 Experiments in columns

Tests were conducted in 50 mm inside diameter glass columns with 260 mm high at constant temperature of 30°C ± 0.5°C. Columns have a perforated plate in the bottom and a layer of glass wool to support the ore sample. Thirty grams of each ore sample with particle size ranging between 10 and 16 mesh were used in every column under flood conditions.

Each column was charged with 300 milliliters of free-iron 9K medium at initial pH 1.8. Percolating solution was recirculated from the open top of the column to the bottom by continuous airflow rate at 1.2 VVM. In order to reach a pH condition compatible with the bacterial growth it was necessary to achieve an acid stabilization before the inoculation. Therefore, the pH value was initially adjusted at 1.8 by adding drops of sulfuric acid solution (4.8 N) and then it was not controlled again. The amount of sulfuric acid added was used to calculate the initial acid consumption. After the pH stabilization, the inoculation was done. Sterile control columns were prepared replacing the same volume of inoculum by a solution of 2% timol in methanol. Additionally, uninoculated control columns were prepared to check indigenous bacterial activity. Sterile distilled water was added to compensate evaporation. Samples from every column were taken at regular intervals.

2.5 Analytical methods

Copper, total iron, manganese and zinc in solution were determined by atomic absorption spectrophotometry. Iron (II) concentration was determined by permanganometry. Bacterial populations in solution were determined using a Petroff-Hausser camera in a microscope with a contrast phase attachment. This determination was not representative of the bacterial growth because the cells attached to the mineral were not determined. Both the redox potential and the pH were measured with specific electrodes Sulfuric acid production was analyzed by titration with sodium hydroxide solution. Solid residues were recovered by filtration and analyzed by X-ray diffraction (XRD) in Rigaku DII-Max equipment.

3. RESULTS AND DISCUSSION

3.1 Detection of indigenous bacteria

After 25 days, ferrous iron was completely oxidized, but no free cells in media were observed at microscope. On the other hand, the pH values in the systems supplemented with sulfur did not decrease. Moreover, the pH remained almost constant in the case of La Silvita (pH 3.5) whereas it increased in La Resbalosa and Mallín Quemado (pH 4.9).

Since it was not possible to detect free bacterial cells, ferrous iron was oxidized more slowly than the usual by *A. ferrooxidans* and cells were not able to oxidize sulfur as energy source, it is possible to suggest that the indigenous bacterial activity was due to the presence of *Leptospirillum ferrooxidans*-like microorganisms. As it is known, these bacteria grow preferentially adhered to surfaces and their physiology is consistent with the result observed [5].

3.2 Experiments in columns

The experiments took one hundred days. After that period, the solid residues were removed from the columns and analyzed by XRD. The main mineralogical species originally present in the ores remained until the end of all leaching process indicating that
the oxidation of sulfides associated with the dissolution of metals was not complete. Additionally, after the leaching processes it was possible to identify sulfur and jarosite from La Silvita and La Resbalosa ores both in the biotic and abiotic systems. Galene present in the ore treated was oxidized to anglesite only in the biotic systems showing that bioleaching processes were important to improve this phase transformation.

The initial sulfuric acid consumption before the inoculation was: 39.2 g H₂SO₄/kg ore to La Silvita, 40.5 g H₂SO₄/kg ore to La Resbalosa and 43.5 g H₂SO₄/kg ore to Mallín Quemado, which indicates the presence of slightly alkaline gangue content such as carbonate minerals.

### 3.3 La Silvita

Figure 1 shows the percentages of zinc and manganese solubilized from La Silvita ore during the test. The highest zinc extraction (75%) was obtained in the column with *A. ferrooxidans*. In addition, zinc solubilization in the column with mixed culture (48%) was significantly higher than the one with *A. thiooxidans* (22%). The performance of the last column was similar to the uninoculated one (21%) throughout the experiment. The zinc extraction in the sterile column only reached 7%. These results suggest that *A. ferrooxidans* plays a key role in the bioleaching process for La Silvita ore contributing to release the zinc from the sphalerite. Meanwhile *A. thiooxidans* as pure or mixed cultures was not so efficient to improve the Zn solubilization. This was probably due to the mineralogical species present in this natural ore, since the extraction obtained in this work was lower than those reported using synthetic sulfide [6].

In contrast with the zinc solubilization, the amount of manganese released from La Silvita ore was higher when *A. thiooxidans* was present in the cultures. Figure 1 shows that the behavior displayed by this pure culture was very similar to the mixed one, reaching in both cases a manganese extraction close to 100%. Since the manganese solubilization was higher in presence of *A. thiooxidans* or mixed cultures and considering that these systems reached pH values lower than the other systems (Fig. 2), manganese could be present as a mineralogical species easily leachable by acid. This hypothesis could not be confirmed by XRD analysis probably due to the low amount of manganese present in this ore.

In Figure 2, the pH evolution can be observed. During the first twenty-five days, all columns showed an increase of pH values indicating that the solubilization of some basic species present in the mineral continued beyond the inoculation. After that period, pH values decreased in all biotic system around 1.7 while the pH value increased to reach a value of 2.6 in the sterile system. These results could indicate that sulfur obtained from the redox dissolution of sulfides and identified by XRD analysis of leached residues, was partially oxidized by bacterial action and contributed to the acid production. Therefore, the whole bioleaching process had a positive acid balance.

Additionally, in Figure 2, the redox potential evolution can be observed. Eh rapidly increased during the first days of the experience in columns inoculated with *A. ferrooxidans* reaching values over than 550 mV. Then, Eh values oscillated around this threshold until the end of the experiment. The uninoculated system took more than twenty days to reach the same final value. In the sterile column Eh remained constant close to the initial value.
Figure 1. Comparison of zinc and manganese extraction from La Silvita ore using pure and mixed cultures of *A. ferrooxidans* (Af) and *A. thiooxidans* (At) with uninoculated and sterile control systems.

Figure 2. Evolution Eh and pH values from the La Silvita under different conditions: *A. ferrooxidans* (Af) cultures, uninoculated system and sterile control.

Figure 3 shows the evolution of total soluble iron and ferrous iron concentrations in columns inoculated with *A. ferrooxidans*, uninoculated and sterile controls. The iron evolution was completely in agreement with Eh values shown in Figure 2. Ferrous iron was rapidly oxidized in *A. ferrooxidans* column and slightly slower in the uninoculated one. On the other hand, the ferrous iron remained reduced in the sterile controls reaching 2.5 g/l. Additionally a significant amount of iron was released from La Silvita ore during the leaching process mainly in *A. ferrooxidans* column (12 g/l).
These results show: i) \textit{A. ferrooxidans} cultures remained active along the experience and the cells in suspension reached a value of $2.5 \times 10^8$ cells/ml after one hundred days of operation. ii) This microorganism contributed to increase the solubilization of pyrite and other iron species. And iii) the bacterial activity detected in the uninoculated column was probably due to another ferrous iron oxidizing \textit{L. ferrooxidans}-like bacteria.

From Figures 2 and 3 the total iron dissolved with the pH evolution can be correlated either in the sterile or biotic systems. The amounts of iron dissolved were minimal when the values of pH were maximum. Correspondingly, an abundant amount of jarosite and other ferric oxyhydroxides covering solid residues was visually observed and then detected by X ray diffraction analysis.

3.4 La Resbalosa

Figure 4 shows the percentages of zinc and manganese solubilized from La Resbalosa ore during the test. In the column with \textit{A. ferrooxidans} the highest extraction of zinc was obtained, reaching 17.5%. In addition, zinc solubilization in the column with mixed culture (12.4%) was slightly higher than the one observed in the column inoculated with \textit{A. thiooxidans} (9.7%) and in the uninoculated system (9.3%). The sterile control only removed 2.6% of zinc initially present in the ore.

Figure 5 shows pH evolution and total iron solubilized throughout the experience. When the pH values increased, the iron in solution decreased. In \textit{A. ferrooxidans} inoculated column the solubilization of different basic mineralogical species present in the ore caused an increase of pH values of over 3.3. After that, an important precipitation onto the mineral surface was observed. Moreover, the zinc extraction and the bacterial counts ($1 \times 10^8$ bacteria/ml) were lower than those obtained from La Silvita. It was probably associated with diffusional barriers due to the large amount of brown amorphous ferric precipitated, which avoided further sphalerite dissolution.

The inoculated systems with \textit{A. thiooxidans}, as pure or mixed cultures reached the highest manganese solubilization (Fig. 4). Although La Resbalosa and La Silvita ores
showed a similar behavior, in the first ore the percentage of manganese extraction was only 70%. This was probably due to the fact that pH values reached in La Resbalosa systems were higher than those obtained in La Silvita.

![Graph showing zinc and manganese solubilization](image)

**Figure 4.** Comparison of zinc and manganese extraction from La Resbalosa using pure and mixed cultures of *A. ferrooxidans* (Af) and *A. thiooxidans* (At) with uninoculated and sterile control systems.

![Graph showing evolution of iron concentration and pH](image)

**Figure 5.** Evolution of total iron and pH from La Resbalosa using pure and mixed cultures of *A. ferrooxidans* (Af) and *A. thiooxidans* (At) with uninoculated and sterile control systems.

### 3.5 Mallín Quemado

Figure 6 shows the evolution of zinc solubilization from Mallin Quemado ores. In contrast to the bioleaching experiences using La Silvita and La Resbalosa ores, Mallin Quemado reached higher Zn extraction when *A. thiooxidans* cultures were present. On the
other hand, the Zn solubilization was very similar in the *A. ferrooxidans* inoculated, uninoculated and sterile systems, and the metal extractions were not relevant in all these flasks. pH values at the end of the test were 2.0 in *A. thiooxidans* pure and mixed cultures and 2.3 in the other biotic systems while the sterile system reached a pH of 3.5. Eh values were increasing according to ferrous iron oxidation (data not shown).

![Figure 6. Comparison of zinc and manganese extraction from Mallín Quemado ore during leaching experiences using pure and mixed cultures of *A. ferrooxidans* (Af) and *A. thiooxidans* (At) with uninoculated and sterile control systems](image)

The manganese extraction was higher in the systems inoculated with *A. thiooxidans* cells. The different percentages of manganese removed could be attributed to the final pH in each system.

The final percentages of Zn and Mn solubilized from Mallín Quemado ore were lower than those obtained using the other minerals. Bacterial counts were also very low (0.6 x 10^8 bacteria/ml) when this high-grade galena ore was tested. These results could indicate that the great lead percentage in the sample may inhibit the bacterial growth. Meanwhile the low solubility of lead (II) reduced the possibility of toxicity when it is present in low-grade ores as La Silvita and La Resbalosa [7].

4. CONCLUSIONS

The bioleaching treatment of La Silvita complex sulfide ore by bioleaching process appears to be technically feasible, since the zinc solubilization was increased ten fold when *A. ferrooxidans* was used as inoculum. Although La Resbalosa tests showed that the bioleaching processes were amenable, they could be improved if a rigorous pH control is done to avoid great oxides precipitation onto the mineral surface. Mallin Quemado tests did not show significant zinc extraction but it was possible to oxidize galene. Further tests should be carried out to analyze the extension of this transformation and the effects of lead toxicity on the bacterial grow.

In summary, the results analyzed here are good enough to consider the metal biohydrometallurgical extraction as a promissory method to be applied for these regional ores to be treated using bacterial heap leaching technique. Moreover, future studies
involving isolation and identification of indigenous bacteria should be done in order to improve the bioleaching treatment of these Argentinean ores.

REFERENCES
Bioleaching of complex gold-lead ores

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Abstract

The present work is relevant to bioleaching of galena from gold-lead ore using Acidithiobacillus such as Thiobacillus ferrooxidans and Thiobacillus thiooxidans in the presence of magnetite as well as under action of DC electric field, which resulted in the acceleration of sulfide dissolution from the natural and synthetic galena. Various electrochemical mechanisms in the leaching process were considered. The electrokinetic properties of the thiobacteria under the conditions of galena microbial leaching were also studied.

Keywords: galena; bioleaching; magnetite; galvanic couple; zeta-potential

1. INTRODUCTION

In recent years, the use of microorganisms for metal solubilization from ores has increasingly attracted the attention of hydrometallurgists and biotechnologists. Microbial leaching is characterized by low cost, and its realization continues to create fewer problems compared to corresponding hydrometallurgical or pyrometallurgical processes. The bioleaching method is based on the ability of some microorganisms to oxidize Fe (II) ions or reduced sulfur compounds. As a result of the accumulation of sulfuric acid in the biosuspension, the decrease in pH and the metal solubilization from sulfides takes place. The cultures Thiobacillus ferrooxidans and Leptospirillum ferrooxidans are most-used [1].

Galena is the main industrially available source of lead. Hydrometallurgical processes for lead leaching have been studied by a number of researchers [2]. Quite a few studies have been dedicated to the use of microorganisms for lead leaching from lead-bearing ores [3, 4]. In gravitation and flotation separation of ores where the gold-bearing minerals are associated with the sulfide minerals of non-ferrous metals one obtains concentrates enriched with these metals, namely, lead. The Muzhiyev gold-mining deposit (Eastern Carpathians, Ukraine) belongs to such an ore deposits. A gravity concentrate with a galena upwards of 40% is produced at the operation. To avoid the formation of matte (melted sulfide), which is capable of dissolving a significant part of the gold during melting, the cleaner concentrate needs to be subjected to either an oxidizing roasting or some other process to remove the sulfur, arsenic, and antimony. Roasting, however, volatilizes sulfur and arsenic. The pyrometallurgical method for processing sulfide concentrates, therefore, has limits as to its application both economically and from the standpoint of environmental safety. Microbial oxidation of sulfide minerals (concentrates) is proposed as a viable alternative to the pyrometallurgical method.
Tomizuka and Yagisawa [4] examined the role of bacteria and proposed a base scheme for galena leaching in the presence of *T. ferrooxidans*; the leaching mechanism includes the following reactions:

\[
\begin{align*}
2\text{PbS} + 2\text{H}_2\text{SO}_4 + \text{O}_2 & \rightarrow 2\text{PbSO}_4 + 2\text{H}_2\text{O} + 2\text{S} \quad (1) \\
2\text{S} + 2\text{H}_2\text{O} + 3\text{O}_2 & \rightarrow 2\text{H}_2\text{SO}_4 \quad (2)
\end{align*}
\]

According to these authors, at low pH values sulfur can serve as the sole energy source for the autotrophic microorganisms. The elemental sulfur is released as a result of the electrochemical reaction with oxygen (reaction 1), and further, sulfur serves as a substrate for thiobacteria which produce sulfuric acid (reaction 2).

The aim of this investigation was to establish whether it is possible to improve the kinetics and the process efficiency of galena microbial leaching from the gold-bearing ore of the Muzhiyev deposit by the addition of natural iron-bearing raw material (for example, magnetite). Attention was also paid to the electrosurface properties of thiobacteria and galena in the microbial leaching process.

### 2. MATERIALS AND METHODS

The middlings of gravity separation (MGS) of the gold-bearing ore from the Muzhiyev deposit, which was produced in a Knelson centrifugal concentrator, was investigated. The phase composition of this sample was determined by four main components: galena PbS (~42%), barite BaSO$_4$ (~20%), pyrite FeS$_2$ (~6%), and quartz. The sample also contained individual gold grains. MGS was very similar to the concentrate and differed from it only by low gold content. The sample was ground -0.063 mm.

The mixed culture, containing mainly *T. ferrooxidans* as well as *T. thiooxidans*, was used for the microbial leaching. The culture was cultured from the samples of the same deposit and was adapted to a 10% (w/v) galena pulp density for 10 weeks. The accumulated culture was grown in basal 9K medium. The experiments were conducted in 0.5-L shaker flasks with 10 g of MGS, 2.5-15 g of magnetite, 50 ml of cell biosuspension (inoculum) and 100 ml of 9K medium without iron at pH 2.1. The stirring speed was 150 rpm. The magnetite sample contained 64.5% of Fe$_3$O$_4$; the moisture content of the product was 10.5%. The efficiency of the microbial leaching (%) was estimated from the sulfide-sulfur content (% w/w) of the dried mineral sample before and after treatment by bacteria; the iodine method of analysis was used. For this purpose the sample was dissolved in HCl; released hydrogen sulfide was absorbed by ammonia solution of zinc sulfate. The precipitate formed was dissolved in the mixture of HCl and titrated iodine solution; the sulfur quantity was determined on the iodine excess in the solution. The Fe$_3$O$_4$ dissolution was assessed on the Fe$^{2+}$ and Fe-total concentration by colorimetric method with ortophenanthroline.

The PbS electrode for rest potential investigations was prepared in the following way. The side surface of a circular graphite electrode with a cross-sectional area 0.3 cm$^2$ was embedded in epoxy insulating glue. The working face surface was first polished and then rubbed with a finely dispersed powder of the synthetic PbS. Excess powder was removed with distilled water. Each electrode was placed in a vessel with 9K medium and bacterial inoculum. Stirring in the vessels with the biosuspension was accomplished with a magnetic-stirrer. The rest potential measurement was made with each electrode using an Ag-AgCl reference electrode. Zero time was considered to be the "control" without the microorganism action (Table 2).
The experiments on the influence of an electric field on the rate of leaching were carried out in the following way. Suspensions containing either MGS or synthetic PbS, the mixed thiobacteria culture, and 9K medium with 44.5 g/L iron (II) sulfate at pH 2.1 were prepared. All vessels containing the suspension were incubated on the shaker. Two vessels, one containing synthetic PbS and one containing MGS, served as controls; no electric field treatment was applied to the control vessels. The graphite electrodes were introduced into the other two vessels, and periodically an electric field with a voltage of 0.5 V/cm (anode potential was +0.22V C.S.E.) was applied. The duration of the electric field treatment was 5 min. followed by a pause of 10 min. The treatment by the discontinuous DC field was conducted in vessels with non-separated cathode and anode chambers.

The electrophoretic mobility of *T. ferrooxidans* M1 was measured by the microelectrophoresis method in different buffers: in the citrate buffer of McIlven and in universal buffer mixture composed of phosphoric, acetic, and boric acids; these measurements were recalculated into the electrokinetic potential ($\zeta$) using the Smolouchowsky formula.

All the said experiments were carried out at least by duplicate.

### 3. RESULTS AND DISCUSSION

#### 3.1 Galena oxidation in mixed biomineral suspension

As seen from data given in Table 1 the MGS sample placed in the iron-free 9K medium was slowly oxidised. The galena oxidation reaction in water is described by this equation:

$$\text{PbS} + 4\text{H}_2\text{O} \rightarrow \text{PbSO}_4 + 8\text{H}^+ + e$$

(3)

The formation of insoluble anglesite (PbSO$_4$) promotes the reaction shift to the right. However, the formation of an insoluble film on the surface of the mineral particles decreased the kinetics of the oxidation process. As a result, about 21% of the sulfides was oxidized from the original mineral sample in iron-free 9K medium in nine days.

The second sample, which contained thiobacteria in the iron-free 9K medium with the MGS sample, was significantly oxidized. In three days about 73% of the sulfide was oxidized; in 9 days 82.5% of sulfide was oxidized. As can be seen, thiobacteria significantly improved both the oxidation kinetics and degradation of the mineral. Chemically produced sulfur (equation 1) served as the substrate for the *T. thiooxidans*, which metabolized it to sulfuric acid (equation 2). The data given in Table 1 for the sulfide oxidation (item 2) indicate that galena can serve as the energy source for the mixed culture resulting in degradation of the galena-containing ore. That is a reason why the degree of the sulfide destruction in the second sample considerably exceeded that of the first sample that did not contain bacteria.

The data on the change in rest potential of lead sulfide with an increase in bioleaching time is testimony to the changes in the sulfide surface composition and the electrochemical properties of the surface. Table 2 gives the values for the electrode made from natural galena [5] and the electrode made from the synthesized PbS from day 0 through 14 days of leaching with the thiobacteria. The interaction between the mineral and thiobacteria was accompanied by the increase in rest potential with respect to that measured in fresh nutrient medium. The positive potential increases with the bioleaching time. Comparing the galena mineral and PbS electrodes, it is seen that pure product was oxidized more intensively because it had higher positive potential values. Note, however,
that the rest potentials for both the mineral and powder electrodes were not large. The reason for this phenomenon was discussed earlier; it is the formation of reaction products on the electrode surface diminishes oxidation.

Table 1. The sulfide oxidation and Fe$_3$O$_4$ dissolution the mixed biomineral suspension

<table>
<thead>
<tr>
<th>No. and sample composition</th>
<th>$S^{2-}$ before leaching (%)</th>
<th>$S^{2-}$ in the oxidised ore (%)</th>
<th>Sulfide oxidation (%)</th>
<th>$\text{Fe}^{2+}/\text{Fe}^{3+}$</th>
<th>$\text{Fe}_3\text{O}_4$ dissol. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In 3 days</td>
<td>In 9 days</td>
<td>In 3 days</td>
<td>In 9 days</td>
</tr>
<tr>
<td>1. MGS</td>
<td>5.6</td>
<td>5.2</td>
<td>4.4</td>
<td>7.2</td>
<td>21.5</td>
</tr>
<tr>
<td>2. MGS + bacteria</td>
<td>5.6</td>
<td>1.5</td>
<td>0.8</td>
<td>73.2</td>
<td>85.7</td>
</tr>
<tr>
<td>3. MGS + 2.5 g of Fe$_3$O$_4$</td>
<td>4.5</td>
<td>3.8</td>
<td>2.8</td>
<td>25.6</td>
<td>37.8</td>
</tr>
<tr>
<td>4. MGS + bacteria + 2.5 g of Fe$_3$O$_4$</td>
<td>4.5</td>
<td>0.6</td>
<td>0.5</td>
<td>86.7</td>
<td>89.0</td>
</tr>
<tr>
<td>5. MGS + bacteria + 5 g of Fe$_3$O$_4$</td>
<td>3.7</td>
<td>0.15</td>
<td>0.13</td>
<td>96.0</td>
<td>96.5</td>
</tr>
<tr>
<td>6. MGS + bacteria + 10 g of Fe$_3$O$_4$</td>
<td>2.8</td>
<td>0.31</td>
<td>0.30</td>
<td>88.9</td>
<td>89.3</td>
</tr>
<tr>
<td>7. MGS + bacteria + 15 g of Fe$_3$O$_4$</td>
<td>2.2</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>86.6</td>
</tr>
</tbody>
</table>

Table 2. Stationary potential of PbS-electrode in the 9K medium with microorganisms

<table>
<thead>
<tr>
<th>Bioleaching time, days</th>
<th>Rest potential of natural galena [5], mV (C.S.E.)</th>
<th>Rest potential of PbS-electrode, mV (C.S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>68</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>140</td>
</tr>
<tr>
<td>14</td>
<td>110</td>
<td>159</td>
</tr>
</tbody>
</table>

Now we shall address the experiments in which magnetite was introduced simultaneously with thiobacteria. It is known that iron (II) as well as the reduced sulfur species can serve as an energy source for thiobacteria; this property is widely used for the microbial leaching of ores that contain sulfides with iron (e.g. pyrite, arsenopyrite, chalcopyrite, and pyrrotite) [6]. The majority of thiobacteria can use these substrates in their metabolism. The role of the added magnetite may iron (II) generation from the dissolution of Fe$_3$O$_4$. The autotrophic bacteria, in turn, form the oxidizing medium by generating the Fe (III) ions.

Galvanic interactions among different minerals are known and sometimes used to promote the leaching of sulfide minerals [7-8]. The electrochemical concept of many galvanic leaching systems is the basis of these processes [9]. In our case the role of the added magnetite in promoting galena leaching from ore may be due to coupling the electrochemical reactions of magnetite reduction and galena oxidation.

The cathodic reaction under acidic conditions could be:

$$\text{Fe}_3\text{O}_4 + 6\text{H}^+ + 2e \rightarrow \text{FeO} + 2\text{Fe}^{2+} + 3\text{H}_2\text{O}$$  \hspace{1cm} (4)
The anodic reaction of galena oxidation to sulfur and then to sulfate could proceed according to polysulfide mechanism [10]:

\[ \text{PbS} + 4\text{H}_2\text{O} \rightarrow \text{PbSO}_4 + 8\text{H}^+ + 8\text{e} \]  

However, the run of such galvanic pair can be retarded due to the formation of sulfur film on surface of galena particles and to low Fe\(^{3+}\) concentration in solution. Indeed, the sulfur leaching from galena in the magnetite presence was higher as compared in the absence of this addition, but in the whole remained still low (item 3, Table 1).

The availability of the autotrophic bacteria and their metabolites promote galena oxidation by providing the Fe (III) generation and increasing the oxidizing conditions in a bioleaching system in the following way:

\[ \text{Fe}^{2+} \xrightarrow{\text{bacteria}} \text{Fe}^{3+} + \text{e}, \]  

as well as accordingly equation 2.

Natural magnetite in 0.5 M sulfuric acid has a wide range, 0.5-1.4 V (S.H.E.), in which complete passivation occurs. Increasing the current during the cathodic scans at more negative potentials of 0.5 to −0.1 V may be necessary for the process described by equation 4.

The mineral suspension, which contained sulfide ore, magnetite and thiobacteria culture had a positive Eh (0.2-0.4 V). Therefore, oxidative destruction of galena as well as microbial sulfuric acid production are possible. Superimposing several processes can have a number of consequences. For example, if the cell concentration is not high and these mechanisms have competitive character, then the appearance of the new substrate (Fe\(^{2+}\)) for autotrophic bacteria can result in the decrease of the sulfuric acid production by microorganisms. On the other hand, the appearance of the new substrate can result in the increase of the cell concentration and then an acceleration of the sulfur oxidation rate. The appearance of the Fe\(^{3+}\) also must be accompanied by the acceleration of the solubilization process and greater dissolution of sulfide.

The results of Table 1 (items 4-7) show that after magnetite introduction in the suspension, which included MGS, bacterial inoculum, and iron-free 9K medium, galena dissolution increased considerably. In three days sulfide oxidation reached ∼86-90%; this level of oxidation was not achieved until day 9 in the suspension without magnetite (sample 2).

A number of experiments with different magnetite content, ranging from 2.5 to 15.0 g in the suspension, have been carried out. The best result was achieved in tests with 4 and 5 g of magnetite. In these tests, the sulfide oxidation level reached ∼96% after three days; little change in the oxidation level was observed with additional incubation. The sulfide content in the solid phase, the total concentration of the dissolved iron, and the Fe\(^{2+}\) concentration in incubation solution were all measured allowing assessment of magnetite degradation. As noted in sample 5 (Table 1), the magnetite was subjected to the highest solubilization level (∼71%) with a corresponding high degree of galena oxidation. The maximum concentration of oxidized iron Fe\(^{3+}\) was also achieved in this test.

The results obtained lead to the conclusion that the addition of 2.5-5.0 g of magnetite for up to 10 g of MGS positively influenced galena leaching from ore. Addition of this mixed iron oxide promoted oxidizing conditions because of iron sulfate generation. The introduction of a larger quantity of magnetite also positively influenced the kinetics and efficiency of the sulfide leaching in relation to the control. However, with greater magnetite addition the Fe\(_3\)O\(_4\) degradation declined as well as the oxidized iron concentration. Hence, it is possible to conclude that the increase in the magnetite
concentration in the suspension is not expedient; this would lead to a decrease in the redox-potential due to the cathodic process activation and will mean non-productive additive use.

### 3.2 Galena electro-bioleaching

We also performed experiments on the influence of an electric field on the rate of the lead sulfide and galena-bearing ore bioleaching. Table 3 shows sulfide analysis results for the four experimental tests. Within two days those tests in which the electric field treatment was applied exhibited sulfide oxidation of 98.9% for the synthetic PbS and 90% oxidation for the MGS. After four days all tests, including the controls, were analyzed for residual sulfide. The difference was notable. In the controls the degree of synthetic galena oxidation was 87% and for MGS the sulfide oxidation was 78%; this is considerably less sulfide oxidation that was observed for the corresponding samples subjected to the field action (Table 3).

**Table 3. Microbial galena oxidation in field 0.5 V/cm**

<table>
<thead>
<tr>
<th>Duration, days</th>
<th>Degree of the sulfide oxidation, %</th>
<th>Without field</th>
<th>In field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MGS</td>
<td>PbS</td>
<td>MGS</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>87</td>
<td>99</td>
</tr>
</tbody>
</table>

We attempt to explain the results. Our previous experiments with iron alum (NH₄Fe(SO₄)₂·12H₂O) showed that during the DC electric field treatment in the electrochemical cell with non-separated electrodes, a decrease in Fe³⁺ ion concentration took place due to the cathodic processes. With an increase in the cathode potential the concentration of reduced iron rose. Thus, under the action of the DC electric field in the inoculated 9K medium, Fe³⁺ was continuously reduced, providing a substrate for microbial oxidation of the Fe²⁺ to Fe³⁺ (equation 6) [11]. The additional substrate stimulated bacterial growth. This, in turn, enhanced sulfide oxidation, resulting in increased dissolution of the lead sulfide and increased sulfuric acid formation. It appears that in the present experiments the system of coupling the electrochemical reactions is similar to that with magnetite, which was described earlier (section 3.1) in this paper. In the tests that employ non-separated electrodes, it is important not to apply too great of an applied potential in the negative direction; such an action could increase the Fe²⁺ concentration too much for the level of microbial activity. It is important to maintain a low voltage field for the biosuspension. The dynamics of the oxidation-reduction potential change indicates that at the beginning of the field action on the biosuspension (about 18 hours) the Fe³⁺ ions were regenerated in excess and the Eh potential increased to the extent expected by thiobacteria activity under the field action. It is likely that the acceleration of the galena oxidation in the biomineral suspension is related to the action of the discontinuous DC electric field.

### 3.3 Electrosurface properties

Some investigations were undertaken to determine if the electrosurface properties of thiobacteria play any role in the interaction of the bacteria with galena and if these properties can be regulated in enhance the bioleaching efficiency [12]. The surface chemical and electrokinetic properties of galena were studied in detail by Pugh [13]; this study focused on establishing optimal conditions for the flotation separation of sulfide ores. Main attention was paid to the mineral properties under neutral and alkaline pH...
values corresponding to the flotation conditions. Only a few studies have been dedicated to the electrical and hydrophilic/hydrophobic properties of the thiobacteria surface [14-16].

The correlation between the oxidation-reduction potential and Zeta-potential of the bacteria during leaching was determined: the minimum $\zeta$-potential values of bacteria corresponded to the maximum values of the Eh. One peculiarity connected with the presence of lead sulfide in the cultivation medium was noted. The $\zeta$-potential value of the bacterial cells grown on 9K medium was higher by 1-2 mV compared to the cells grown in the same medium with MGS added. This was possibly due to the presence of highly dispersed particles of PbSO$_4$ or PbCO$_3$, which do not possess a high electrokinetic potential [17]. During the electrocoagulation of these minerals with the cells the decrease in the electrokinetic potential of the latter may have taken place.

The interaction between a mineral and a cell depends on the pH value. Fig. 1 shows the Zeta-potential dependence with pH for galena as observed by Pugh [13] and our data for thiobacteria. In an acidic, oxidizing medium (NaNO$_3$) the synthetic galena was positively charged (curve 4); under the same acidic, oxidizing conditions the natural Swedish galena was negatively charged (curve 5) and the value of the electrokinetic potential at pH 2 to 2.5 was very close to the $\zeta$ values of the cells *T. ferrooxidans* M1 (curve 1-3). The difference in the surface properties of various samples of galena is associated with its surface oxidation products including PbSO$_4$ and PbCO$_3$.

As seen in Fig. 1, in the interval of pH 2.5-3.75 the Zeta-potential values closely coincide in both buffer solutions (curves 1-3). In the citrate buffer the maximum Zeta-potential value was achieved at pH 3.75, however, the charge was not changed even at pH 1.75. It is likely that for the given culture the isoelectric point (IEP) does not exist. For two of six *T. ferrooxidans* strains studied by Skvarla and Kupka [13] the IEP was also not achieved. It is likely that the pK value observed at 2.60-2.70 represents the mixed value of two pKs related to the dissociated phosphate groups of phosphatidic acid and carboxyl groups of gluconic acid in the composition of the lipo-polysaccharide cell wall [18]. Consequently, the cellular envelope of *T. ferrooxidans* M1 bacteria, grown in the medium containing iron as an energy source, consists of acidic material determined by phosphate and carboxyl groups. The number of these groups changes depending on the growth phase, the bacterial oxidative activity, and the intensity of the exchange processes on the membrane.

Electro-bioleaching experiments may be a proof of this. In the tests described in Section 3.2 the bacterial cells were separated from the solutions, two times washed off in 5 N H$_2$SO$_4$, re- suspended in McIlvien buffer, and after the mentioned preparation they were subjected to $\zeta$-potentials measurement. As seen in Fig. 2, the cells separated from the suspension subjected to the DC field action had higher negative $\zeta$-potential values compared to non-DC field-treated cells. The $\zeta$-potential of the cells not subjected to the electric treatment during the initial incubation period decreased slightly at first and then increased. The cells grown under the Fe$^{2+}$ ion electrochemical regeneration and electrostimulation conditions showed a stable increase of $\zeta$-potential as the cells aged. On the 9$^{th}$ day of the incubation (approximately 216 hours), the electrokinetic potential increased nearly two times in both the DC field-treated and non-treated cultures.

The electrokinetic potential of the thiobacteria changed with increases of electrolyte concentration at a constant pH. Changes in the electrokinetic potential could not be attributed to compression of the electric double layer with increase of the electrolyte concentration. As observed in Fig. 3 (curve 1), with an increase in the Fe (II) concentration from 10$^{-6}$ to 10$^{-2}$ M the negative potential increased by 5 mV, indicating the
higher affinity between *T. ferrooxidans* cells and iron. However, the cells proved practically indifferent to added calcium ions (curve 3); an insignificant decrease in the $\zeta$-potential value was observed when Ca(NO$_3$)$_2$ was added over a broad concentration range. Lead increased the negative charge of the cell surface at high Pb(NO$_3$)$_2$ concentrations ($10^{-3} - 10^{-2}$ M). This same increase in the negative charge was not reached in pulp during galena leaching due to the extremely low solubility of the lead sulfate that was formed (curve 2). The aforementioned property of the *T. ferrooxidans* culture may indicate that the bonding locations of Fe (II) on the cell surface are not accessible to other cations and that its oxidation mechanism is protected from metals in the surrounding medium [19].

Figure 1. Zeta-potential versus pH plots of *T. ferrooxidans* M1 (1-3) and galena (4-6). The experimental conditions: 1 – growing in 9K medium with MGS (measurements in universal buffer mixture); 2 – growing in 9K medium, (measurements in McIlven buffer); 3 – growing in 9K medium with MGS (measurements in McIlven buffer); 4 – PbS in $2\times10^{-3}$ M NaNO$_3$ solution; 5 – natural Swedish galena in $2\times10^{-3}$ M NaNO$_3$ solution; 6 – natural Swedish galena in $2\times10^{-3}$ M NaNO$_3$ solution plus $1\times10^{-5}$ M Pb(NO$_3$)$_2$. Curves 4-6 according to Pugh and Bergström [13]

Figure 2. Zeta-potential versus incubation time plot of *T. ferrooxidans* M1 bacteria in McIlven buffer. Previously incubated under the 0.5 V/cm DC field treatment (1); and without the DC field treatment (2)

Figure 3. Zeta-potential versus electrolyte concentration plot of *T. ferrooxidans* M1 bacteria dispersed in FeSO$_4$ (1), Ca(NO$_3$)$_2$ (2), and Pb(NO$_3$)$_2$ (3). The $\zeta$-potential value without electrolyte addition was $-13.5$ mV
5. CONCLUSIONS

1. The addition of magnetite to lead sulfide in the ratio of 1-2:4 in the microbial leaching system enhanced the efficiency and kinetics of sulfide degradation.

2. The thiobacteria substantially changed the galena surface, surface composition and increased the mineral rest potential during the leaching study.

3. Application of a discontinuous DC electric field with low voltage application to the galena bioleaching system improved the kinetics of the sulfide degradation.

4. The thiobacteria cell and the natural galena surfaces possess the same electrokinetic potential signs but low in value, which promotes their heterocoagulation. The electrokinetic potential of the thiobacteria changes during galena leaching and under the action of the DC electric field.

REFERENCES

Bioleaching of electronic scrap material by *Aspergillus niger*

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**Abstract**

This work reports on the bioleaching of electronic scrap material (ESM) from a local waste recycling company. The most abundant elements present in the ESM dust were oxygen and silicon, followed by various base metals with concentration exceeding 10,000 mg/kg. Precious metals gold, silver and palladium were found in trace amounts (<1,000 mg/kg). The fine ESM particles showed a heterogeneous matrix and a low specific area. Bioleaching experiments were carried out using *Aspergillus niger*, at various ESM pulp densities (0.1%-2.0% w/v). Using a two-step bioleaching process, the fungus was able to grow at up to 1.0% w/v, with the optimum pulp density at 0.1% w/v, under which about 35% Sn, 65% Pb, Zn, Al, and Mn and more than 70% Fe, Ni, and Cu were mobilized. It was also observed that various organic acids produced by the fungus during the leaching process paralleled the leaching of the heavy metals. Higher pulp density led to a decrease in acids produced due to the inhibitory effect of the toxic metals. The use of spent medium resulted in higher metal leaching efficiency than the two-step bioleaching at all pulp density (with the exception of Fe and Al at 0.1% w/v), due presumably to higher concentration of citric acid and lower concentration of oxalic acid in the spent medium which enhanced metals dissolution. Metals solubilization in spent medium leaching was not attributed to extracellular enzymes, but was mainly due to the action of the organic acids. Chemical leaching confirmed that citric, gluconic and oxalic acids were the responsible leaching agents in the bioleaching processes in removing heavy metals from the ESM. Compared with chemical leaching at 0.1% w/v, *A. niger* achieved similar leaching efficiency for Al, and was more efficient in the extraction of Fe, Sn and Au.

**Keywords**: Bioleaching, electronic scrap materials, Aspergillus niger

1. **INTRODUCTION**

Bioleaching may be described as an interaction between metals and microorganisms that causes the solubilization of metals. This process is based on the ability of microorganisms to transform solid compounds, and result in soluble and extractable elements which could be recovered [1]. Microbial leaching of metals may have been practiced as early as the 15th century, but the role of microorganisms in the leaching process was more clearly defined only from 1947 when bacterial catalyzation of iron oxidation and sulfuric acid formation in mine waters was demonstrated [2]. Microbial
leaching technologies have gained more attention and have been used on an industrial scale for the recovery of copper, gold, uranium and zinc from low-grade ores or from low-grade mineral resources [2].

Electronic waste, a new emerging and fast-growing waste stream, could be considered as an "artificial ore" due to the presence of intrinsically valuable heavy metals [2]. Recycling of the waste confers two possible benefits: removal of the potentially hazardous substances present in the waste, as well as the recovery of valuable metals in the waste. The use of bioleaching for the treatment of solid wastes may provide a more economic and environmentally friendly alternative to conventional technologies in the recovery of the elements.

A variety of microorganisms are known to be capable of carrying out the metal mobilization processes. This includes chemolithoautotrophic bacteria, chemolithoautotrophic archea and heterotrophic fungi. Among the heterotrophic fungi, the genera Aspergillus and Penicillium are the most important microorganisms used in bioleaching [3]. Of these, Aspergillus niger and Penicillium simplicissimum are probably the most widely used. Metal leaching by heterotrophic microorganisms generally involves an indirect process with microbial production of organic acids, amino acids and other metabolites. Four mechanisms have been identified [4]: (i) acidolysis (ii) complexolysis (iii) redoxolysis and (iv) bioaccumulation. Metal solubilization in fungal bioleaching can also occur via the production of enzymes, e.g. phosphatases, which can solubilize metal phosphates [5].

The purpose of this study was to determine some aspects of the physical and chemical properties of electronic scrap material (ESM) and investigate the use of heterotrophic filamentous fungi Aspergillus niger in the leaching of heavy metals from the ESM under different pulp densities and bioleaching conditions. Commercial inorganic acids were also used to leach ESM and the results were compared with bioleaching.

2. MATERIALS AND METHODS

2.1 ESM

The ESM sample was collected from a local waste recycling company specializing in electronic waste. The ESM collected, in dust-like form, was generated during shredding and other separation processes in the mechanical recycling of the waste. The as-received ESM was screened with a series of testing sieves and the fraction < 0.212 mm was used in the experiments.

2.2 Characterization of ESM

The composition of the ESM was determined through acid digestion/ICP-AES. The sample was acid digested using aqua regia/hydrogen fluoride/hydrogen peroxide in a microwave oven following the method of Das et al. [6]. The result was compared with the elemental determination through Energy-Dispersive X-ray Fluorescence Spectrometer (XRF) and Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX). pH buffering capacity of the ESM was measured according to the method of Chandler et al. [7] and Crawford [8]: individual 5g samples of ESM were mixed in 30 ml nitric acid of varying strength and agitated for 48 hours before monitoring of the pH. The particle size distribution of the ESM was determined using Particle Size Analyzer (Coulter LS 230). The specific gravity of ESM was determined using specific gravity bottle (Bibby). Brunauer-Emmett-Teller (BET) multipoint method (QuantaChrome, Nova 3000) was used...
to obtain the specific surface area of the ESM. Image of the ESM was obtained using a Scanning Electron Microscope (Jeol JSM-5600LV).

2.3 Microorganisms and spore inoculum preparation

_A. niger_ used in this study was supplied by Dr H. Brandl (University of Zürich, Switzerland). The fungus was maintained on potato dextrose agar (PDA) in petri dishes, incubated at 30°C for 7-10 days. Spores were harvested from the PDA surface by suspension in sterile deionised water. A haemocytometer was used to enumerate the number of spores. 1 ml of spore suspension (approximately 1 x 10^7 spores) was then added to 100 ml of culture medium with the following composition (g/l): sucrose (100), NaNO₃ (1.5), KH₂PO₄ (0.5), MgSO₄·7H₂O (0.025), KCl (0.025), yeast extract (1.6). No pH adjustment was made for the medium. The cultures were incubated in a water bath with rotary shaker at 30°C and 120 rpm.

2.4 Bioleaching procedure

Bioleaching experiments were performed at 0.1%, 0.5%, 1.0% and 2.0% w/v of ESM under two-step bioleaching and spent medium leaching. In two-step bioleaching, the fungus was incubated for two days before the ESM was added into the culture. In spent medium leaching, the ESM was added to the filtered cell-free spent medium wherein the fungus had been incubated for 18 days. Two control experiments (fresh medium and deionised water leaching) were also set up under identical incubation conditions. At intervals, samples were withdrawn from the flasks for the analyses of pH, sugars concentration, acids concentration, metals concentration and the biomass. The experiments were terminated after 18-26 days.

2.5 Chemical leaching

Chemical leaching of ESM was carried out at a concentration of 0.1% w/v pulp density, with 100 ml of different molarities of individual sulphuric and nitric acids (30 mM, 50 mM and 100 mM) as well as a mixture of commercial citric, gluconic and oxalic acids at equal molarity with the acids biogenically-produced in bioleaching processes. Samples were withdrawn after 10 days incubation and analyzed for soluble metals.

2.6 Analytical methods

The heavy metals in the solutions were quantified by Inductively Coupled Plasma Atomic Emission Spectroscopy (Perkin Elmer Optima 3000V). Glucose and sucrose were analyzed using a Biochemistry Analyzer (YSI 2700). The concentrations of the organic acids and fructose were determined by High Pressure Liquid Chromatography (Hewlett Packard 1100 Series). The separation was carried out with an Aminex HPX-87H cation exchanger column (Bio-rad); mobile phase: 5 mM H₂SO₄; injection volume: 20 µl; flow rate: 0.4 ml/min; temperature: 30°C and detected using UV at 210 nm and Refractive Index (RI) detector. For biomass determination, the culture broth with ESM was dried at 80°C for 24 hours, followed by ashing at 500°C for 4 hours; the biomass was determined gravimetrically.

3. RESULTS AND DISCUSSION

3.1 Characterization of ESM

Table 1 shows the elemental composition of ESM determined by acid digestion/ICP-AES, XRF and SEM/EDX. The most abundant elements in ESM are oxygen and silicon,
comprising 80,925 mg/kg and 64,625 mg/kg, respectively. It is known that refractory oxides typically constitute about 30% of electronic waste and the main component of refractory oxides is silica. Base metals, such as Fe, Cu, Pb, Al, Sn and Zn fall in the major element group (>6,000 mg/kg); most of these elements were found at a concentration higher than 10,000 mg/kg based on the result of digestion/ICP-AES and XRF analyses. Compared to the analyses of electronic scrap by Brandl et al. [9], the major elements found (Al, Cu, Pb, Sn and Zn) were similar with the ESM used in this work. Precious metals such as Ag, Au and Pd were found at lower concentration (<1,000 mg/kg). All subsequent metal analyses were performed using ICP-AES and calculations of metals leaching efficiencies were based on the digestion/ICP-AES results. The ESM was found to have a pH buffering capacity at a range from 4.6 to 1.2. This shows that the ESM is reasonably well buffered and can moderately resist changes in pH.

**Table 1. Elemental Analysis of ESM**

<table>
<thead>
<tr>
<th>Element (mg/kg)</th>
<th>Acid digestion/ICP-AES</th>
<th>XRF</th>
<th>SEM/EDX</th>
<th>Element (mg/kg)</th>
<th>Acid digestion/ICP-AES</th>
<th>XRF</th>
<th>SEM/EDX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si Average 64625.00</td>
<td>100000.00</td>
<td>16625.00</td>
<td>Sn Average 11212.50</td>
<td>60000.00</td>
<td>10400.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%) 2.40</td>
<td>1.47</td>
<td>22.72</td>
<td>RSD (%) 6.91</td>
<td>6.79</td>
<td>28.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu Average 49600.00</td>
<td>33000.00</td>
<td>7200.00</td>
<td>Au Average 876.25</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%) 3.06</td>
<td>0.42</td>
<td>37.46</td>
<td>RSD (%) 4.38</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe Average 36000.00</td>
<td>19800.00</td>
<td>9700.00</td>
<td>Ni Average 745.00</td>
<td>1200.00</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%) 6.82</td>
<td>0.51</td>
<td>33.84</td>
<td>RSD (%) 4.21</td>
<td>3.35</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al Average 27062.50</td>
<td>9000.00</td>
<td>nd</td>
<td>Mn Average 575.00</td>
<td>1000.00</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%) 3.14</td>
<td>4.25</td>
<td>-</td>
<td>RSD (%) 7.68</td>
<td>7.02</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb Average 22600.00</td>
<td>20000.00</td>
<td>6650.00</td>
<td>Ag Average 475.63</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%) 1.43</td>
<td>0.92</td>
<td>39.34</td>
<td>RSD (%) 3.18</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn Average 21688.00</td>
<td>19500.00</td>
<td>9875.00</td>
<td>O Average nt</td>
<td>nd</td>
<td>80925.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%) 2.18</td>
<td>0.66</td>
<td>15.61</td>
<td>RSD (%) -</td>
<td>-</td>
<td>11.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

nd: not detected; nt: not tested

Using the Particle Size Analyzer, it was found that particles with the size range 10-200 µm constitute the major portion of the ESM, with a mean at 64.43 µm. The fine ESM has a low specific surface area (1.95 m²/g) probably due to its non-porous structure. The SEM micrographs revealed a significantly heterogeneous matrix, with both rough and smooth surfaces, as well as particles of different shapes (Figure 1). Nevertheless, the ESM particles are typically smooth rods and spheres with many fine crystals and condensed flakes attached on its surface.

**Figure 1. SEM micrographs of ESM (at 500 times magnification)**
3.2 Bioleaching of heavy metals from ESM

Results showed that the fungus was able to grow at an ESM pulp density of up to 1.0% w/v in two-step bioleaching. The inhibition of growth at 2.0% w/v was due to increased concentration of toxic metals in the ESM. Figure 2 illustrates the growth of *A. niger*, at 0.1% w/v. Glucose and fructose were consumed simultaneously by the fungus, and the biomass increased over time and attained a maximum of 20.3 g/l. At higher pulp densities, slower rate of sugar consumption and biomass formation were observed (data not shown). For instance, a longer lag phase in biomass production (5-18 days) was noted at 0.5% and 1.0% w/v pulp densities. The toxicity of the metals present in the ESM was evident, even at a low concentration of 0.5% w/v.

![Figure 2. Growth of *A. niger* at 0.1% w/v ESM](image)

During bioleaching, pH decreased from an initial value of 3.0 to 2.30 at the end of incubation, and revealed that the amount of protons produced exceeded the demand for the solubilization reaction. In spent medium leaching however, pH marginally increased along the incubation period for all the pulp densities tested (ranging from 2.52-2.71; see Figure 3). The increase in pH was due to the reaction between organic acids and the ESM that consumed protons during the leaching process. The result suggested that acidolysis played an important role in the leaching which converts the insoluble metal compounds to soluble metal salts. The control tests showed relatively constant pH over the acidic range at 5.0-5.6 during the leaching process. In the two-step bioleaching at 0.5% and 1.0% w/v, the pH remained constant as the fungus grew under these pulp densities (Figure 4). This may be attributed to the buffering capacity of the ESM.

Table 2 summarizes the concentrations of the organic acids in the two-step and spent medium leaching. Compared with spent medium leaching, higher pulp density led to a lower acid production by the fungus. A significant decrease in citric acid was observed when pulp density increased from 0.1% to 0.5% w/v. This is due to the presence of increased manganese in the medium, which inhibited the biosynthesis of the acid even though low pH of the medium favors its production. The strong inhibition effect of manganese on citric acid synthesis has earlier been reported by Röhr and Kubicek [10]. Similar to the two-step bioleaching, the 18-day spent medium contained citric acid as the dominant leaching agent, followed by small amounts of gluconic and oxalic acids. The low pH and deficiency of manganese in the medium led to an accumulation of citric acid in the pure fungal culture, as has been reported by others [6, 11, 12].
Table 2. Organic acids in two-step bioleaching and spent medium leaching

<table>
<thead>
<tr>
<th>Organic acid (mM)</th>
<th>Two-step bioleaching</th>
<th>Spent medium leaching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1% w/v</td>
<td>0.5% w/v</td>
</tr>
<tr>
<td>Citrate</td>
<td>75.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Gluconate</td>
<td>22.0</td>
<td>31.2</td>
</tr>
<tr>
<td>Oxalate</td>
<td>31.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Figure 3. pH profiles in spent medium leaching under various pulp densities

Figure 4. pH profiles in two-step bioleaching under various pulp densities

Figure 5. Metals leaching efficiency in various leaching processes at 0.1% and 0.5% w/v
Figure 5 shows the leaching efficiency of various leaching processes at 0.1% and 0.5% w/v. The optimum pulp density for bioleaching for nearly all the heavy metals investigated was 0.1% w/v. Under two-step bioleaching at 0.1% w/v, 90-100% Fe, 60-65% Al and Mn and 30-40% Sn removal were achieved. In general, metal extraction yields decreased as pulp density increased; an increase in pulp density to 0.5% w/v, led to a significant decrease in leaching efficiency. High leaching efficiencies (70-90%) for Ni, Cu and Pb were also obtained under the optimum pulp density. On the other hand, spent medium was able to leach Ni (75-95%), Cu, Pb, Zn and Mn (50-80%), Fe (5%-75%) and Sn (15-40%), depending on the pulp density.

As a comparison, spent medium leaching generally gave higher metal extraction efficiencies than the two-step bioleaching at all pulp densities, with the exception of Fe and Al at 0.1% w/v. The spent medium contained higher concentrations of the organic acids than the two-step bioleaching at 0.5% w/v and 1.0% w/v (see Table 2). The lower pH of spent medium at these two pulp densities may have contributed to the higher leaching efficiency since a low pH is important in maintaining the availability of metal ions in the solution [13]. The higher concentration of oxalic acids in two-step bioleaching, compared with the spent medium, led to the formation of insoluble oxalate complexes and reduced metal leaching efficiencies. Fe and Al are exceptions, since the remarkable selectivity of oxalic acid for these metals and the solubility of the metal complexes may have contributed to its higher leaching yields [14, 15].

In the two-step bioleaching, the production of the organic acids and the concentration of the heavy metals increased with the growth of the fungus. Using Fe and Al at 0.1% w/v pulp density as examples, Figure 6 illustrates the parallel increase in metal leachability and the concentration of the organic acids, and shows that the leaching efficiency of the heterotrophic microorganisms depends on the extent of the production of organic metabolites. The organic acids have the dual effect on increasing metal solubilization by lowering the pH and complexing the metals into soluble organo-metallic complexes [16]. In spent medium leaching, the concentration of the heavy metals reached a plateau after a time period of 1-2 days of leaching.

In order to determine if extracellular enzymes were involved in the spent medium leaching, a further experiment was conducted. ESM (0.5% w/v) was subjected to leaching by spent medium (without autoclaving), as well as autoclaved spent medium (at 121°C for 20 min.). As can be seen from Figure 7, similar metal extraction yields for both processes were noted for all the metals tested. This confirmed that the metal solubilization in spent medium leaching was not due to action by extracellular enzymes, but may be attributed to certain metabolites (principally organic acids) contained in the medium.

3.3 Comparison between chemical leaching and biological leaching

As Figure 8 shows, the metal leaching efficiency of commercial organic acids was of the same order of magnitude as those obtained with biogenically produced organic acids of A. niger. The results confirm that citric, gluconic and oxalic acids were dominant leaching agents in the leaching processes, which effected heavy metals dissolution from the ESM. Table 3 shows the efficiency of bioleaching compared to chemical leaching using sulphuric and nitric acids. Both inorganic acids were able to completely solubilize Ni, Cu and Zn in the ESM. Lower Pb extraction by sulphuric acid compared to nitric acid was due to low solubility of the PbSO₄ formed [17].

Chemical leaching showed a significantly higher leaching yield than bioleaching of A. niger in the case of Ni, Cu, Pb and Zn. Complete solubilization of these heavy metals were achieved in chemical leaching, compared to 40-90% extraction in biological leaching. The
only exception is low Pb extraction by sulphuric acid (as explained earlier). *A. niger* was found to be more efficient in solubilising Fe (76-92%) due to the production of oxalic acid that favors the metal leaching. Together with Fe, higher extraction of Sn and Au were also achieved in bioleaching. The leaching efficiency of Al was generally similar for chemical and biological leaching. However, both leaching processes were unable to efficiently leach Ag from the ESM; a low extraction yield of less than 10% was obtained.

![Figure 6. Organic acids and metals leached in two-step bioleaching (0.1% w/v)](image1)

![Figure 7. Metals leaching efficiency of native and autoclaved spent medium (0.5% w/v)](image2)

![Figure 8. Comparison of metals leaching efficiency between (a) spent medium and (b) two-step bioleaching and chemical leaching at equal molarity of mixture acids (0.1% w/v)](image3)
Table 3. Metals leaching efficiency between bioleaching and chemical leaching (0.1% w/v)

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<thead>
<tr>
<th>Metal Extraction (%)</th>
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<td>Two-step bioleaching</td>
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<td>Fe</td>
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<td>Sn</td>
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<td>Au</td>
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4. CONCLUSIONS

The ESM used in this study is a fine particle with low specific surface area due to its non-porous structure. It contains high concentration of base metals and trace amount of precious metals. The optimum pulp density for the bioleaching was 0.1% w/v. Under this condition, 75-94% Fe, Ni and Cu and 45-67% Pb, Zn, Al and Mn were solubilized. Except for Fe and Al, spent medium leaching was more efficient than two-step bioleaching in removing heavy metals from ESM. The metal leaching efficiency of chemical and biological leaching varies according to the heavy metal being extracted, the type of acid used and the leaching process. Metals solubilization in spent medium leaching was not attributed to extracellular enzymes, but was mainly due to the action of the organic acids. Chemical leaching confirmed that citric, gluconic and oxalic acids were the responsible leaching agents in the bioleaching processes in removing heavy metals from the ESM.

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REFERENCES

Bioleaching of metallic sulphide concentrate in continuous stirred reactors at industrial scale – Experience and lessons

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Abstract

From laboratory to industrial size, scale-up of bioleaching of sulphide concentrate in continuous stirred reactors derives from the properties of the reactions and of the medium as measured at lab-scale. Literature is abundant in emphasising the influence of the main operating parameters of this process from small-scale testwork, and provides modelling approaches for simulation and performance predictions.

Experience gained in real case situation shows that a very critical aspect is the control of the performances and how to evaluate the efficiency of the process on real time. Gas analysis is applicable to performance control and can be integrated to the process control system. Even if such a method gave relative values in the case mentioned in this paper because of the difficulties of gas sampling, it is a reliable way to follow up bacterial activity on real time, which can be improved to give accurate figures.

The operating ranges of parameters like pH, air flowrates, temperature, etc. are reviewed and discussed by comparing information obtained from literature, laboratory testwork, and industrial practice.

Eventually, indications are provided concerning the information that a practical model should give as relevant to process operation at industrial scale and questions that remain unanswered although they have a key influence on process performances and costs.

Keywords: bioleaching, sulphide concentrate, pyrite, process control

1. INTRODUCTION

Industrial-scale bioleaching has known a relatively rapid growth in the last twenty years with the start-up of about ten plants using bioheap leach, seven plants treating sulphide concentrates in agitated tanks [1] and countless pilot-scale operations.

The process is reliable, efficient, simple, user-friendly and safer for the environment than other conventional techniques. Bioleaching is known to be a more cost-effective process to the extent that the very conservative decision-makers in mining and metallurgy have dared to invest in industrial-scale bioleach operations. However, successful operations do not mean that there are no technical difficulties or requirements for further improvement. Imaginative engineering addresses the main concept issues of design. However, the operators’ experience is invaluable in highlighting the pros and cons of a technique and it is a source of information that cannot be ignored.
In that context, this paper mainly aims at giving a practical examination of some of the critical technical points of the bioleach treatment of a sulphide concentrate in continuous stirred tank reactors (CSTR) at industrial scale. The experience of bioleach treatment at this scale presented in this paper refers to the Kasese cobalt producing plant operation in Uganda [2]. The production operation started in June 1999 and was shut down in August 2002 as a result of the persistently low cobalt prices.

2. EXPERIMENTAL

The Kasese Cobalt Company Limited (KCCL) bioleach plant was designed to treat 10.2 t per hour of a cobaltiferous sulphide concentrate.

The concentrate contains average grades of 80% pyrite and 1.4% cobalt. Cobalt is finely disseminated in the pyrite crystalline matrix. Copper, and nickel also occur as sulphides at low grades; 0.14% and 0.12% respectively. The non-sulphide components are silicates. No carbonates were found in the concentrate.

The design and operating characteristics of the bioleach unit are summarised as follows:

- A primary stage with three tanks, and secondary and tertiary stages with one tank each. Launder and gravity transfer ensured pulp flowing from one stage to the next.
- Every tank had the same total operating volume of 1,380 m$^3$. Tanks were made of stainless steel 304L (BS).
- Mixing and aeration in the tanks was provided by a rotating shaft system, equipped with two upper impellers and one bottom turbine. Air was injected under the turbine. The turbine was a disc with flat blades on the lower face. The size and layout of the blades were designed to provide the required air dispersion with the least energy consumption possible. About 60% of the energy for agitation is used by the turbine rotation. Contrary to what was observed on a small scale, the air injection does not reduce this value. This system was designed, scaled up and supplied by Robin Industries, France. BRGM tested and provided technical assistance to the design of this system called BROGIM®.
- Air supply was provided by five blowers, which could supply up to 20,000 Nm$^3$/h air to every tank. The operating airflow rates in the tanks were in the range of 10,000 to 15,000 Nm$^3$/h in the primary stage reactors and between 5,000 and 10,000 Nm$^3$/h in the secondary and tertiary stage reactors. Flowmeters on every air feed pipe provided airflow rates in Nm3/h.
- Nominal temperature was 42°C. Heat removal was ensured by internal stainless-steel cooling coils connected to a cooling tower
- pH was kept constant in every tank by a continuous addition of limestone slurry at a controlled rate. The nominal values were 1.4 to 1.5 and 1.6 to 1.8 respectively in the primary tanks and in the secondary/tertiary tanks.
- Solids concentration in the feed was 20% (solids wt / pulp wt).

The follow-up of the performances in the tanks was ensured by cobalt and sulphur assays in liquids and solids. Cobalt in solution was determined by atomic absorption after dilution in acidic solutions. Cobalt in solids was obtained from cobalt concentration in solution after acid digestion of the solids. Sulphate analysis by barium sulphate gravimetry in liquids and after hydrochloric acid digest for the solids provided the sulphide oxidation rate. The proportion of elemental sulphur has always been quantitatively negligible.
Another way of measuring the performances was to analyse the composition of the off-gas of the bioleach tanks. The method consists of sampling the off-gas from the tanks by means of a vertical 13-m pipe with open slots at different levels. The pipe is fixed in the tank, near the tank wall and its length goes down to about half a meter from the bottom. Assuming that the tank is perfectly mixed, the gas taken by this way was supposed to be representative of all the off-gas. The sampling well was also used to measure the oxygen concentrations in the slurry (using an oxygen probe) at the level of the open slots. Gas taken from each tank was driven through a network of pipes by a suction pump to a dewatering device and then to two analysers. The oxygen and carbon dioxide concentrations in the inlet and outlet gas of each reactor were measured using a paramagnetic analyser and an infrared analyser respectively (ADC 7000 Gas Analysers).

Mass balance of oxygen allowed determination of the oxygen uptake rate for each tank in milligrams of oxygen per litre of slurry per hour by assuming the nitrogen flow in the air fed to the reactor to remain unaffected in the off-gas. It also takes into account the variation of carbon dioxide concentration in the off-gas due to the dissolution of limestone used for pH control. The gas measurements were carried out once a day.

The Oxygen Uptake Rate, OUR, in mg.L⁻¹.h⁻¹ from the values of the flow rates of oxygen in mol L⁻¹ can be expressed as:

\[
\text{OUR} = \frac{32}{1000xV_o}(n_o^I - n_o^O)
\]

(1)

where:
- \(n_o^I\) is the inflow rate of \(O_2\), mol h⁻¹
- \(n_o^O\) is the outflow rate of \(O_2\), mol h⁻¹
- \(V_o\) is the operating volume of slurry in the tank considered, m³. This volume is about 1300 m³ depending on the airflow rate and the gas hold-up in the tank.
- 32 is the molar mass of \(O_2\), g

Then,

\[
n_o^I = 1000 \times \frac{Q_o^I}{22.414}
\]

(2)

where \(Q_o^I\) is the flowrate of oxygen in the air fed into the tank in m³ h⁻¹ in normal conditions (\(T = 0\)°C and \(P = 1\) Atm), and 22.414 is the volume of one mol of gas expressed in litres,

\[
n_o^O = 1000 \times \frac{Q_o^O}{22.414}
\]

(3)

where \(Q_o^O\) is the normal flow rate of oxygen in the air going out of the tank in m³ h⁻¹.

Moreover,

\[
Q_o^I = Q_{air} \times %O_2^I \quad \text{and} \quad Q_o^O = Q_{air} \times %O_2^O
\]

(4) and (5)
where $%O_2^i$ and $%O_2^o$ are the oxygen concentrations in the air flowing in and out of the tank in volume fractions respectively. $Q_{air}^i$ and $Q_{air}^o$ are the airflow rates in and out of the tank respectively in m$^3$h$^{-1}$ in normal conditions.

In the same way, for nitrogen: $Q_{air}^o = Q_{air}^i \times %N_2^o$ and $Q_{N_2}^i = Q_{air}^i \times %N_2^i$

where $%N_2^i$ and $%N_2^o$ are the nitrogen concentrations in the air in and out respectively, in volume fractions.

As $Q_{N_2}^i = Q_{N_2}^o$, then:

$Q_{air}^i \times %N_2^o = Q_{air}^i \times %N_2^i \qquad (6)$

$%N_2^i = 1 - %O_2^i - %CO_2^i$ and $%N_2^o = 1 - %O_2^o - %CO_2^o \qquad (7)$ and (8)

where $%CO_2^i$ and $%CO_2^o$ are the carbon dioxide concentrations in the air flowing in and out of the tank in volume fractions respectively.

Therefore:

$Q_{air}^o = Q_{air}^i \times \frac{1 - %O_2^i - %CO_2^i}{1 - %O_2^o - %CO_2^o} \qquad (9)$

From equations (1), (2) & (3) the Oxygen Uptake Rate (OUR) can be defined as:

$OUR = \frac{32}{V_o} \times \frac{1}{22.414} \times \left(Q_{air}^i - Q_{air}^o \right) \qquad (10)$

and combining (10) and (4) and (5), it comes:

$OUR = \frac{32}{V_o} \times \frac{1}{22.414} \times \left(Q_{air}^i \times %O_2^i - Q_{air}^o \times %O_2^o \right) \qquad (11)$

Then, combining (11) and (9):

$OUR = \frac{32}{V_o} \times \frac{Q_{air}^i}{22.414} \times \left( %O_2^i - \frac{1 - %O_2^i - %CO_2^i}{1 - %O_2^o - %CO_2^o} \times %O_2^o \right) \qquad (12)$

From this value of the OUR, the oxygen transfer rate in steady conditions (OTR) will be:

$OTR = V_o \times OUR \; \text{preferably expressed in kg O}_2 \; /h \qquad (13)$

In the same way, the oxygen transfer efficiency (OTE) is:

$OTE = \frac{n_{O_2}^i - n_{O_2}^o}{n_{O_2}^i} \times 100 \qquad (14)$

That can also be written:

$OTE = \frac{Q_{air}^i - Q_{air}^o}{Q_{air}^i} \times 100 \qquad (15)$

And then:
A comparison was established by determining oxygen uptake rate by the two methods, i.e. the pyrite oxidation and the gas mass balances. In the chemical balance method based on sulphide oxidation, it was assumed that oxygen reacts according to the stoichiometry of the following reaction:

$$4\text{FeS}_2 + 15\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}_2(\text{SO}_4)_3 + 2\text{H}_2\text{SO}_4 \quad (1)$$

Due to the fact that some flows were partly recycled inside the unit, it was not possible to make a reliable comparison for each bioleach tank and consequently, the comparison is established for the entire bioleach circuit.

Dissolved oxygen was measured by means of an Au/Ag electrochemical Orbisphere probe.

3. RESULTS AND DISCUSSION

3.1 Oxygen uptake rate measurement by $\text{O}_2$ and $\text{CO}_2$ measurements in the off-gas

As shown in figure 1, the sulphide biooxidation activity was particularly variable during the first six-months of 2001. The series of stages of the unit worked well only after the first three months, as it is only after this period that the OUR of the primary stage could be clearly distinguished from the values of the other stages. The unit was, on a number of occasions, affected by mechanical and electrical problems resulting in an interruption of the normal operations of the bioleach tanks.

Even after the beginning of April, operating problems like power failures for several hours affected the units’ performance. The sharp drop in activity recorded at the beginning of June is a typical consequence of that kind of problem. However, it is also noticeable that the bioleach primary tanks reacted in a relatively similar way to incidents affecting all the tanks at the same time like at the end of the study period.

Figure 1. OUR as measured by off-gas analysis vs. time in the bioleach tanks of the KCCL plant from January to June 2001
The results showing the comparison between the two methods of determining OUR for the period are shown in figure 2. Generally, it appears that the OUR provided by the gas method applied in the conditions described in this paper is overestimated when compared to the value obtained by material balance. When the operation was more stable, namely after mid-March, the two trends were rather similar, demonstrating that gas analysis as measured in those conditions provided a good method even for a relative picture of the biological activity in the tanks. It must be confessed that the system as installed at the plant suffered several limitations of which the main ones are the following:

- Gas measurements were carried out batch wise and only once a day, which means that the results could be affected by uncontrolled variations of the operating parameters like air flowrate or limestone addition. The gas measurement method gave a snapshot of the performances, whereas material balance was assumed to be more representative of the performances for a period in the range of the residence time in the tank. Moreover, it is assumed that steady state takes place and the calculation by the material balance in the slurry does not take into account the retention time in the bioleach circuit. Actually, as shown by the scattering of the data the steady state was far from being ensured on such a long period.

- The OUR data by gas analysis as given in figure 2 are the average values for all the bioleach tanks of the unit. These values do not take into account the errors involved in the process of sampling and measurement.

The sampling procedures were perfectible for the gas and for the slurry. In particular, sampling of the gas near the wall of the tank was not the appropriate place if gas was not evenly dispersed through the tank. Sampling the entire off-gas would have been the right way, but impracticable. The collection of the off-gas along a radius from the centre to the periphery of the tank would be feasible and probably satisfactory in terms of sampling accuracy.

![Figure 2. Values of OUR as obtained by material balance of the sulphide oxidation and by the gas balance for the entire bioleach unit.](image)

Eventually, it is reasonable to think that the ratio between the amount of oxygen consumed and the pyrite oxidised was not strictly equal to the stoichiometrical factor as in reaction 1. In particular, the consumption of oxygen by other minor sulphides and the
biological consumption of oxygen was not taken into account. However, the nearness of the two trends for the last bioleach tank in the cascade (fig. 3), hints that the gas measurement can lead to a good indication of the performances.

Figure 3. Values of the OUR as obtained by the material balance method compared to that obtained by the gas analysis method in the last tank of the bioleach unit

3.2 OUR vs. air flow rate

The air supply is the largest operating cost item of a bioleach unit and optimising the airflow rate is vital for the plant economy. The gas analysis method can be used to assess the influence of the airflow rate on biological activity.

In order to determine the minimum air flow rate in nominal conditions of operation, the OUR was measured in one of the bioleach tanks by the gas analysis method for a range of air flow rates from 7,000 to 15,000 Nm$^3$.h$^{-1}$. Figure 4 shows the results of the test work.

Figure 4. OUR measured by the gas analysis method vs. airflow rate in a tank of the bioleach unit

It appears that an airflow rate between 11,000 and 12,000 Nm$^3$.h$^{-1}$ is the minimum value to obtain a maximum OUR level. The maximum OUR values obtained are in the
range from 1,350 to 1,400 mg L^{-1} h^{-1}, meaning an Oxygen Transfer Rate (OTR) of about 1,750 kg O_2 h^{-1} and an Oxygen Transfer Efficiency (OTE) of ca. 50%. Such a high OTE value was above the original predictions.

Measurements of dissolved oxygen at the top of the bioleach tank showed that oxygen concentration was in the range of 1.5 to 2.0 ppm for flow rates from 10,000 to 12,000 Nm^3 h^{-1}. In this range of flow rates, the oxygen concentration is above the value of 1.5 ppm. This value of 1.5 ppm is generally agreed as being the minimum oxygen concentration acceptable for the maintenance of stable aerobic conditions required by the bacterial population in bioleach processes [3], though the system would still be efficient at as low as 0.1 ppm [4]. Furthermore, the bacterial growth was, apparently, not affected by a tip speed of the turbine of about 4.5 m s^{-1}.

It must be mentioned that a critical operating aspect for the aeration was the difficulty to equilibrate the air pressure in all the bioleach reactors. Differences in pressure load from one reactor to another frequently resulted in blower trips.

3.3 pH

Acidity level of bioleaching medium results from the balance of protons between net-consuming reactions (oxides/carbonates dissolution, arsenopyrite/pyrrhotite/iron oxidation, etc.) and reactions of sulphuric acid production and iron hydrolysis.

The optimal pH range is variable from one system to another and one microorganism to another [5]. On one hand, the higher the pH, the easier the acid-producing reactions. However, a relatively high pH, between 1.8 and 2, may lead to the precipitation of iron hydroxide in excess. This would further lead to an increase in the proportion of sterile surface that would interfere with the interaction of the bacteria with the sulphides, increasing the slurry viscosity and making mixing and oxygen transfer less efficient. On the other hand, a low pH value, close to 1.0, of course is harmful to the microorganisms metabolism and can be very selective for acid-tolerant species making the biological system fragile.

Other aspects can also be taken into consideration when pH is low. At low pH, ferric iron in solution from iron sulphides increases in concentration, which can reduce bacterial growth of species sensitive to this ion [6]. The lower the amount of a neutralising agent like limestone being added, the lower the amount of carbon dioxide available in situ for stimulating bacterial growth and the less the amount of gypsum being precipitated, which may have consequences on the retention time in neutralisation operation. In the case of refractory gold concentrates, it is observed that low pH reduces the risk of gold being encapsulated and being less soluble during cyanidation treatment [7].

Another aspect is related to nutrients as some authors think that too much calcium could result in less phosphate being available in solution for the bacterial metabolism needs [8]. This could be relevant for ammonium as well, because the formation of jarosite is enhanced by the addition of neutralising agent. It is true that one must be especially aware of nutrients availability in the primary stage of the bioleaching treatment in stirred tanks. The major part of the bacterial growth occurs in the primary stage of bioleaching, and the growth is reduced in the subsequent stages. This is the reason why pH can be increased from the primary to the following stages.

A pH range of 1.4-1.6 is probably a good compromise between the risks mentioned above and the technical feasibility of controlling pH in huge stirred tanks. The fact is that considering the evident and unavoidable inefficiency of mixing systems at microscopic
scale, the range of local pH in a tank is actually very large in any situation, from less to 1 to more than 7 probably.

3.4 Temperature

The bioleach unit was originally designed to work at 42°C. At laboratory scale, it was shown that the maximum temperature was 46°C. Above this value, biological growth and bioleaching activity were significantly reduced.

In practice, it happened that the cooling system was damaged in one of the bioleach tanks and the temperature was left to rise up to almost 50°C in this tank. Actually, the slurry temperature in the bioleach tank could only be kept in the range from 46 to 50°C. However, no significant reduction of the bioleach activity could be observed and the performances were quite similar to the performances in nominal design conditions. It therefore seems that the bacterial system is flexible to the point that new properties can appear when the system has to adapt to new operating conditions. It would confirm the great bacterial diversity in bioleach tanks, especially resulting from the microorganisms naturally occurring with the substrate itself. Unfortunately, no investigation was carried out to identify the change in bacterial population, while temperature had increased.

3.5 Modelling and prediction

Modelling the biochemical system in bioleach units is an extremely difficult task and the gain of a possibly efficient mathematical model is unpredictable. A modelling approach with a detailed representation of the medium content is not required.

On the other hand, a pragmatical model based on experimental data just for simulation as used for the engineering design of a plant and for the cost estimate is vital and sufficient.

Moreover, the ability to understand the phenomena occurring at the reaction interfaces could be quite essential. Indeed, it is, for instance, known that nutrients like ammonium do not only play a role for bacterial growth. They also form compounds with iron covering the sulphides, which compounds do not inhibit the oxidising reactions contrary to what is too often said when no other argument can be used, but these compounds are probably essential components of the catalytic process of biochemical transformations. During the operation at Kasese, a correlation between bioleaching efficiency and ammonium concentration was observed, even when the available amount in solution was presumably largely enough for the bacterial growth (results not shown in this paper). The compromise between the necessary concentration of ammonium for a good bacterial growth, the amount to be precipitated to play the optimum catalytic role in the biochemical process and a reasonable rate of consumption remains to be established at the real scale.

4. CONCLUSION

Operating a bioleach unit at industrial scale reveals the limits of the conclusions made from test work done on small scale. It must be admitted that generally a bioleach plant has better capacity to tolerate changes in operating conditions than expected. In particular, when the biomass is really established it is observed that changes in the normal operating conditions like absence of feed, and other problems related to cooling, mixing, aeration, etc. do not necessarily lead to major production problems. Actually, bioleach ecosystems have a great flexibility and own unknown resources, particularly in the composition of their bacterial population. Already, bioreactors are appropriate confined vessels where new micro-organisms may be isolated like Ferroplasma acidiphilum, an acidophilic,
autotrophic, cell-wall-lacking, mesophilic new micro-organism discovered by serial dilution of the aqueous phase of a bioreactor of a pilot plant treating a gold-bearing arsenopyrite/pyrite concentrate [9]. The diversity of the biological content and the positive selection that bioreactors operate, make that a continuous bioleach unit is a very robust industrial system.

However, practise at this scale also shows the limitation of the available tools to monitor the activity. Trouble-shooting is difficult before the consequences of the problem become really serious and result in significant loss of production. The gas analysis system as tested in the frame of the KCCL project was not designed for working in an industrial context. However, it was helpful enough to the operators for the monitoring of the daily bacterial activity. A fully automatic system connected to the general process control system would be easy to design and could inform the operators on critical parameters in real-time. The automatic system would provide the sulphide oxidising performances in steady state operating conditions.

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Bioleaching of natural zeolite – the processes of iron removal and chamfer of clinoptilolite grains

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Abstract

Natural zeolite, including clinoptilolite, often contains iron and manganese which decrease the whiteness of this sharp angular material.

The biological treatment of zeolite enables its use as the substitute for triplyphosphates in wash powders which have to comply with strict requirements as far as whiteness is concerned and rounded off grain content. Insoluble Fe$^{3+}$ and Mn$^{4+}$ in the zeolite could be reduced to soluble Fe$^{2+}$ and Mn$^{2+}$ by silicate bacteria of Bacillus spp. These metals were efficiently removed from zeolite as documented by Fe$_2$O$_3$ decrease (from 1.37% to 1.08%) and MnO decrease (from 0.022% to 0.005%) after bioleaching. The whiteness of zeolite was increased by 8%. The leaching effect, observed by scanning electron microscopy, caused also the chamfer of the edges of sharp angular grains. Despite the enrichment by fine-grained fraction, the decrease of the surface area of clinoptilolite grains from the value 24.94 m$^2$/g to value 22.53 m$^2$/g was observed. This fact confirms the activity of bacteria of Bacillus genus in the edge corrosion of mineral grains.

Removal of iron and manganese as well as of sharp edges together with the whiteness increase should give a product which is fit for industrial applications.

Keywords: zeolite, bioleaching, Bacillus sp., silicate bacteria

1. INTRODUCTION

Natural zeolite and its synthetic counterparts are used as filters, sorbents and ion exchangers with the characteristics of superior selective adsorption properties. Also, many researchers have found applications in air and wastewater pollution control, gas purification, petroleum refining and oxygen concentration. Because a dependable supply of zeolite is important, the synthesis of zeolite was developed by scientists. Before a large amount of natural zeolite was found in rock formations from the Cenozoic age, volcanic and sedimentary rocks of volcanic origin, only synthetic zeolite was used in the areas of pollution control, petroleum refining and gas purification processes due to the convenient
supply. If the physical and chemical properties of natural zeolite are defined and improved by special modification processes, adequate supplies for commercial users and a reduction in costs could be achieved. Moreover, it would be viewed as a very competitive material in many fields [1].

In the West Carpathians, deposits of zeolites including clinoptilolite, mordenite, and analcime occur exclusively in Miocene silicate volcanoclastic rocks. Two areas of zeolite occurrences, each having a different genesis and economic significance, can be distinguished in the territory of Slovakia:

(a) the East Slovakian basin with regionally widespread zeolitization associated with the Lower Badian sequence of rhyodacite, the so-called Hrabovec tuffites,
(b) the southwestern margin of the Kremnické vrchy Mountains having a distinct zone of about 2km² of Upper Sarmatian – Pannonian zeolitic tuffs [2].

Natural zeolites could be far more utilized if the modification and purification techniques for these materials made faster progress. With natural zeolites, in addition to acidity modification, the H3PO4 treatment can assist in elimination of impurities, such as carbonates or Fe. Iron is one of the most significant impurities in natural zeolites [3, 4] and it would be of great practical interest to reduce its content without crystal lattice destruction.

Berthelin et al. [5] have described the important role of bacteria in iron reduction when an enzymatic mechanism similar to dissimilative nitrate reduction should be involved in this reaction. Fe³⁺ is mobile only at very low pH values (pH<3). Reduction enables the formation of Fe²⁺, which is mobile in the normal range of soil pH. Consequently, if microorganisms and plants are able to reduce Fe³⁺, they can have advantage in competition for available iron. In the case of silicates, an increase in Fe solubility generally occurs with acid and complex secretions, and feldspars and micas can be destroyed [6].

*Bacillus* spp. play an important role in silicate biodegradation during the process of rock disintegration [7, 8]. The results of such activity involve both geochemical and structural changes in silicate minerals and rocks. Tešič and Todorovič [9] have proposed that so-called "silicate bacteria" belong to the *Bacillus circulans* group. The mechanism of microbial destruction of silicates and aluminosilicates by these bacteria is not understood yet. However, it is known, for example, that their activity leads to a decrease in Si content of bauxites of lower quality [10], and to the extraction of Al, Ti, U, Au and other elements from silicates and aluminosilicates [11].

This paper describes the treatment of natural clinoptilolite by bioleaching. Clinoptilolite, the most abundant natural zeolite, is a member of the heulandite group of the natural zeolites.

### 2. MATERIALS AND METHODS

#### 2.1 Zeolite sample

Zeolite sample (NI) from Nižný Hrabovec deposit is composed of clinoptilolite (51-68%), quartz + cristobalite (9-20%), feldspar (4-13%) and mica (13-22%). The iron-bearing minerals decrease the quality of this raw material. Its chemical characteristics are shown in Table 1.
2.2 Bacteria and media

Two bacterial strains (Bacillus cereus and Bacillus pumilus) were isolated from a kaolin quarry in Horná Prievrana by a colony reisolation on Nutrient agar No.2 (Imuna, Šarišské Michaľany) plates to obtain pure strain cultures. They were identified by means of the BBL CRYSTAL ID panel (Becton-Dickinson, USA). This panel contains 29 enzymatic and biochemical substrates and a fluorescence control on tips of plastic prongs. The resulting pattern of the 29 reactions is converted into a ten-digit profile number that is used as the basis for identification of a wide variety of microorganisms. For the species identification, the strains were cultivated on Columbia agar plates according to recommendation of the panel producer. For experiment, the bacterial strains were grown in Nutrient broth No.2 (Imuna) at 37 °C for 18 hours. Bacterial cells were subsequently centrifuged at 4000 rpm for 15 min, subsequently washed twice with saline solution (0.9% NaCl) and added in a concentration of $10^{10}$ cells per ml to modified Bromfield liquid medium [12]. Bioleaching of the samples was carried out in 3000 ml Erlemeyer flasks containing 2000 ml of modified Bromfield medium (NaH$_2$PO$_4$ – 0.5g/l, MgSO$_4$7H$_2$O - 0.5g/l, (NH$_4$)$_2$ SO$_4$ – 1.0g/l, NaCl – 0.2g/l, glucose – 10g/l) inoculated with a mixture of both Bacillus cereus and Bacillus pumilus strains. The flasks were incubated statically for 85 days at 28°C. The abiotic controls were cultivated under the same conditions. After incubation, the culture solutions were separated from the biomass by means of membrane filtration. The presence of vegetative bacterial cells in Erlemeyer flasks and their morphology were regularly examined by light microscopy after Gram staining.

2.3 Chemical analyses

Quantitative changes of samples (solid and liquid phases), investigated from the view of element composition stability, were evaluated by standard analytical method – atomic absorption spectrometry on a VARIAN spectrometer AA - 30 apparatus (Varian, Australia) after dissolution of the samples by standard procedure.

2.4 Grain size analysis

The particle size distribution of zeolite sample was measured by the laser radiation scattering on a Laser - Particle - Sizer Analysette 22 (Fritsch, Idar – Oberstein, Germany).

2.5 X-ray diffraction analysis

The qualitative characterization of the bacterially leached samples was investigated on a Philips XPERT powder diffractometer with CuKa radiation (40kV, 40mA), equipped with an automatic divergence slit, sample spinner, and a graphite secondary monochromator. For the data collections the range 2-72 deg2theta, a step width of 0.02 deg, and a counting time of 3sec/step were selected.

2.6 Scanning electron microscopy

The morphological changes in the surfaces of individual minerals were investigated by SEM (scanning electron microscopy) and the changes of chemical composition by energy-dispersion microanalysis (EDS). All mineral samples were coated with carbon and subsequently examined in a Tesla BS 340 scanning electron microscope.

3. RESULTS AND DISCUSSION

The bioleaching system studied in this work involves Bacillus bacteria and their metabolic products, zeolite particles and the leaching medium. First, we monitored the
elements extraction by bacterial leaching from NI sample using atomic absorption spectrophotometry (Fig. 1). It is demonstrated that bacterial growth and elements extraction from zeolite particles are coupled through biochemical interactions, which involve the different components in this system. The leaching rate of mineral particles was dependent on the fermentation of the organic compound (glucose) during bioleaching discontinuous processes. When the bacteria of *Bacillus spp.* were inoculated into zeolite, it was observed that gases were produced after a short adaptation phase due to the fermentation of organic compounds during elements extraction. The element extraction in zeolite began gradually increased with the generation of these fermentative gases and ceased to progress when gas generation or colour and pH change of medium was no longer observed. However, very low elements extraction was observed in the flasks without bacterial inoculum (data not shown). This fact suggests that elements extraction is bacterially mediated.

![Figure 1. Kinetics of elements extraction as followed by AAS](image)

In the presence of the zeolite during bacterial growth, the pH of the Bromfield medium was decreased from 6.5 to about 4.0 within 5 days, because organic acids were accumulated from the fermentation reaction of glucose. That is why this pH decrease was continually neutralized to pH 6.5 during bacterial leaching. Most of the bacteria were adsorbed on the mineral surfaces during discontinuous bioleaching as shown by light microscopy. Therefore, the bulk solution was exchanged with fresh glucose containing Bromfield medium nine times during 85 days without significant loss of active bacteria. The initial Eh was 160 mV and then was decreased to -490 mV during fermentative processes, indicating an anaerobic environment where the reduction reaction elements such as Fe$^{3+}$ and Mn$^{4+}$ performed well. The refinement of zeolite was carried out through these processes.

Fe (III) can generally serve as an electron acceptor in microbial metabolism around -182 mV [13]. The microbial reduction was more active in ferric hydroxide than in goethite. The other form – hematite, was difficult to reduce by microorganisms. It appears that the lower the degree of crystallization, the higher is the probability of reduction [14].

Chemical composition of zeolite before and after bioleaching is shown in Table 1. The insoluble Fe$^{3+}$ and Mn$^{4+}$ was reduced to soluble Fe$^{2+}$ and Mn$^{2+}$ by silicate bacteria of
Bacillus spp. The content of Fe\textsuperscript{2+} in solid samples after bioleaching was increased because it was probably adsorbed by zeolite and bacterial cells. The removal of total iron content was not to exceed 21%. The whiteness of zeolite was increased in 8% after bioleaching and grey-green colour before bioleaching was changed to white-green after bioleaching.

Table 1. Effect of bioleaching of zeolite samples on elements removal

<table>
<thead>
<tr>
<th>Chemical composition w.t.(%)</th>
<th>Sample before bioleaching</th>
<th>Sample after bioleaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO\textsubscript{2}</td>
<td>67.02</td>
<td>64.44</td>
</tr>
<tr>
<td>Al\textsubscript{2}O\textsubscript{3}</td>
<td>12.25</td>
<td>11.24</td>
</tr>
<tr>
<td>K\textsubscript{2}O</td>
<td>3.12</td>
<td>2.83</td>
</tr>
<tr>
<td>Fe\textsubscript{2}O\textsubscript{3}</td>
<td>1.19</td>
<td>0.74</td>
</tr>
<tr>
<td>FeO</td>
<td>0.16</td>
<td>0.31</td>
</tr>
<tr>
<td>MnO</td>
<td>0.022</td>
<td>0.005</td>
</tr>
<tr>
<td>TiO\textsubscript{2}</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>CaO</td>
<td>3.23</td>
<td>1.64</td>
</tr>
<tr>
<td>MgO</td>
<td>0.65</td>
<td>1.30</td>
</tr>
<tr>
<td>Na\textsubscript{2}O</td>
<td>0.65</td>
<td>1.29</td>
</tr>
<tr>
<td>Li\textsubscript{2}O</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>SZ*</td>
<td>7.73</td>
<td>12.05</td>
</tr>
<tr>
<td>SS*</td>
<td>3.62</td>
<td>3.67</td>
</tr>
<tr>
<td>S*</td>
<td>99.82</td>
<td>99.67</td>
</tr>
</tbody>
</table>

SZ* loss by ignition (900°C), SS* loss by drying, S* total amount (%)

Microbial production of organics by fermentation, or reductive dissolution of Fe – Mn mineral phases can greatly accelerate weathering rates of aluminosilicate minerals [15, 16, 17].

The mineral composition of zeolite sample is shown on X-ray diffraction pattern (Fig. 2). This pattern indicates that clinoptilolite, cristobalite, feldspar, celadonite and quartz are major constituents of the NI sample. The portion of quartz and feldspar was lowered by bioleaching as documented by chemical and mineralogical X-ray analyses. Moreover, the changes in Ca, Na, K, and Mg contents in clinoptilolite sample were visible on X-ray pattern because they caused the intensity decrease of individual peaks. This fact suggests a transformation of structural plains of clinoptilolite (hkl –422, 441).

Powder X-ray diffraction pattern reveals the clinoptilolite as a predominant mineral, however, the monoclinic habit of this mineral is not easily recognized in the scanning electron microscope. The raw material contained sharp angular grains after pulverizing and before bioleaching (Fig. 3). The plates or blades of clinoptilolite are visible after bioleaching and are generally less than 20\(\mu\)m in length (Fig. 4). The leaching effect, observed by scanning electron microscopy, caused also the chamfer of the edges of sharp angular grains and coating of fine-grained particles on grain surfaces (Fig. 4). The amount of finest-grained fraction from 0.9 to 5.0 \(\mu\)m and the distribution of fraction with particle size from 51\(\mu\)m to 103\(\mu\)m was in NI sample increased, and on the other hand, the distribution of fraction with particle size from 6.0 to 103.0 \(\mu\)m was decreased (Fig. 5). Despite the enrichment of the sample by fine-grained fraction, there was observed the decrease of the surface area of clinoptilolite grains from the value 24.94 \(m^2/g\) to value 22.53 \(m^2/g\). This fact confirms the activity of bacteria of Bacillus genus in the edge corrosion of mineral grains. We are sure that so-called "silicate bacteria" include not only
Bacillus circulans group as proposed by Tešič and Todorovič [9] but also many other Bacillus spp. described in last years.

Zeolitic tuffite from Nižný Hrabovec, earlier described as rhyodacite tuffite, was found to contain approximately 40-56% clinoptilolite. The zeolite was formed from volcanic ash as a product of its diagenetic alteration. The crystals of clinoptilolite are impregnated by amorphous phases and cristobalite [13]. Probably, these amorphous phases of zeolite matrix were particularly destructed by bioleaching because this devitrification was observed also on X-ray pattern as a partial increase of intensities of some diffraction lines of clinoptilolite (Fig 2).

Figure 2. X-ray diffraction pattern of the zeolite sample (S-celadonite, C-cristobalite, Q-quartz, F-feldspars, unsigned peaks - clinoptilolite)

Figure 3. Zeolite particle before bioleaching

Figure 4. Zeolite particle after bioleaching
Depending on physical and chemical properties, the zeolite rock from Nižný Hrabovec (Slovakia) should be useful also in many other industrial and agricultural fields (waste water treatment, soil conditioner, absorbent for water removal from refrigerants, for \( \text{SO}_2, \text{CO}_2, \) and \( \text{NH}_3 \) removal processes from waste gases, etc.) as described by Kozáč et al. [18].

Removal of iron and manganese as well as of sharp edges together with the whiteness increase should give a product which is also fit for industrial applications as the substituent for tripolyphosphates in wash powders which nowadays contaminate the environment.

Complex phosphates were identified as major contributors to the de-oxygenation of inland waters. Phosphonates cause the same problems, albeit requiring a greater quantity to do the same damage. Since few companies in the UK are equipped to remove phosphates, they persist in waterways. A phosphate ban was introduced in some Scandinavian countries, and many UK companies now use builder systems based on zeolite, which is similar to pumice stone, with or without polycarboxylic acid, sodium citrate, or carbonates. Zeolite is inert, but it has a positive environmental impact [19].

ACKNOWLEDGMENTS

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Bioleaching of pyrite by defined mixed populations of moderately thermophilic acidophiles in pH-controlled bioreactors

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Abstract

Pure and defined mixed cultures of moderately thermophilic acidophiles were compared for their abilities to accelerate the oxidative dissolution of pyrite oxidation in pH-controlled bioreactors run at 45°C. Three species of acidophiles used were; a thermophilic Leptospirillum isolate (MT6), Acidimicrobium ferrooxidans (strain ICP) and Acidithiobacillus caldus (strain KU). Microbial populations were analyzed using a combination of cultivation techniques (plate counts on selective solid media) and a molecular approach (fluorescent in situ hybridisation: FISH). Pyrite oxidation in mixed cultures of the two iron-oxidizers was greater than that by Am. ferrooxidans alone, but slightly less than by pure cultures of Leptospirillum MT6, suggesting no synergistic interaction between these bacteria. In contrast, mixed cultures of Am. ferrooxidans and At. caldus were the most effective system tested. Results from FISH and plate counts showed that, in contrast to earlier reports, numbers of iron-oxidizing bacteria were frequently greater than those of At. caldus in mixed cultures, and that the most efficient cultures contained more bacteria and dissolved organic carbon than relatively inefficient pure cultures and consortia. These results indicate that autotrophic At. caldus stimulate mineral dissolution by the "heterotrophically-inclined" Am. ferrooxidans by providing the latter with organic carbon as well as by oxidizing sulfur and polythionates.

Keywords: Leptospirillum; mixed cultures; pyrite; synergism; thermophiles

1. INTRODUCTION

Biological oxidation of sulfidic minerals may be mediated by a variety of pure and mixed cultures of acidophilic microorganisms. Frequently, such systems have been categorized on the basis of their optimum temperatures. "Moderately thermophilic" acidophiles are those that grow optimally between ~45-55°C, and include iron-oxidizing bacteria (e.g. Sulfobacillus spp., Acidimicrobium ferrooxidans) sulfur–oxidizing bacteria (e.g. Acidithiobacillus caldus and Sulfobacillus spp.), and heterotrophic bacteria (Alicyclobacillus-like) and archaea (Thermoplasma and Picrophilus spp.) [1]. Some Ferroplasma-like isolates (iron-oxidizing archaea) can also grow at 45°C. In contrast to mesophilic species, the majority of characterized thermophilic iron-oxidizers listed above are either mixotrophic or heterotrophic. A notable exception is Leptospirillum thermoferrooxidans, which was reported to grow at up to 55-60°C [2], though the original (and sole) isolate has subsequently been lost. A thermotolerant Leptospirillum isolate
(MT6) was isolated from a 45°C pilot-plant pyrite bioleaching operation by Okibe et al. [3]; this iron-oxidizer had a temperature optimum of 43°C and grew at up to 50°C. Phylogenetic analysis showed that it was most closely related to the newly designated species, *L. ferriphilum* [4]. *At. caldus*, *Ferroplasma*- and *Sulfobacillus*-like prokaryotes were also isolated from the stirred tanks, and relative numbers of microorganisms fluctuated as mineral leaching progressed.

Given the increasing importance of biological mineral processing, it is somewhat surprising that there are still relatively few detailed accounts of the microflora present in commercial mineral leaching operations [5]. However, it is recognized that consortia rather than pure cultures of acidophiles are involved in mineral biooxidation, and that interactions between microorganisms are of fundamental importance in determining the efficiencies of the process [6, 7]. In this paper, we describe pyrite oxidation by defined pure and mixed cultures of three species of moderately thermophilic acidophiles. Changes in microbial populations as mineral oxidation progressed were monitored using a combination of cultivation (plating) and molecular (FISH) techniques.

2. **MATERIALS AND METHODS**

2.1 Bacteria and bioleaching protocols

Three species of moderately thermophilic acidophiles were used in the present study: (i) *Leptospirillum* MT6 [3]; *Acidimicrobium ferrooxidans* (strain ICP) which was kindly provided by Dr. Paul Norris (Warwick University, U.K.) and *Acidithiobacillus caldus* (strain KU) which is maintained in the Acidophile Culture Collection at Bangor University. Bacteria were routinely subcultured in 2% (w/v) pyrite (*Leptospirillum* MT6), 2% pyrite + 0.02% (w/v) yeast extract (*Am. ferrooxidans*) and 5 mM potassium tetrathionate (*At. caldus*) liquid media (pH 2.0).

2.2 Shake flask experiments

Bioleaching of pyrite (obtained from the Cae Coch mine, north Wales [8]) by various combinations of the three moderately thermophilic acidophiles was compared in shake flasks, containing 100 ml of 2% (w/v) of finely ground ore. Cultures were inoculated (2%, v/v) and incubated, shaken (130 rpm) at 45ºC. Samples were withdrawn at regular intervals to determine concentrations of soluble iron (total and ferrous), sulfate, pH, dissolved organic carbon (DOC) and to enumerate bacteria.

2.3 Pyrite bioleaching in pH-controlled bioreactors

Bacteria, grown in batch culture as described above, were used to inoculate 2 L bioreactors (P350; Electrolab, U.K.) fitted with temperature, pH and aeration control. The working volume of the bioreactor was 1.5 L, and 2 x 10⁹ cells of each acidophile (enumerated in a Thoma counting chamber) were added at the start of each bioleaching experiment. Again, various combinations of the three acidophiles were used, and on each occasion two bioreactors were run in parallel to compare either a pure and a defined mixed culture, or else consortia containing different combinations of moderate thermophiles. Cultures were maintained at 45ºC throughout, and aerated at 0.2 L/minute. The initial bioreactor pH was fixed at 1.5, though in the later phases of each experiment this was lowered to 1.2, and finally 1.0 (by addition of sulfuric acid). Samples were withdrawn at regular intervals to determine ferrous iron, sulfate, DOC and microbial populations. The amounts of acid or alkali required to maintain cultures at the pre-determined pH were also recorded.
2.4 Analysis of microbial populations using solid media

Mineral leaching samples were vortexed thoroughly to dislodge loosely adhering bacteria from mineral surfaces, a dilution series prepared and inoculated onto solid media. The latter were ferrous sulfate (for *Leptospirillum* MT6) and ferrous sulfate/tetrathionate (for *Am. ferrooxidans* and *At. caldus*) overlay media; these had previously been shown to be highly efficient in promoting colony growth of these bacteria (data not shown). Full details of these solid media are given elsewhere [9]. Plates were incubated at 45°C for up to 10 days. Colonies were readily distinguished from each other (*Leptospirillum* MT6 as small Fe$^{3+}$-encrusted colonies; *Am. ferrooxidans* as ferric iron-stained “fried egg”-like colonies, and *At. caldus* as cream/white-colored colonies.

2.5 Analysis of microbial populations by fluorescent *in situ* hybridization (FISH)

The protocols used for FISH analysis are described elsewhere [10]. To analyze relative numbers of microbes using FISH, fixed cells were hybridized with a Cy3-labelled probe that targeted a specific acidophile, and simultaneously with a fluorescein-labelled eubacterial probe (EUB338) that targeted all eubacterial cells. Numbers of a specific acidophile were compared to total numbers of eubacterial cells targeted by the EUB338 probe, and also to total numbers of microorganisms stained with 4’,6’-diamidino-2-phenylindole (DAPI) in the same field of view, to work out the relative abundance of a particular acidophile. Probes used to target *Leptospirillum* MT6, *Am. ferrooxidans* ICP, *At. caldus* KU were LF655 [11], ACM995 (P.R. Norris, unpublished) and THC642 [12], respectively.

2.6 Miscellaneous analyses

Ferrous iron was determined colorimetrically using the ferrozine assay [13] and total iron by atomic absorption spectrometry. Sulfate was determined turbidometrically as barium sulphate (Hydrocheck; Cambridge, U.K.). DOC was determined using a Protoc DOC analyzer (Pollution & Process Monitoring Ltd., U.K.).

3. RESULTS

3.1 Shake flask experiments

Both *Leptospirillum* MT6 and *Am. ferrooxidans* oxidized pyrite, though the latter was not very effective in pure cultures (data not shown); iron solubilization in pure cultures of *At. caldus* was similar to that in abiotic controls (Fig. 1a). In contrast, mixed cultures of *Am. ferrooxidans* and *At. caldus* oxidized pyrite at similar rates as mixed cultures that also contained *Leptospirillum* MT6 (Fig. 1b). Pyrite leaching by mixed cultures of *Leptospirillum* MT6 and *At. caldus* was very similar to those by the iron-oxidizer alone for the first 35 days, though concentrations of soluble iron in the mixed culture were greater after this time (Fig. 1a). This contrasts with culture pH, which was much lower in the mixed cultures than in pure cultures of either acidophile throughout. DOC concentrations were greater in mixed (104 +/-13 mg/L) than in pure cultures (17+/-1 for *At. caldus*; 88+/-6 mg/L for *Leptospirillum* MT6).
Figure 1. Oxidation of pyrite in shake flask cultures. (a) pure and mixed cultures of *Leptospirillum* MT6 and *At. caldus* (key: ●, O *Leptospirillum* MT6; ▲, △ *At. caldus*; ■, □ *Leptospirillum* MT6/*At. caldus*). (b) pure and mixed cultures of *Am. ferrooxidans* (key: ●, *Am. ferrooxidans*; ▲, *Am. ferrooxidans*/*Leptospirillum* MT6; ■, *Am. ferrooxidans*/*At. caldus*; X, *Am. ferrooxidans*/*At. caldus*/*Leptospirillum* MT6.

Data from leaching experiments carried out in controlled pH bioreactors are shown in Fig. 2. *Am. ferrooxidans* was highly ineffective in leaching pyrite in pure culture, while the mixed culture of this iron oxidizer and *At. caldus* displayed the most rapid rate of pyrite dissolution of all those tested during the early phase (days 6-20) of the experiment, though this declined dramatically after day 20 (Fig. 2a). The mixed culture containing both iron-oxidizers (*Leptospirillum* MT6 and *Am. ferrooxidans*) also oxidized pyrite at a relatively slow rate, and the addition of *At. caldus* to this consortium resulted in a marked increase in mineral dissolution (Fig. 2b). The consortium of all three moderate thermophiles was also superior in leaching pyrite to the mixed culture containing only *Leptospirillum* MT6 and *At. caldus* (Fig. 2c).

Acid production in mixed cultures of pyrite-oxidizing moderate thermophiles was determined from the amounts of alkali that were required to maintain culture pH at the pre-set levels. No alkali was consumed in the pure culture of *Am. ferrooxidans* though, in contrast, 400 mmoles of NaOH was added to the mixed *Am. ferrooxidans*/*At. caldus* culture during the first (pH 1.5) phase of the experiment, though no more was consumed when the culture pH was subsequently lowered (data not shown). With other mixed cultures, greater amounts of alkali were consumed in the consortia containing all three moderate thermophiles than in mixed cultures containing only two. Interestingly, no alkali was consumed in the mixed culture of *Leptospirillum* MT6 and *At. caldus*.

Microbial populations in two of the bioreactor cultures, as determined by plate counts and FISH analysis, together with DOC data from these cultures, is shown in Fig. 3. Plate counts indicated that numbers of both *A. ferrooxidans* and *At. caldus* (in the culture containing only these two bacteria) increased during days 1-17, and subsequently declined. There was generally a good correlation between plate counts and relative numbers determined by FISH with this culture; e.g. at day 17 both techniques showed that *Am. ferrooxidans* was more numerous, whereas at day 28 the two acidophiles were present in similar numbers. Concentrations of DOC increased steadily throughout incubation, and there was a marked increase when culture pH was lowered to 1.0. In contrast, colonies of *At. caldus* were only observed on two occasions from the *Leptospirillum* MT6/*Am. ferrooxidans*/*At. caldus* consortium, even though this moderate thermophile was detected (at 23-64% of the total population) by FISH analysis. The iron-oxidizers were numerically dominant in this culture, except when the culture pH was lowered to 1.0, when *At. caldus*
comprised 64% of the total microbial population (Fig. 3b). Again, there was a gradual, though more spasmodic, increase in DOC concentrations throughout incubation, and a large increase when culture pH was adjusted to 1.0.

Figure 2. Oxidation of pyrite in bioreactor cultures (●, O iron; ▲, △ sulfate-S) (a) *Am. ferrooxidans* (O, △); *Am. ferrooxidans/At. caldus* (●, ▲). (b) *Am. ferrooxidans/Leptospirillum MT6* (O, △); *Am. ferrooxidans/Leptospirillum MT6/At. caldus* (●, ▲). (c) *Leptospirillum MT6/At. caldus* (O, △); *Leptospirillum MT6/At. caldus/Am. ferrooxidans* (●, ▲). (i) pH 1.5; (ii) pH controlled removed; (iii) pH 1.2; (iv) pH 1.0.
4. DISCUSSION AND CONCLUSIONS

Whilst it is acknowledged that mixed cultures of acidophiles are involved in mineral biooxidation processes, there are relatively few reports detailing microbial populations in commercial operations. The "primary" microorganisms involved are, in many cases, iron-oxidizers such as *Leptospirillum* spp., since some sulfidic minerals (such as pyrite) are acid-stable, but are oxidized by the ferric iron that these prokaryotes produce. Sulfur-oxidizing bacteria, such as *At. caldus*, whilst having no direct role in the process, exploit the elemental sulfur and polythionates formed as by-products [14]; since the free energy available from oxidizing sulfur and/or polythionates far exceeds that available from ferrous iron oxidation, sulfur-oxidizers may be present in far larger numbers than iron-oxidizers in mineral leachate liquors [15]. Since elemental sulfur may form on the surfaces of oxidizing sulfides and inhibit mineral oxidation, *At. caldus*-like bacteria have been considered to have a beneficial role in biomining operations (e.g. [7]). Another way in which acidophilic microorganisms interact is in the production (by autotrophic species) and consumption (by heterotrophic and mixotrophic species) of soluble organic compounds [6]. This is particularly pertinent at moderately thermophilic temperatures (40-50°C) where the many of the known iron-oxidizers appear to have limited capacities for fixing CO\(_2\), which restricts their capacities for mineral oxidation when grown in pure cultures without extraneous organic carbon. For example, *Sb. thermosulfidooxidans* was found to oxidize pyrite effectively in mixed culture with *At. caldus* or in pure cultures amended with yeast extract, but not in organic C-free medium [7].

Of the two iron-oxidizing moderate thermophiles used in the present experiments, one (*Leptospirillum* MT6) was an obligate autotroph, and the other (*Am. ferrooxidans*), whilst having the capacity to fix CO\(_2\), is relatively ineffective at this and was originally classified as a heterotroph [16]. This was illustrated by the lack of pyrite oxidation by pure cultures of *Am. ferrooxidans*, in contrast to the mixed culture with *At. caldus*, where the organic C requirement of the iron-oxidizer was presumably provided as soluble compounds originating from the sulfur-oxidizer. Concentrations of DOC in the latter were greatest of those measured in any bioreactor culture; it might be expected that that not all of the cell exudates and lysates originating from *At. caldus* would be utilized by *Am. ferrooxidans*. In contrast, the mixed culture of the two iron-oxidizers was less effective at oxidizing pyrite, whilst the culture that also contained *At. caldus* was superior in this respect. Interestingly, the mixed *Leptospirillum* MT6/*At. caldus* culture was also a relatively poor pyrite-oxidizing consortium (and was inferior to a pure bioreactor culture of *Leptospirillum* MT6 that was run on a separate occasion; data not shown). One possible reason for this is that these autotrophs competed for CO\(_2\), the solubility of which decreases with decreasing pH and increasing temperature, and is potentially a limiting factor to their growth. Interestingly, numbers (from plate counts) of both *Leptospirillum* MT6 and *At. caldus* were less than in the parallel culture that also contained *Am. ferrooxidans* (data not shown).
Figure 3. Microbial populations and DOC concentrations in consortia of moderate thermophiles. The pie graphs show data from FISH analysis, and the line graphs data from plate counts ( ■, *Am. ferrooxidans*; ▲, *At. caldus*; ○, *Leptospirillum MT6*), and DOC (♦).

A variety of chemical reactions may occur in mineral leachate liquors that can either consume or generate protons. At the pH values at which the bioreactors were run, there was no hydrolysis of ferric iron, and no observable formation of jaroites or other secondary ferric iron minerals. The major acid-generating reaction was therefore the oxidation of sulfur and/or polythionates to sulfuric acid, primarily by *At. caldus* (though this may also be achieved abiotically with ferric iron). Measurements of the amounts of
alkali required to neutralize proton production in the pH-controlled bioreactors was therefore a useful indication of the extent of (microbial) sulfur oxidation. The fact that the greatest amounts of alkali were consumed in bioreactors that contained both \textit{At. caldus} and \textit{Am. ferrooxidans} (and which were the most effective leaching consortia) suggests that interactions involving transfer of carbon and oxidation of sulfur/polythionates were important in promoting pyrite oxidation.

The combination of plate counts and FISH analysis to assess microbial populations gave useful insights into how these evolved during mineral oxidation, and also when subjected to low pH stress. In many cases, the relative abundances of the different bacteria were similar when analyzed by either method, further validating the use of “overlay” media to assess populations of acidophiles. On some occasions, however, no colonies of \textit{At. caldus} were recovered, though FISH analysis confirmed that the sulfur-oxidizer was present. The reason for this is unclear, though it may be caused by the physiological state (stress etc.) of \textit{At. caldus} in the leach liquors. What was apparent from these analyses was that, contrary to earlier reports, numbers of iron-oxidizers in mineral leachates often exceeded those of the “secondary” sulfur-oxidizers. It was also apparent that the most efficient pyrite-oxidizing consortia contained far greater numbers (from plate counts) of iron-oxidizers than the relatively inefficient bioreactor cultures that were run in parallel.

One other notable fact from the present study was the finding that \textit{Am. ferrooxidans} (strain ICP) is an important mineral-leaching organism, though only when grown in mixed cultures containing \textit{At. caldus}. Mineral oxidation in cultures containing \textit{Am. ferrooxidans} began far earlier and was more rapid than those where \textit{Leptospirillum} MT6 was the primary iron-oxidizer. In cultures containing both iron-oxidizers, \textit{Am. ferrooxidans} was frequently more numerous and presumably had a major role in promoting mineral dissolution. One potentially negative characteristic of \textit{Am. ferrooxidans}, from the point of view of mineral leaching, is its lower tolerance of ferric iron than some other iron-oxidizers [16]. This may be the reason for the cessation of pyrite oxidation beyond day 20 in the mixed culture of \textit{Am. ferrooxidans} and \textit{At. caldus} which, until that point, was the most efficient pyrite-oxidizing system of all those tested.

This study has shown that some, though not all, mixed cultures of acidophiles are highly effective at oxidizing pyrite. One limitation of the study is that consortia permutations have involved only three moderate thermophiles. Additional experimental work, incorporating other iron-oxidizers such as \textit{Sulfobacillus} spp. and \textit{Ferroplasma} spp. would shed further insights into microbe-microbe and microbe-mineral interactions in such environments.

\textbf{ACKNOWLEDGEMENTS}

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\textbf{REFERENCES}

Biolixiviation of Cu, Ni, Pb and Zn using organic acids produced by Aspergillus niger and Penicillium simplicissimum

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\textsuperscript{c} Graduate student, Department of Civil Eng., Laval University, Quebec, Canada, G1K 7P4 R

Abstract

In order to assess extraction potential and identify the mechanisms of solubilisation, washing tests were performed using organic acids produced from the culture of Aspergillus niger and Penicillium simplicissimum. Two types of mining residues were studied: one coming from a Zn and Pb mine in New Brunswick, Canada and the second from a Ni mine in New Caledonia. Metals: Cu, Fe, Mn, Ni, Pb and Zn were in high concentration in the residues from the Canadian mine (from 1130 to 590,000 mg/kg of residues). Both residues are from different mineral backgrounds. For instance, Noranda residues are pyritic with a high sulfur concentration (267,569 mg/kg of residues). The results showed that various factors controlled the efficiency of the extraction. These factors arise from the mineral origin of residues and from the physico-chemical characteristics of the washing solution, which is a mixture of organic acids. Metal distribution and mineral origin of the residues were evaluated using SSE and SEM. The individual concentrations of acids constituting the mixture (citric, mactic, gluconic) were monitored using HPLC. These acids were produced under different periods of incubation, pH. Finally, in order to reduce the process cost, various organic residues were used as substrate (fruit skin or juices). Results show very encouraging efficiencies. The maximum solubilization obtained for Cu was close to 30% for the residues coming from New Caledonia.

Keywords: heavy metals, biolixiviation, organic acids, geochemistry

1. INTRODUCTION

1.1 The mining residues problem

In Canadian mines, large amounts of soil and rocks that don’t contain commercially attractive amounts of precious metals (less than 0.5% w/w) will be exposed to the atmosphere. At the surface of the rock piles, wind, rainfall and other weathering factors (i.e. temperature) will produce exhaustive leaching of metals. These leachates are acid and contain high concentrations of heavy metals (e.g. cadmium, lead, copper) that may threat
the health of plants, fish and humans. In Canada, it has been estimated that over 12,500 ha of tailings and 750 million tons of waste rock exist, and are potentially acid generating [2, 6]. Over the world, similar conditions are found. Notably, in mines in South America and Africa where the Canadian Mining Industry is largely present. To rehabilitate these acid generating tailings and waste rock dump sites in Canada, more than $10 billion dollars will have to be invested over the next 20 years [7]. Currently, the non-existence of reliable, predictable and cost-effective technologies challenges both the industry and the government management strategies to provide long term environmental protection against the potential risks of mining waste. Therefore, the valorization of mining residues could lead to important breakthroughs towards the sustainable use of mineral resources.

1.2 Current mining residues management strategies

Until 1998, mining waste management strategies were mostly focused on the confinement of mining residues (e.g. underwater disposal, surface flooding, dry barriers and porous covers). Bioleaching processes have just recently been considered for metal recovery from mining residues. These bioprocesses are new, under development and, at most, are at batch scale with no proper technico-economical study been done. The preferred organisms for bioleaching are bacteria. For instance, experimental theory on the use of chemolithotrophic bacteria such as the *Thiobacillus* species for metal leaching has, to limited extent, been documented [1, 4]. Only few natural scale operations have been assayed. In most biological process applications, the process is developed under the exclusive study of the organism growth and its relationship with the aqueous medium in which it develops. Although, in the case of solid residues, geochemistry is fundamental to the success of microorganism growth it is almost never studied or taken in consideration. Bioleaching using fungi is originally new and agrees with sustainable development. Fungi bioleaching relies on the solubilization of metal by means of washing with the organic acids produced by the fungal activity. In many technical aspects, fungi growth is easier to achieve than bacteria. Various groups of native fungi microorganisms are known to produce organic acids. For instance, *Aspergillus niger* and *Penicillium simplicissimum* produce high complexing organic acids (e.g. citric, oxalic, gluconic) [5]. Fungi growth can be attained by providing optimal nutrient conditions and optimal substrates. Minerals contained in the mining residues can provide with excellent nutrients (e.g. Fe, Ca, Mg). When geochemistry of residues is known and taken in consideration, nutrients can be provided by the residues. Some organic wastes, such as some food or agricultural wastes, can be excellent providers of carbon. With some minimal waste manipulations carbon from these wastes can be easily available for fungi consumption.

In the light of the expected shortage of non-renewable resources, increased efforts are absolutely necessary to seek new sources of raw materials with the aid of new or improved technologies. Bioleaching technology will enhance the release of precious metals to a concentration more commercially attractive and thus will give value to mining residues. The major innovation of this project lies in the facts that it will solve 3 important environmental problems: 1) reduce mining waste, 2) revalorization of mining residues and 3) revalorization of organic waste. Also, it will advance knowledge in organic acid production from fungi, it will incorporate fundamental geochemistry into the comprehension of the bio-leaching process and will systematically develop an industrial bio-leaching process from batch to bench, from column to pilot scale.
2. OBJECTIVES OF THIS STUDY

The objectives of the study are to: (1) identify and evaluate the parameters (biological and geochemical) that affect the production of organic acids by *Aspergillus niger* and *Penicillium simplicissimum*, (2) study the potential use of new carbon sources for fungi growth and evaluate its impact on organic acid production, (3) study the influence and variation of metal geochemistry on the solubilization or immobilization of metals during bioleaching under two percolation setups (growth of fungi within the solid matrix, and growth of the fungi outside the solid matrix).

3. SITES OF STUDY

This study comprised two different solid matrices of two actual mining sites. The First one from New Brunswick, Canada, which is a ground mine of Zn and Pb and a Ni sky cut mine from New Caledonia both belonging to the Noranda Company.

<table>
<thead>
<tr>
<th>Table 1. Residues characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Water content (%)</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
</tr>
<tr>
<td>Total S (mg/kg)</td>
</tr>
<tr>
<td>Cu</td>
</tr>
<tr>
<td>Fe</td>
</tr>
<tr>
<td>Mn</td>
</tr>
<tr>
<td>Ni</td>
</tr>
<tr>
<td>Pb</td>
</tr>
<tr>
<td>Zn</td>
</tr>
</tbody>
</table>

4. MINING RESIDUE GEOCHEMISTRY

Mine deposits are different in their geological and mineralogical nature. Their mineralogical composition determines their economical potential by determining the ease of precious metal extraction. Thus, any process seeking the extraction of metals from their solid matrix must take in consideration the geochemistry of its minerals and non-mineral components. Metals are retained in the soil under various forms as shown in figure 1. Manipulating pH, redox and acidity, a selective dissolution procedure can release in one step metal nutrients (e.g. Fe, Mg, Ca) and in a second step precious heavier metals (Pb, Zn, Cu, Ni, etc.). The first step can be done through a chemical washing procedure. The second step can be achieved using organic acids in an optimal ratio of concentrations and dosages (citric:oxalic:gluconic).

Metal bioavailability greatly determines metal recovery rates. Also, it is known that heavy metals are in higher or lower scale, toxic to fungi (e.g. Ag > Hg > Cu > Cd > Cr > Ni > Pb > Co > Zn > Ca, Fe). In this sense, the presence of metals in either a soluble or exchangeable phase could significantly affect the biosynthesis of organic acids while using an in-situ bioaugmentation process. Thus, this toxicity needs to be controlled and
studied. On the other hand, strongly sorbed metals could increase operation costs since more organic acids would be needed to dissolve solid phases contributing to metal retention. Metal speciation in mining wastes before and after bioleaching were determined by using a selective sequential extraction (SSE) method [3]. Figure 2 shows the initial metal distribution of the 2 mining residues before bioleaching.

Figure 1. Partitioning of metals in soils under aerobic conditions (from (8)).

Figure 2. Metal distribution under natural pH for the 2 residues
(Résiduel = Residual, M. Organique = Organic Matter, Oxide/Hydroxides, Carbonates, Échangeable = Exchangeables and Solubles)
Bioleaching using fungi organisms is principally based on the following mechanisms: acidolysis (consisting of the solubilisation of the matrix by pH reduction), complexolysis (consisting of the complexation of metals by the excreted organic acids or amino acids and shown in Fig. 3), redoxolysis (the reduction of ferric iron which is mediated by the oxalic acid) and bioaccumulation of metals by the organism's mycelium. Metal bioleaching rates depend on acid concentration, complexation kinetics, contact time, pH, and metal geochemistry.

![Citric Acid](image)

\[ \text{RH} \rightarrow \text{R}^- + \text{H}^+ \]

\[ K = \frac{[\text{R}^-][\text{H}^+]}{[\text{RH}]} \]

\[ \log K = \log (\text{R}^-) + \log (\text{H}^+) - \log (\text{RH}) \]

\[ pK = \log (\text{RH}) - \log (\text{R}^-) \]

\[ pK_1 = 3.5 / pK_2 = 4.8 / pK_3 = 6.4 \]

**Figure 3. Complexation of heavy metals by organic acids**

5. **BIOLEACHING PROCESS**

Bioleaching studies were funded by Natural Resources Canada and were recently performed at the environmental laboratory in Laval University. Organic acid production can be carried out within the mine residue or produced elsewhere in a reactor. Thus, in this study, organic acids and residues were mixed directly or indirectly using either beaker or column reactors as shown in Figure 4. Each option presents specific advantages and were tested in a preliminary activity. While in-situ bioaugmentation can accelerate the overall treatment process, ex-situ production of organic acids can facilitate the isolation of preferred compounds for more efficient application on the piles and can avoid difficulties related to keeping optimum culture conditions in the field.

Indirectly, acids (supernatants) can be separated from fungi growth (biomass) and mixed with various concentrations of residues (1, 5, 7, 10 and 15% w/v). During preliminary experiments, fungus were grown in the presence of the same concentrations of residues up to 15 days.

Various bioleaching results are presented in table 2 and figure 4. As it can be seen, indirect bioleaching give in some cases low extraction ratios than direct bioleaching, this is due to the purity and sterile condition in indirect bioleaching were organic acid production is optimized. Targeted metals for extraction were Cu and Pb from the New Brunswick mine and Cu, Ni and Zn from the New Caledonia mine.
a) Acid production phase for indirect leaching

*P. simplicissimum*  
*Aspergillus niger*

b) Direct and indirect bioleaching in column

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Direct Bioleaching
```

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Indirect Bioleaching
```

Microorganisms culture

Mining Residues

```
New Caledonia residues
```

```
New Brunswick residues
```

Figure 4. Bioleaching layouts
Table 2. Results in % mass extraction from column bioleaching

**New Brunswick**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Aspergillus Niger</th>
<th>Penicillium simplicissinum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Indirect</td>
</tr>
<tr>
<td>Cu</td>
<td>8.9</td>
<td>12.3</td>
</tr>
<tr>
<td>Fe</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Mn</td>
<td>78.7</td>
<td>83.7</td>
</tr>
<tr>
<td>Ni</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Pb</td>
<td>19.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Zn</td>
<td>6.0</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**New Caledonia**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Aspergillus Niger</th>
<th>Penicillium simplicissinum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Indirect</td>
</tr>
<tr>
<td>Cu</td>
<td>12.5</td>
<td>31.3</td>
</tr>
<tr>
<td>Fe</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Mn</td>
<td>35.4</td>
<td>25.6</td>
</tr>
<tr>
<td>Ni</td>
<td>63.5</td>
<td>51.1</td>
</tr>
<tr>
<td>Pb</td>
<td>2.8</td>
<td>100</td>
</tr>
<tr>
<td>Zn</td>
<td>27.0</td>
<td>75.7</td>
</tr>
</tbody>
</table>

Figure 4. Bioleaching results for *Penicillium S.*
Results showed that organic acid production was not inhibited by the presence of mining residues, at least in the range of soil/liquid ratios under study. Preliminary tests were run to obtain optimal soil/liquid ratios for fungi growth.

An analytical procedure combining HPLC and GC-MS was developed in order to dose the various organic acids produced. As shown in figure 5 Gluconic acid was produced at the highest concentration followed by citric acid.

In a second part of the study, batch experiments evaluated continuous washing with the same initial organic acid load. After five washings, the metal efficiency extraction was: lead, 42%; cadmium, 96%; chromium, 85%; copper, 60% and zinc, 77%. Since pH influence the solubilization/precipitation of some metal forms (exchangeable, oxides, carbonates), the study included a pH monitoring of both, acid and percolated metal containing effluent solutions. A slightly increase in metal extraction was observed with a decrease in the pH of the acid produced solution. It was also concluded that metal geochemistry played a significant role in metal bioleaching.

![Figure 5. Organic acids production as function of time for indirect bioleaching](image)

Indeed, the SSE results not only allowed to determine metal distribution within the soil matrix but also to conclude that carbonate and amorphous forms of metals were among the more difficult metal particulate species to destroy before extraction was possible. In revenge, exchangeable and oxides were more easily attacked by the acids. Also, a slight redistribution of metal particulate species after bioleaching was observed. We want to further explore the interrelations between metal species distribution, metal species re-adsorption and acid components (e.g. gluconic, citric) selectivity. By developing the application of the SSE method, the information gained will allow a better conception of reliable, and predictable methodologies for on-site metal bioleaching.

### 6. USAGE OF ORGANIC WASTES AS SOURCE OF CARBON

Parallel experiments were run under direct and indirect leaching conditions in order to evaluate different carbon sources. These experiments were run using a concentration of 10% residues and use only *aspergillus niger*’s produced acids. Various Carbon sources
included molasses, corncobs, and brewery wastes. These experiments lasted for about three weeks. The results are shown in Figure 6. It is clear that sucrose solution gave the best leaching results under both direct and indirect conditions. The pH was low (pH 3.6) as in the previous set of experiments. Molasses yielded lower leaching results than the sucrose and better than the corncobs. Corncobs yielded reasonably good results (up to 24% copper removal) under indirect leaching conditions. They contain simple sugars that can be used by *A. niger*. Brewery waste gave poor results. The grains would require pretreatment to release simple sugars for it to be a suitable substrate.

![Graph showing leaching results](image)

Figure 6. Effect of C source on Cu extraction using indirect (a) and indirect (b) conditions

7. DISCUSSION AND CONCLUDING REMARKS

Indirect leaching was clearly more favorable for metal removal. When the flasks were not sterilized, faster growing bacteria took over, causing problems for the fungus and decreasing acid yields.

Another important parameter is the degree of selectivity of the organic acid mixture for precious metals rather than for non-precious metals (e.g. Ca, Mg, Na, K). Therefore, the bioleaching process must be conceived in such way of maximizing precious metal extraction, minimizing non-precious metal extraction, optimal organic acids ratios within the mixture, optimal pH to avoid early metal precipitation and optimal T°. The process must also include a pre-screening geochemical characterization that will determine the real potential of metal extraction.

In conclusion, metal recovery by fungi bioleaching is feasible, however, further applied research must be performed to provide greater understanding on the nature of complexes and bioleaching selectivity, on the influence of metal geochemistry on bioleaching as well as on the reuse of organic acids.

REFERENCES

Biooxidation of pyrite by *Acidithiobacillus ferrooxidans* in single- and multi-stage continuous reactors

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Abstract

The objective of this work was to study the extent of the bacterial attack on pyrite in the biooxidation of a refractory gold concentrate in continuous stirred tank reactors (CSTR). Two laboratory-scale biooxidation systems were installed. One of them consisted of a single stage 4-litre CSTR; the other one was a four-stage system with a total volume of 4 L. *Acidithiobacillus ferrooxidans* R2 was used; the concentrate, containing 66.7% pyrite, was suspended in 9K medium without ferrous sulfate.

The single-stage CSTR was operated with residence times between 3.5 and 10 days, with Eh values of 600-650 mV. Up to 51% of iron solubilization was obtained, with negligible ferrous iron levels. This result suggests that the bacterial ferrous iron oxidation proceeded at a higher rate than the pyrite attack.

The four-stage system operated with total residence times between 7 and 14 days, with maximum iron solubilization of 66% and Eh of 525 to 675 mV. Again, almost no ferrous iron was detected. Decreasing residence times had a large effect in diminishing the bacterial activity especially in the first stage because its low residence time. Oxygen demands measured in each stage revealed that decreasing total residence times caused a displacement of the main microbial activity towards the last stages.

These results show that increasing residence times favor the multistage configuration, resulting in higher degrees of pyrite oxidation than in the single CSTR. This is because in the latter case the positive effect of residence time tends to saturation.

*Keywords*: refractory gold concentrate, reactor configuration, CSTR, pyrite biooxidation

1. INTRODUCTION

Continuous biooxidation of refractory gold concentrates in tank reactors is currently a technologically and economically feasible technology that is being applied in several large-scale operations the world over [1, 2]. Reactors present significant advantages over heaps for bioleaching operations, in particular related to a more homogeneous reacting mass and the possibility of exerting close control on the main process variables.

The biooxidation of gold concentrates, as well all other bioleaching processes, is as a whole an autocatalytic complex reaction. This autocatalytic character is given by the fact

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that both cells and ferric iron act as reactants and products. This being the case, the optimal reactor configuration that minimizes the reaction volume for a given conversion is a continuous stirred tank reactor (CSTR) followed by a tubular reactor (TR) [3]. Conversely, given a defined volume, the multi-stage arrangement will render higher degrees of conversion. Because the need to maintain the solids in suspension and to supply oxygen and carbon dioxide rules out the operation of an actual TR, its kinetic behavior is simulated with a battery of CSTR’s connected in series [1, 4, 5]. This configuration is used in large-scale operations utilizing mesophilic bacteria [6] and has been tested at laboratory and pilot scale with extreme thermophiles [7, 8].

The objective of this work was to quantitatively characterize the behavior of a reaction system composed of four CSTR’s in series and compare it with the performance of a single CSTR of a volume equal to the total volume of the series arrangement.

2. MATERIALS AND METHODS

2.1 Microorganism, concentrate and culture medium

*Acidithiobacillus ferrooxidans* R-2 was used. This strain was cultivated in the presence of the same gold concentrate for over one year before performing these experiments. The refractory gold concentrate contained 15 g gold/tonne, 67.6% pyrite and 9.0% chalcopyrite. It contained 36.1% Fe, 39.7% S and 3.3% Cu. A fraction of particle size less than 75 µm was used.

Before each run the concentrate was washed with a 10% v/v aqueous solution of acetone, rinsed with diluted sulfuric acid pH 1.8 and dried overnight at 100°C. 9K medium was used, replacing the ferrous sulfate with the concentrate at a pulp density of 6% w/v.

2.2 Analytical methods

Ferrous ion iron was measured by the modified o-phenanthroline method [9] and total soluble iron was determined by reducing the ferric ion to ferrous ion with hydroxylamine and assaying with phenanthroline. Ferric iron was calculated as the difference between the two. Sulfate was assayed by turbidimetry [10]. Eh was monitored with an Ag/AgCl probe and dissolved oxygen was measured with a polarographic probe. Oxygen consumption rate was determined in each stationary state by the gassing-out method [11].

2.3 Bioreactor systems

Two continuous biooxidation systems were set up, each one of a total working volume of 4 litres. One was a single 4-L continuous stirred tank reactor (CSTR) and the other consisted of four CSTR’s connected in series. The first stage has a working volume of 1.75 L and the other three, which represent a TR, are 0.75 L each. The reactors were made of acrylic plastic and each one has four baffles, heating jacket, variable speed agitator with one pitched-blade turbine pumping down and Eh, pH and dissolved oxygen probes. The CSTR’s had constant geometrical ratios of H/L/T = 1.0 and D/T = 0.30. Aeration was supplied by means of a perforated annular sparger.

The tanks were fed and discharged by peristaltic pumps. Because of the very low flow rates involved, the pumps were turned on and off periodically by a programmable switch. Fresh pulp was fed from an agitated feed tank.
2.4 Operation conditions

The reactors were operated at 33ºC, with agitation of 600 rpm and aeration of 0.5 volumes of air per volume of liquid per minute (vvm). pH was adjusted to 1.8 with sulfuric acid at the start of the batch phase operation. Pulp density was 6% w/v. These constant conditions were used in all runs of both biooxidation systems.

The 4-L CSTR was operated at pulp residence times between 3.5 and 10 days. The four-stage system was operated at residence times in the range of 7 to 14 days. The reactors were operated continuously for at least three residence times before any measurements were done. The different steady states were always established by increasing the pulp flow rate.

3. RESULTS AND DISCUSSION

3.1 Single stage reactor

Figure 1 depicts the results of the operation of the single stage reactor. Under the experimental conditions used in this work, 50% of the pyrite was solubilized as revealed by the total soluble iron measurements. At all times the concentration of ferrous ion was negligible, a fact compatible with the high Eh values attained. The absence of Fe²⁺ is an indication that the rate of bacterial oxidation of ferrous ion was higher than the rate of ferrous production in the ferric leaching of the concentrate. The low Eh value at a residence time of 3.5 days could be due to the high solids content of the reactor due to the low extraction at that operation condition. This effect has been repeatedly observed by the authors under similar circumstances.

Table 1 presents the production rates, oxygen consumption rates and ferric/sulfate ratios obtained at the different residence times. Maximum rates are obtained in the range of residence times of 5.5-7 days, while the consumption rate of oxygen decreases steadily with increasing residence times. The Fe³⁺/SO₄²⁻ ratio varies between 0.60 and 0.69, higher than the stoichiometric value of 0.47 predicted by equation 1. It can also be noted that the oxygen consumption rate decreases with increasing ferric/sulfate ratios. These results

![Figure 1. Continuous solubilization of pyrite concentrate in 4-L CSTR at 6% pulp density, 0.5 vvm and 600 rpm](image)
suggest that the pyritic sulfur is not completely oxidized to sulfate. This uncoupled behavior of the solubilization and oxygen consumption rates arises from significant changes in cell physiology mediated by the dilution rate.

**Table 1. Production rates, oxygen consumption rates and ferric/sulfate ratios in the single-stage reactor**

<table>
<thead>
<tr>
<th>Residence time, (d)</th>
<th>Fe production, (g/L·d)</th>
<th>SO$_4^{2-}$ production, (g/L·d)</th>
<th>O$_2$ consumption rate, (g/L·d)</th>
<th>Fe$^{3+}$/SO$_4^{2-}$ w/w ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.86</td>
<td>1.43</td>
<td>3.54</td>
<td>0.60</td>
</tr>
<tr>
<td>4.5</td>
<td>1.33</td>
<td>2.00</td>
<td>3.34</td>
<td>0.67</td>
</tr>
<tr>
<td>5.5</td>
<td>1.36</td>
<td>2.27</td>
<td>2.70</td>
<td>0.60</td>
</tr>
<tr>
<td>7</td>
<td>1.37</td>
<td>2.00</td>
<td>2.06</td>
<td>0.69</td>
</tr>
<tr>
<td>10</td>
<td>1.10</td>
<td>1.60</td>
<td>1.79</td>
<td>0.69</td>
</tr>
</tbody>
</table>

* Dissolved oxygen concentration was higher than 40% saturation at all operation conditions

4 FeS$_2$ + 15 O$_2$ + 2 H$_2$O → 2 Fe$_2$(SO$_4$)$_3$ + 2 H$_2$SO$_4$  

(1)

**3.2 Multi stage system**

Steady state results are presented as a function of cumulative residence time in each reactor for the four total residence times considered.

The dissolution of pyrite is presented in Figure 2. No ferrous ion was detected in the liquid. The first stage operated at residence times between 3 and 6 days. The increase of solubilization obtained in that range was over seven fold, significantly higher than the one obtained in the single CSTR under similar conditions (Figure 1).

The solubilization that took place in the first CSTR increased steadily with residence time. Blank runs made with non-inoculated pulp showed that chemical leaching was almost nil, so the very low leaching that occurred at 3 days residence time must be due to the activity of a small bacterial population, as revealed by microscopic examination. By the other hand, the contribution of the three 0.75-L reactors was more important at low residence times than at high ones. As a whole, these data confirm the importance of the multi-stage design, pointing to an adequate operation residence time around 9 days.

It is worth noting that in the intermediate range of residence times such as 8 or 9 days, highest solubilizations were obtained with two and three stages rather than with the four reactors. This result could be influenced by the fact that some solids accumulated in each stage because of difficulties in attaining a homogeneous pulp because of the small scale of the reaction system.

Biooxidation expressed as percent iron extraction is shown in Figure 3. A maximum extraction of 66% is obtained at a cumulative residence time of 14 days. As a rule, the multi-stage system rendered higher extractions than the single vessel. This observation is consistent with the fact that the Eh values were also higher, as can be seen in Figure 4 as compared to Figure 1. The production of sulfate, presented in Figure 5, shows a similar pattern to the iron extraction, although the advantage of the four-stage system is apparent at lower residence times.
The biological oxidation generated ferric/sulfate ratio values of 0.45 to 0.68, lower than the ones obtained in the single reactor unit, pointing to a more complete oxidation of sulfur in the complex arrangement.
The production rates of each stage are presented in Table 2. Except for stage 1, the rates increase with decreasing total residence times. The residence time in stage 1 at total residence time of 7 days across the system is 3 days; this is probably a too short time and did not allow the establishment of a stable cell population of a sufficient size. Data from Table 2 regarding sulfate production rate suggest that the bacterial activity is displaced successively from the first to the following stages as the residence time decreases. This effect can also be appreciated in Table 3 that shows the oxygen consumption rates. From the results of Tables 2 and 3 it can be concluded that the main variation in the behavior of the system is produced in the transition from 7 to 8.5 days of total residence time.

The cumulative production rates of iron and sulfate in each stage are presented in Figures 6 and 7. Iron solubilization is less affected by residence time in stages 3 and 4 than in the first two stages, while sulfate production rate in each reactor increases up to a maximum and then decreases steadily with residence time. Cell activity is proportional to the slope of the curves of each stage. Similar behavior can be seen in the first three reactors, in which most of the pyrite solubilisation takes place. This saturation pattern could be due to the exhaustion of available active sites on the surface of the concentrate particles [12].

Table 2. Iron and sulfate production rates in the four-stage system (g/L·d)*

<table>
<thead>
<tr>
<th>Residence time** (d)</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe SO₄²⁻</td>
<td>Fe SO₄²⁻</td>
<td>Fe SO₄²⁻</td>
<td>Fe SO₄²⁻</td>
</tr>
<tr>
<td>7</td>
<td>0.43</td>
<td>0.67</td>
<td>1.67</td>
<td>3.05</td>
</tr>
<tr>
<td>8.5</td>
<td>0.86</td>
<td>1.61</td>
<td>1.25</td>
<td>1.88</td>
</tr>
<tr>
<td>10</td>
<td>1.19</td>
<td>2.51</td>
<td>1.44</td>
<td>2.13</td>
</tr>
<tr>
<td>14</td>
<td>1.63</td>
<td>2.45</td>
<td>0.76</td>
<td>1.14</td>
</tr>
</tbody>
</table>

* Calculated as the ratio of the increase of production in each stage to the residence time. Dissolved oxygen concentration was higher than 40% saturation at all operation conditions

** Considering the total reaction volume of 4 litres

Table 3. Oxygen consumption rate in the four-stage system (g/L·d)

<table>
<thead>
<tr>
<th>Residence time* (d)</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.32</td>
<td>1.41</td>
<td>3.12</td>
<td>2.66</td>
</tr>
<tr>
<td>8.5</td>
<td>1.91</td>
<td>1.58</td>
<td>1.47</td>
<td>1.07</td>
</tr>
<tr>
<td>10</td>
<td>1.98</td>
<td>1.68</td>
<td>1.48</td>
<td>1.41</td>
</tr>
<tr>
<td>14</td>
<td>1.80</td>
<td>1.75</td>
<td>1.50</td>
<td>1.30</td>
</tr>
</tbody>
</table>

* Considering the total reaction volume of 4 litres
4. CONCLUSIONS

The biooxidation of a pyritic refractory gold concentrate has been compared using a single stage CSTR and a reactor arrangement consisting of four CSTR’s in series of equivalent volume. It is concluded that increasing residence times favor the multistage configuration, resulting in higher degrees of pyrite oxidation than in the single CSTR.

ACKNOWLEDGMENTS

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REFERENCES

Chemical chalcopyrite leaching and biological ferric solvent production at pH below 1

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Abstract

Chalcopyrite ferric leaching experiments were conducted at pH 1.0 to 2.5 at 50°C and 65°C under air and nitrogen atmospheres. Copper leaching yields were higher (97%) at pH 1.0 than at pH 1.8-2.5 at both temperatures. Increase in the temperature increased the initial rate of leaching, but resulted in lower yields indicating high jarosite precipitation rates. The composition of the gas phase did neither affect redox potentials nor leaching rates. Jarosite and iron hydroxide precipitates were not formed and copper yields increased by using a ferric solution at pH 1.0. In tank reactor, similar copper leaching yields were obtained with different ferric supply regimes. Biological generation of ferric solution at low pH was studied in batch and continuous-flow fluidized-bed reactors (FBR). In batch assays at 35°C, biological iron oxidation rate was not affected by pH of 0.9-1.5 but started to decline at 0.7. The pH of the FBR was gradually decreased to 0.9 without changes in iron oxidation; the maximum iron oxidation rate at pH 0.9 was 10 g Fe$^{2+}$ dm$^{-3}$ h$^{-1}$. The results indicate that biologically produced ferric solvent at pH 0.9 results in high chalcopyrite leaching yields.

Keywords: chalcopyrite, ferric leaching, iron oxidation, passivation

1. INTRODUCTION

One of the major problems in hydrometallurgical applications is the formation of a hindering diffusion layer on the mineral surface or a contact hindrance between the leaching solution, microbes and mineral. A better understanding of the surface speciation under leaching conditions is a key factor in improving dissolution kinetics and yields in bioleaching.

Passivation layer in sulphide mineral leaching with ferric iron has been proposed to consist of iron hydroxy precipitates or elemental sulphur formed as end products [1,2,3]. Polysulphides may act as transient intermediates of leaching [1]. Iron in the leaching solution precipitates as iron oxides or basic iron salts like jarosite [2,3,4]. Leaching conditions such as pH, temperature and ionic composition and concentration of the medium affect the formation of iron hydroxy precipitates [5].

Ferric iron precipitation diminishes the available ferric iron in the leach solution, forms kinetic barriers and tends to block pumps and valves. The iron precipitation highly
depends on the pH and temperature. [5] *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* are the most important mesoacidophilic microorganisms involved in ferrous iron oxidation. The optimum pH for bacterial iron oxidation is generally 2.0 to 2.5. [6] *L. ferrooxidans* tolerates lower pH values than *T. ferrooxidans*, which usually does not survive below a pH of 1.0 [5,6]. Even though microorganisms may adapt to physicochemical changes such as pH in their environment, there are limits to the extent to which this may occur [7].

In this study the influence of temperature, pH and atmospheric oxygen on indirect ferric leaching rates and surface precipitates were studied. Since the jarosite precipitation can be prevented at a very low pH, the potential of biologically producing ferric solvent at low pH was studied in batch and continuous flow reactors. The maximum iron oxidation rate at pH of 0.9 was determined. Further, different ferric leach solutions were used to maximize the copper leaching and minimize the iron precipitation.

2. MATERIALS AND METHODS

2.1 Influence of pH on the leaching rate

The influence of pH on the leaching rate of chalcopyrite (Outokumpu Ltd, Pyhäselmi, Finland, Cu 25.4%; Fe 27.9%) was studied at 50°C and 65°C in 150 cm³ erlenmeyer flasks using 3% (w/v) solids concentration. Leaching solution consisted of 0.4 g dm⁻³ (NH₄)₂SO₄, 0.25 g dm⁻³ KH₂PO₄·2H₂O, 0.25 g dm⁻³ MgSO₄·7H₂O, 0.02 g dm⁻³ yeast extract and a stoichiometric amount of ferric iron as Fe₂(SO₄)₃ (Eq. 1).

\[ \text{CuFeS}_2 + 4 \text{Fe}^{2+} \rightarrow \text{Cu}^{2+} + 5 \text{Fe}^{2+} + 2 \text{S}^0 \]  

(Eq. 1)

The pH was adjusted with H₂SO₄ or NaOH. Experiments under nitrogen atmosphere were conducted in order to study the influence of atmospheric oxygen on the precipitation. The leaching solution was sparkled with N₂ before the addition of chalcopyrite (15 min) and after sampling (5 min). Samples (5 mL) were centrifuged (5000 rpm, 12 min) and the supernatants were used for analyses. Dissolved copper and iron concentrations were analyzed by inductively coupled plasma connected to mass spectrometry (ICP-MS) and the ferrous iron concentration spectrophotometrically by ferrozine method [8]. Ferric iron concentration was determined as the difference of total and ferrous iron concentration. The pH was measured with WTW Sentix 42 electrode and redox potential an ORION combination electrode 9678BN. Samples for SEM/EDX and XRD were centrifuged (4000 rpm, 3 min) and pellets were dried at 40°C. SEM/EDX-analyses were conducted with JEOL JSM-5600LV scanning electron microscope. In addition some of the residues were examined with x-ray diffraction (Cu anode, Kα radiation, λ = 1.54).

2.2 Effect of the ferric addition on chalcopyrite leaching

The experiments were conducted in 2 L stirred tank reactors (Figure 1). The reactor conditions were as follows: liquid volume 1.5 L, solids concentration 10% (w/v), and temperature 50°C. The leach solution and a stoichiometric amount of Fe₂(SO₄)₃ was added in either in 1, 2 or 5 shares. The pH of the leach liquor was adjusted to 1.5 with H₂SO₄ and NaOH at the beginning and at the sampling time. Redox potential was measured using Hamilton Pt-ORP electrode and pH using Orion model SA720 pH-meter after filtration (0.45 µm). The concentrations of dissolved copper and iron were measured using ICP-MS. The ferrous iron and SEM/EDX-analyses were performed as described above. The evaporation losses were accounted for in the results.
2.3 Influence of pH on iron oxidation

The influence of pH on iron oxidation was studied in duplicate 120 mL serum bottles in 60 mL of total volume at 150 rpm in a rotary shaker at 35°C. The mineral medium was autoclaved (121°C, 0.1 MPa, 20 min) before the addition of FeSO₄·7H₂O after sterile filtration (0.2 µm). The final concentration of the mineral medium was 0.35 g dm⁻³ (NH₄)₂HPO₄, 0.05 g dm⁻³ K₂CO₃ and 0.05 g dm⁻³ MgSO₄. The pH was adjusted with H₂SO₄ to 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5 with 7 g dm⁻³ Fe²⁺ in the solution. The bottles were inoculated with 1 mL of an enrichment culture of iron-oxidizers from a fluidized-bed reactor [9]. In the chemical control bottles, sterile H₂O was used instead of inoculum.

After batch assay determinations of the pH tolerance limits, the pH in the fluidized-bed reactor with activated carbon as the biomass carrier [9] was gradually decreased to 0.9. The composition of the feed solution was 34.75 g dm⁻³ FeSO₄·7H₂O, 0.35 g dm⁻³ (NH₄)₂HPO₄, 0.05 g dm⁻³ K₂CO₃ and 0.05 g dm⁻³ MgSO₄ in tap water at pH of 0.9. The maximum iron oxidation rate was determined in the fluidized-bed reactor at the pH of 0.9 with 21 g dm⁻³ ferrous iron in the feed solution.

The ferrous iron concentration was determined using the Shimadzu UV 1601 spectrophotometer by the colorimetric ortho-phenantroline method [10] modified as follows: 2 mL of 1,10-phenantroline (10 g/L) and 1 mL of ammonium acetate buffer were added to 3 mL of sample. Dissolved oxygen and temperature measurements were made using WTW OXI96 meter at the sampling time.

3. RESULTS

3.1 The influence of pH, temperature and gas phase composition on leaching and precipitation

Leaching experiments were conducted at different pH values under air and nitrogen atmospheres at 50°C and 65°C (Figure 2). Copper yields increased with decreasing pH; at 50°C they were 95%, 35% and less than 10% at pH 1.0, 2.0 and 2.5, respectively. At
65°C, the initial rate of leaching was higher, but resulted in lower yields than at 50°C indicating rapid precipitation. Iron precipitation was significant at pH of 1.8-2.5, which affected the leaching efficiency (Figure 3). At pH 1.0, the ferric iron did not precipitate, but was reduced to ferrous iron during leaching. Redox potentials decreased in the course of leaching, being highest at low pH values. The composition of the gas phase did not affect the redox potentials or leaching rates.

Figure 2. Copper leaching yields from chalcopyrite at 50°C (right) and at 65°C (left)

In all experiments, SEM/XRD revealed jarosite precipitation layers on the surfaces of the CuFeS₂. The layer consisted of large and small regular-shaped cubes with the average composition as presented in Table 1. The mineral surface was much less covered by the precipitation layer at pH 1.0 than at pH 1.5 and above (Figure 4). The surface of the ore (25% Cu, 16% Fe, 33% S) was partly covered by amorphous sulphur rich layer (60-97% S) typically formed under the cubic jarosite precipitates. Temperature and pH influenced the leaching yields, but not the composition of the residue-precipitates.

Table 1. The range of elemental-% composition (EDX) of separate layers on CuFeS₂ surfaces after 17 to 19 days of ferric leaching at pH 1.8-2.5

<table>
<thead>
<tr>
<th>Mineral surface, unleached</th>
<th>Theoretical composition of jarosites</th>
<th>Surface composition after leaching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Larger precipitates</td>
</tr>
<tr>
<td>O, %</td>
<td>---</td>
<td>44-50</td>
</tr>
<tr>
<td>S, %</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Fe, %</td>
<td>11-22</td>
<td>33-35</td>
</tr>
<tr>
<td>Cu, %</td>
<td>14-24</td>
<td>---</td>
</tr>
<tr>
<td>Na/K %</td>
<td>---</td>
<td>~0</td>
</tr>
</tbody>
</table>
Figure 3. The fate of iron in chalcopyrite leaching at 50°C under the air atmosphere at A) pH 1.0, B) pH 1.8 and C) pH 2.0. (▲) calculated dissolved iron concentration (Fe from Fe$^{3+}$-solvent plus Fe dissolved from CuFeS$_2$), (x) dissolved iron (measured concentration), (♦) Fe$^{2+}$ concentration, (■) Fe$^{3+}$ concentration

Figure 4. The mineral surface shown by scanning electron microscopy after 17-19 days of ferric leaching at 50°C A) at pH 1.0 and B) at pH 1.8
3.2 Effect of the ferric addition on chalcopyrite leaching

Ferric iron was added to the 50°C stirred tank reactor at pH 1.6 in five, two or one doses and resulted in similar copper leaching yields (approximately 40%) with all supply regimes ferric iron becoming the limiting factor (Figure 5). The initial leaching rate was highest, and redox potential and pH decreased fastest when ferric solution was added in one dose in the beginning. The iron precipitation was highest with one dose and lowest with five doses. SEM/EDX revealed elemental sulphur layer on the surface of the mineral at the end of the experiment, but showed also mineral surfaces without passivating layer (Figure 6).

![Fig 5](image)

**Figure 5.** The yield, pH and redox potential with 1 (♦), 2 (▲) and 5 (■) ferric solution additions (shown by arrow). Dissolved iron (◊), Fe³⁺ (Δ), Fe²⁺ (□) and (x) calculated dissolved iron (Fe from Fe³⁺-solvent plus Fe dissolved from CuFeS₂) concentrations. Addition of ferric solution in five (A), two (B) or one (C) doses.
3.3 Effect of pH on iron oxidation

The iron oxidation rate in the batch experiments remained unaffected at pH of 0.9-1.5 but started to decline at 0.7 (Figure 7). The lag phase of three weeks occurred at pH 0.5 and iron oxidation was strongly affected. The pH of the iron oxidizing fluidized-bed reactor was gradually decreased to 0.9 without changes in performance (Figure 8). The maximum iron oxidation rate at pH 0.9 was 10 g Fe^{2+} dm^{-3} h^{-1} (Table 2). The results show that the high-rate ferric production and regeneration in the FBR was possible at pH 0.9.
Figure 8. The influence of pH decrease on iron oxidation rate in a fluidized-bed reactor. pH in FBR (■), pH in feed (□), load g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1} (△) and oxidation rate g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1} (*).

Table 2. Iron oxidation rate in the fluidized-bed reactor at 35°C. pH of the feed solution 0.9

<table>
<thead>
<tr>
<th>Period</th>
<th>Length of the period (days)</th>
<th>Number of data points</th>
<th>Fe oxidation max (%)</th>
<th>Fe oxid. min-max (g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1})</th>
<th>Fe oxid. mean (g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>10</td>
<td>7</td>
<td>94.6</td>
<td>7.3-9.7</td>
<td>8.1</td>
</tr>
<tr>
<td>Period 2</td>
<td>20</td>
<td>4</td>
<td>99.2</td>
<td>9.7-10.4</td>
<td>10.0</td>
</tr>
<tr>
<td>Period 3</td>
<td>14</td>
<td>3</td>
<td>99.5</td>
<td>9.2-10.4</td>
<td>9.8</td>
</tr>
</tbody>
</table>

4. DISCUSSION

In this work, jarosite precipitates were typically found on top of the sulphur rich layer indicating the passivation of the mineral surface first by sulphur followed by jarosite precipitates. Increase in temperature from 50°C to 65°C increased the initial copper leaching rate, but resulted in reduced copper yields. This was likely due to faster precipitation at high temperature. Chalcopyrite leaching with ferric iron was initially fast regardless of pH, but slowed down or completely stopped due to intense precipitation. Decrease of the leaching pH resulted in less precipitates and copper yields close to 100%. Leaching proceeded similarly under air and nitrogen atmospheres. The results of this work demonstrated that stepwise adding of ferric iron did not improve the copper yields.

For biological ferric regeneration, the pH of the iron oxidation reactor needs to be maintained low. In general the mesophilic iron oxidizers do not survive below pH 1.0 [5, 7], at which the ferric leaching experiments indicated copper yields close to 100%. The other mesophilic iron oxidation studies have been carried out at pH of 1.1-3.2 [11-23]. In this study, the iron oxidation rate remained unaffected at pH of 0.9-1.5. The maximum iron oxidation rate in a fluidized-bed reactor dominated by *Leptospirillum* like organisms (Kinnunen et al., paper in preparation) at pH 0.9 was 10 g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1}, which was similar to the iron oxidation rate at pH 1.4 in the same reactor (8.2 g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1}), when air was used for aeration [9]. The iron oxidation rate of this study compared favourably with the mesophilic iron oxidation rate (0.9 g Fe\textsuperscript{2+} dm\textsuperscript{-3} h\textsuperscript{-1}) in the fluidized-bed reactor with
activated carbon as the carrier material at pH of 1.35-1.5 [23]. Ferric regeneration at 35°C was chosen to this study, because the moderately thermophilic iron oxidation rate at 60°C was considerably less than that of mesophilic [24].

In conclusions, the combination of high-rate biological ferric production at 35°C and pH 0.9 and below followed by chemical chalcopyrite leaching at 50°C to 65°C is promising for chalcopyrite leaching.

REFERENCES

Comparative study of the bioleaching of two concentrates of chalcopyrite using mesophilic microorganisms in the presence of Ag(I)

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Abstract

A bioleaching study of two copper concentrates from Sonora and Zacatecas, (Mexico), containing basically chalcopyrite, was performed. X-ray diffraction studies, XRD, showed certain variations in the composition of the concentrates. One of them, C1, showed a greater secondary copper sulfide presence and pyrite (23.2% Cu and 20.6% Fe). The concentrate C2, practically did not have secondary copper sulfides and it had very little pyrite amount (21.85% Cu and 31.14% Fe). Mesophilic microorganisms at 35°C in presence and absence of Ag(I) as catalytic agent were used. The Ag(I) ion showed an important catalytic effect on concentrate C1 bioleaching, whereas on concentrate C2 the effect was not noticed, and an smaller copper extraction was observed. The copper extraction increased doping C2 concentrate with pyrite and chalcocite.

Keywords: bioleaching, chalcopyrite, pyrite, chalcocite, catalytic effect, mesophilic

1. INTRODUCTION

The environmental requirements imposed on pyrometallurgical processes of sulfide mineral concentrates in the copper industry have forced the development of hydrometallurgical routes as alternatives for the conventional treatment of sulfide minerals concentrates in order to avoid the SO₂ production. Those processes involve sulfide oxidation either to sulfur or sulfate using oxidating agents such as O₂ or Fe(III) ions or by a direct anodic oxidation in an electrolyte. This oxidation can be considered as an electrochemical reaction (1), with the cathodic reduction of the oxidant and the anodic oxidation of sulfide (2). The first idea that the existing chemical interactions on the surface of minerals could be of electrochemical nature was proposed by Salamy and Nixon (3).

Bacterial leaching is an economical and widely used method for metal extraction, but until now its application has been limited to low metallic content minerals (4). During the last two decades, this process has been successfully applied to refractory mineral of Au and Ag treatment, and recently has been used for cobalt recovery from pyritic material in Uganda (5).

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Nevertheless, in many cases, the slow kinetic of the biooxidation processes has limited its commercial application. This slowness is attributed to different parameters such as biological, physicochemical, electrochemical and mineralogical factors (6). Among mineralogical factors affecting the bioleaching systems is the initial composition of the material that, will cause that the systems will respond different ways to the same treatment conditions.

As an attempt to accelerate the kinetics of chalcopyrite dissolution, the use of catalytic agents has been proposed. The catalytic effect of silver, Ag(I), during the chalcopyrite leaching has been reported (7, 8, 9). The improvement in the dissolution rate is attributed to the formation of a film of Ag₂S on the surface of chalcopyrite particles (8).

According to the semi-conducting characteristics of sulfide minerals (6), the electrochemical interactions (galvanic pairs formation) originated among different sulfide minerals in a same bioleaching system could improve the selective dissolution of the most active minerals. Although these interactions have been known for some time, it is complicated either to explain or predict their effects on the bioleaching rate in a sulfide mineral mixture (10).

In this work the results of bioleaching tests with mesophilic microorganisms, in absence and in the presence of Ag(I) of two concentrates of chalcopyrite, as well as the results of bioleaching of the concentrate C2 doped with pyrite and chalcocite, also in absence and in the presence of Ag(I) are presented.

2. MATERIALS AND METHODS

2.1 Minerals

Two concentrates of chalcopyrite from Sonora and Zacatecas (Mexico), C1 and C2, respectively, were used. The samples of C2 were doped with 10% of pure minerals of pyrite (Py) or chalcocite (Ct), also from Zacatecas, Mexico.

2.2 Cultures

The mesophilic microorganisms used were mixed cultures obtained from the own microflore of the concentrates, adapted by successive steps and developed in 100 mL of nutrient medium and 5g of concentrate. Three successive steps were carried out.

2.3 Bioleaching tests

The bioleaching studies were made in an orbital incubator with temperature and stirring controlled to 35°C and 150 rpm, respectively. The bioleaching tests were made with 90 mL of medium, 10 mL of inoculum and 5g of concentrate. All the tests were made in Erlenmeyer flasks of 250 mL.

2.4 Monitoring and control techniques

Periodic measurements of pH and redox potentials, ORP, were made. The pH was fixed to the necessary value by addition of a diluted solution of H₂SO₄. The bacterial growth evolution was obtained determining the cellular concentration in solution samples counting the cells using an optical microscope with a Neubauer chamber. The analysis of metallic values in solution (Cu and Feₜot) was conducted by atomic absorption spectrophotometry.

The main mineral phases present in the concentrates were identified by means of x-rays diffraction, XRD.
3. RESULTS AND DISCUSSION

3.1 Chemical analyses and characterization of the samples

The chemical analyses of the resulting solutions of an acid attack to the concentrates allowed determining the following concentrate composition:

Table 1. Chemical composition of the concentrates

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>23.20%</td>
<td>20.60%</td>
</tr>
<tr>
<td>C2</td>
<td>21.85%</td>
<td>31.14%</td>
</tr>
</tbody>
</table>

Studies of XRD showed variations in the initial composition of the concentrates, exhibiting one of them, C1, a greater secondary copper sulfide and pyrite presence. The other concentrate, C2, did not have secondary copper sulfides, and contained very little pyrite and an important amount of arsenopyrite, figures 1A and 1B.

Figure 1A. Diffractogram of C1, and Figure 1B. Diffractogram of C2. (Cv: covellite, SiO₂: silica, Cp: chalcopyrite, Py: pyrite y As: arsenopyrite)

In figures 1A and 1B a clear difference between C1 and C2 is observed. With respect to the secondary copper sulfide, covellite, it was present in the C1 sample but not in the C2 sample. The pyrite presence in C1 was greater than in C2. The presence of arsenopyrite in C2 was not detectable in C1. Diffractograms of synthetic composites (results not shown) presented the signal corresponding to pyrite (sample C2Py) and chalcocite (sample C2Ct).

3.2 Copper and iron extraction

Figure 2A shows an important difference between the reactors of C1, with and without Ag(I), results of reference (12), and the reactors of C2, with and without Ag(I). These last ones practically did not differ between each other. It is necessary to mention that the concentrate C1 contained secondary copper sulfides, which are less recalcitrant to the acid dissolution, present a faster dissolution which was on a greater copper extraction: in 22 days 45% without Ag(I) and 62% with Ag(I). For C2 a copper extraction of only 16% in almost 80 days was reached in both cases.

In figure 2B it can be observed that the iron extraction in systems with C1 and C2 was very similar with the exception of the C1 reactor with Ag(I), where a greater extraction in a smaller residence time than with C2 was reached: 25% of extraction in 30 days. The recalcitrance of C2 with respect to C1 was greater.

In the reactors with C1, figure 2A, an important amount of copper came from secondary sulfides, because in the dissolution of the chalcopyrite equal amounts of both
copper and iron are practically dissolved. Except for the C1 with Ag(I), all of them had a very similar behavior for iron dissolution.

Figures 2A and 2B. Cu and Fe dissolution respectively. ▲ C1 without Ag(I), • C1 with Ag(I), ■ C2 without Ag(I) and ♦ C2 with Ag(I)

Under the testing conditions, a slower dissolution of C2 was obtained, with a residence time of 80 days.

Because of the poor results obtained in the bioleaching tests of C2, it was decided to doped systems of this concentrate with pure minerals of pyrite and chalcopyrite in order to corroborate the influence of these sulfides on the copper extraction using Ag(I).

Figures 3A and 3B show the copper and iron extraction in the bioleaching systems under different testing conditions: pyrite and chalcopyrite doped C2 systems in presence of Ag(I).

In figure 3A it is observed that, at the beginning of the experiments, the reactors with greater copper dissolution were the chalcopyrite-C2 doped systems, due to the acid attack given by:

$$\text{CuS} + 2\text{H}^+ \rightarrow \text{Cu}^{2+} + \text{H}_2\text{S} \quad (1)$$

Figures 3A and 3B. Cu and Fe extraction respectively. ♦ C2 + Ag(I), ■ Pyrite-C2 doped + Ag(I) and ▲ Chalcocite-C2 doped + Ag(I)

The massive dissolution of the chalcopyrite initiates after 70 days, obtaining a substantial increase in the copper extraction in the pyrite-C2 system reaching around 50% in 84 days.

In all systems a greater copper dissolution is observed around day 70, agreeing this with the dissolution of iron, attributed, therefore, to the attack of the chalcopyrite. The higher copper recovery is obtained in the pyrite-C2 doped system, 50% in 84 days; surpassing by almost 20% that of the system also doped but with chalcopyrite for the same residence time.
The iron extraction curves, figure 3B, present a slightly different behavior from the curves of copper extraction, being at the end of the tests the reactors pyrite-C2 doped and C2 those reached the greater extraction.

In the chalcocite-C2 doped systems and in the systems containing only C2, it can be seen a slight increase in the iron dissolution. A possible reason for this behavior is the acid attack only on chalcocite, and therefore, the attack to the crystalline structure of the chalcopyrite was almost negligible.

Figures 4A and 4B show the copper and iron extraction in the bioleaching pyrite-C2 and chalcocite-C2 doped systems in absence of Ag(I).

![Figures 4A and 4B. Cu and Fe extraction respectively. ♦C2, ■ Pyrite-C2 doped and ▲ Chalcocite-C2 doped](image)

It can be seen, in figure 4A, the acid attack to chalcocite crystalline structure occurred based on the high copper extraction in that chalcocite doped system, almost the same that with the pyrite doped system, 31% in 84 days.

This result verifies the fact that the Cu in solution in these systems came mainly from the secondary sulfides that were added, and in the case of the system containing only C2 it resulted from the slight attack to the chalcopyrite structure. Results of MEB (not shown) demonstrated a preferential attack on secondary copper sulfides greater than on the chalcopyrite particle surface.

In the case of the pyrite doped system without Ag(I) a slight reduction in the time needed to initiate the massive iron dissolution, 5 days with respect to others systems was observed. The same behavior was observed in the copper extraction curve of C2, figure 4A and 4B.

This agrees with observations made by other authors (6,10,11) in the sense that, in a bioleaching system where different sulfide minerals are present, their respective rest potentials (E_{rep}) will create galvanic interactions that will influence the selective dissolution of the most active minerals.

### 3.3 pH and redox potential, ORP

In figures 5A, 5B, 6A and 6B the results corresponding to representative curves of redox potential, ORP, and pH evolution for pyrite-C2 and chalcocite-C2 doped systems with and without Ag(I), respectively, are showed. Previous work have showed the effect of the presence of Ag(I) on the redox potential behavior on bioleaching systems (12).

In figure 5A it is observed that the ORP evolution was affected by the presence of Ag(I) in the catalyzed systems.
The way in which Ag(I) affects the chalcopyrite dissolution is depicted by the following electrochemical reaction between Ag(I) and chalcopyrite:

\[
\text{CuFeS}_2 + 4\text{Ag}^+ \rightarrow 2\text{Ag}_2\text{S} + \text{Cu}^{2+} + \text{Fe}^{2+} \quad (2)
\]

Later, the silver sulfide generated in (2) reacts with the ferric ion, Fe(III), to form:

\[
\text{Ag}_2\text{S}_{(\text{sup})} + 2\text{Fe}^{3+} \rightarrow 2\text{Ag}^+ + 2\text{Fe}^{2+} + \text{S}^0 \quad (3)
\]

This reaction consumes Fe(III) ion driving a decrease of the ORP value corresponding to Nernst equation on the Fe(III)/Fe(II) ratio.

In the representative curves of catalyzed doped systems pH, figure 5B, a very similar behavior in all the cases was observed, except on chalcocite doped systems, where a slight increase of pH was recorded during the first 5 days due to the H⁺ consumption by the acid attack on chalcocite (reaction 1).

\[
\text{Cu}^2+ + \text{Ag(I)}, \quad \text{Pyrite-C2 doped + Ag(I)}, \quad \text{Chalcocite-C2 doped + Ag(I)}
\]

From curves 6A it can be appreciated that the system that reached the highest value of ORP, and also with greater iron dissolution, figure 4B, was the pyrite-C2 doped system. In the other cases the values of redox potential are rather similar and only in those systems in which chalcocite was added the values of ORP were slightly low.

In the final stage of the experiments, figure 6B, the reactors showed a slight increase in the pH value due to the ability of protons to attack the chalcopyrite (13) and due to a decrease in bacterial activity.

\[
\text{6A} \quad \text{6B}
\]

Figures 6A and 6B. Redox potential and pH of pyrite-C2 and chalcocite-C2 doped bioleaching systems in absence of Ag(I)
4. CONCLUSIONS

- The initial composition of sulfide minerals affected the kinetics of bioleaching, which was influenced by the galvanic interactions generated when putting in contact minerals of different rest potentials.
- The galvanic interactions follow the thermodynamic prediction of galvanic series of sulfides.
- The chalcocite presence did not improve the copper extraction from chalcopyrite.
- The presence of pyrite improved the chalcopyrite bioleaching.
- The catalytic effect of the Ag(I) was affected by the presence (or absence) of pyrite in the bioleaching systems.
- The highest copper extraction was reached in the pyrite-C2 doped system in the presence of Ag(I): 50% in 84 days.

ACKNOWLEDGEMENTS

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REFERENCES

Comparison of air-lift and stirred tank batch and semi continuous bioleaching of polymetallic bulk concentrate

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Abstract

Metal bioextraction from GMDC polymetallic bulk concentrate was studied in stirred tank and air-lift reactors with a working volume of 5 dm\textsuperscript{3} and 20\% (w/v) pulp density. The inoculum used in these studies was a consortium mainly consisting of \textit{Acidithiobacillus ferrooxidans}, \textit{Leptospirillum ferrooxidans} and \textit{Acidithiobacillus thiooxidans}. Under the optimum conditions air-lift reactor gave 41, 75 and 65\% of copper and zinc extraction and galena oxidation as compared to the STR giving 76, 70 and 80\% respectively in 5 to 9 d of incubation time. During the process, 590 and 547 mV redox potential, 0.05 and 0.07 g/l of soluble ferrous and pH of 1.55 and 1.78 were recorded in air-lift reactor and STR respectively. When the total 20\% (w/v) pulp density was added in four equal fractions in STR study, instead of one lot addition, the metal extraction rate increased by 1.22 and 1.1 fold for copper and zinc respectively. Use of semi-continuous fed-batch STR process further enhanced the copper and zinc extraction rate by 1.6 and 1.54 fold as compared to batch STR process. The highest metal extraction of 92 and 87\% of copper and zinc respectively was achieved in semi continuous process. Acid consumption was 75\% less in fed-batch semi continuous process as compared to batch process. Optimisation of the process and use of developed inoculum reduced the bioleaching time from 30 d to as low as 5 and 7 d for copper and zinc respectively with overall increased percent metal extraction. The reduction in contact time could make the polymetallic bulk concentrate bioleaching process economically viable.

Keywords: polymetallic concentrate, semi continuous, air-lift, stirred tank

1. INTRODUCTION

Bioleaching has generated intense research activity in late nineties which, resulted in important findings in the field, such as the development of economics and engineering factors of biometal extraction [1]. Bioextraction processes are now applied on commercial scale for the extraction of copper, cobalt, gold and nickel from refractory ores and concentrates [2, 3]. Microbial leaching has been used at laboratory scale for the base metal sulphides of Co, Ga, Mo, Ni, Pb and Zn. Biohydrometallurgical processes are developed

* Corresponding author: S.R. Dave, E-mail: shaileshrdave@hotmail.com
for extraction of nickel and cobalt from their sulphidic concentrates using mesophilic iron and sulphur oxidising bacteria in stirred tank reactor [2].

Several factors such as oxygen requirement, nutrient availability, agitation speed and inoculum size plays an important role in metal extraction. However, the operating solid concentration constitutes one of the most critical parameter of a bioleaching process in terms of size of the equipment. At higher operating pulp concentration the above mentioned factors could limit the bioleaching efficiency [4-6]. The previous laboratory pilot scale studies were mainly carried out only up to 10% solids with about 50 to 80% metal extractions in 15 to 20 days [1, 7, 8]. The indirect two-stage bioleaching process from complex sulphidic Cu-Pb-Zn concentrate is developed in which, metal extraction is done at 70°C temperature [9]. But, the direct bioextraction process for such complex polymetallic bulk concentrate is poorly developed.

India has well scattered small reserves of polymetallic ores and Ambamata Mine situated in Gujarat is one of them. Biomineral processing holds great potential for such reserves in India. In India, in spite of cheap labour, liberalised market, vast consumer base and strong foundation of hydrometallurgy, there exists a wide gap between the existing potential and the potentials to be exploited for economic metal growth [10, 11].

In biohydrometallurgical processes the stirred tank and air-lift reactors are widely used at laboratory scale but pulp density studied usually is upto 12.5% [12]. In this context, in the presented work air-lift and stirred tank batch and semi continuous bioleaching of polymetallic bulk concentrate was carried out after optimisation at shake flask leaching scale. Comparative bioleaching profiles of both these reactors at laboratory scale with 20% pulp density is discussed.

2. MATERIALS AND METHODS

2.1 Polymetallic concentrate

Polymetallic concentrate was procured from Gujarat Mineral Development Corporation (GMDC), Ambamata Multimetal Project, Gujarat, India. Major constituents of the concentrate were sphalerite, chalcopyrite, galena and pyrite. The concentration of copper, lead, zinc, iron and sulphur in the concentrate were 2.5, 13, 30, 9 and 27% respectively. Detailed composition is given elsewhere [13]. The bulk concentrate received was of mixed particle size ranging between +150-325 # B.S.S. and was used as received. In all the experiments 20% (w/v) pulp density was used.

2.2 Inoculum

The inoculum used for extraction of metals was developed from shake flask leaching experiment in the form of leachate. Leachate used in the experiment mainly consisted of a consortium of acidophilic chemolithotrophic auto- and heterotrophic iron and sulphur oxidisers [14]. All the experiments were carried out with 20% (v/v) leachate as inoculum and M-2 modified medium [15] prepared in tap water.

2.3 Reactors

Bioextraction studies were compared at laboratory scale with two reactors. First, an Air Lift Glass Reactor (ALGR) which was fabricated in the laboratory consisting 5 dm³ working volume. Detailed design set up and configuration is given elsewhere [12]. The other bioreactor tested was a laboratory scale Stirred Tank Reactor (STR) with axial turbine type impellers which was designed in our laboratory and fabricated by Texfab
Manufacturers, India. The reactor consisted 5 vessels (3 in cascade and 2 in series) each 5 dm$^3$ capacity with working volume of 3 dm$^3$. The rate of agitation, aeration and CO$_2$ supply was 300 rev/min, 0.5 l/min/v and 0.2% (v/v) of compressed air respectively. The detail configuration is quoted elsewhere [10, 11, 15]. Temperature during the investigation was 30 ± 5°C, i.e. ambient room temperature.

2.4 Bioleaching study

In both the reactors typical batch leaching trial was operated until the logarithmic growth and steady leaching conditions were well established. In STR the pulp addition mode was tested with the four equal parts i.e. instead of addition of pulp 20% (w/v) at the 0th h, it was added in four fractions of 5% (w/v) each at 24 h of interval. The semi continuous bioleaching study was carried out in STR with 7 d of residence time. The detail design is given in the previous article [5].

2.5 Analysis

Supernatant of the leaching system was analysed periodically. pH and redox potential (Eh) was determined using Systronics Digital μ pH system 361 with platinum SCE couple electrode. Soluble ferrous iron was estimated by potassium dichromate titrimetric method. Copper, zinc and lead were estimated by spectrophotometric (Systronics UV–Vis spectrophotometer 119), polarographic (ELICO Polarograph Model CL-25 D) and titrimetric (tannic acid as external indicator) methods respectively from the leached solutions [16]. The analysis was confirmed by Atomic Absorption Spectrophotometer (Varian AA-175 model).

3. RESULTS AND DISCUSSION

After the amenability and bioleaching optimisation study of GMDC polymetallic bulk concentrate at shaken flask level [14, 17], the batch and semi continuous fed batch processes were performed with 20% (w/v) pulp density in a laboratory scale reactor. The performances of two reactors were compared for batch leaching with airlift glass reactor and stirred tank reactor, which were challenged with the consortium developed at shake flask as 20% (v/v) leachate. As can be seen from the data presented in Table 1, in ALGR highest copper extraction achieved was 40.1% while, lead and zinc were 65 and 75% respectively at 9 d of contact time in batch leaching. Throughout the study, the extraction of lead represent oxidation of galena to lead sulphate, which remain in insoluble form with the pulp. The system was stabilised after 6 d of residence time which can be noted from the pH, redox potential and soluble ferrous being 1.55, 590 mV and 0.05 g/l respectively at the end of 9 d. The total acid consumption recorded was 19.3 g sulphuric acid/Kg concentrate. This acid requirement was to bring pH back to 2.0 could be due to the acid consuming material present in the pulp and the chemical oxidation of metal sulphide [12].

\[
\text{MS} + \text{H}_2\text{SO}_4 + \frac{1}{2}\text{O}_2 \rightarrow \text{MSO}_4 + \text{H}_2\text{O} + \text{S}^0
\]

Thereafter, the gradual decrease in the pH indicates the enhancement in biooxidation of sulphide content of the concentrate.
Table 1. Batch bioleaching profile of Air Lift Glass Reactor

<table>
<thead>
<tr>
<th>Leaching profile</th>
<th>Before inoc.</th>
<th>After inoc.</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.0</td>
<td>2.35</td>
<td>2.7</td>
<td>2.6</td>
<td>1.6</td>
<td>1.55</td>
<td>1.55</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>308</td>
<td>320</td>
<td>330</td>
<td>370</td>
<td>520</td>
<td>580</td>
<td>590</td>
</tr>
<tr>
<td>Soluble Fe(^{2+}) (g/l)</td>
<td>0.62</td>
<td>0.97</td>
<td>1.06</td>
<td>0.09</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Acid consumption (g/Kg concentrate)</td>
<td>-</td>
<td>2.9</td>
<td>6.4</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper (%)</td>
<td>2.2</td>
<td>4.8</td>
<td>11.22</td>
<td>24.36</td>
<td>41.0</td>
<td>31.6</td>
<td>40.1</td>
</tr>
<tr>
<td>Zinc (%)</td>
<td>12.91</td>
<td>15.92</td>
<td>24.38</td>
<td>50.35</td>
<td>64.98</td>
<td>71.48</td>
<td>75.0</td>
</tr>
<tr>
<td>Lead (%)</td>
<td>4.5</td>
<td>4.9</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>65.0</td>
</tr>
</tbody>
</table>

n.d.: not determined; inoc.: inoculation

When the leaching profile of STR and ALGR batch studies were compared (Table 2), STR performance was proved better than that of ALGR. The maximum copper, lead and zinc extraction recorded in STR were 76, 70 and 80% in 6, 9 and 9 d which comes out to be 2.84, 1.06 and 1.07 folds higher as compared to ALGR. The acid consumption was 10.7 g acid/Kg concentrate which was 1.8 times lower than the ALGR. This indicates highly stabilised system, which was due to the positive effect of aeration and agitation system adopted during the process [18]. The observed high chalcopyrite leaching indicates dominance of biological leaching over chemical leaching as chalcopyrite was very difficult to leach chemically.

Table 2. Bioleaching profile of batch STR process

<table>
<thead>
<tr>
<th>Leaching profile</th>
<th>Before inoc.</th>
<th>After inoc.</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.0</td>
<td>2.52</td>
<td>2.5</td>
<td>2.29</td>
<td>1.84</td>
<td>1.78</td>
<td>1.78</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>326</td>
<td>344</td>
<td>354</td>
<td>382</td>
<td>429</td>
<td>528</td>
<td>547</td>
</tr>
<tr>
<td>Soluble Fe(^{2+}) (g/l)</td>
<td>0.66</td>
<td>1.22</td>
<td>1.53</td>
<td>0.99</td>
<td>0.73</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Acid consumption (g/Kg concentrate)</td>
<td>-</td>
<td>5.73</td>
<td>4.97</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper (%)</td>
<td>3.8</td>
<td>8.4</td>
<td>34.36</td>
<td>57.14</td>
<td>76.0</td>
<td>66.8</td>
<td>65.96</td>
</tr>
<tr>
<td>Zinc (%)</td>
<td>8.4</td>
<td>20.68</td>
<td>39.36</td>
<td>48.68</td>
<td>62.93</td>
<td>72.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Lead (%)</td>
<td>5.1</td>
<td>5.5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>70.0</td>
</tr>
</tbody>
</table>

Looking to the promising leaching result of STR, further experiment was performed with fractional pulp addition and results are shown in Table 3. As can be seen from the data 92.7% copper extraction was achieved in 140 h of contact time followed by 83.62 and 83% zinc and lead extraction respectively in 215 h of reactor run. The high metal extraction during this fractional addition of pulp could be due to the less shear effect generated and better gas exchange rate owing to low pulp density at any particular time throughout the process. The high biological activity throughout the process was responsible for constant neutralisation of the alkaline gangue present in the fraction pulp added at that time, which resulted in 1.96 times less acid consumption compare to the STR batch process. Even when 30% (w/v) pulp was added in 10+10+10% (w/v) fractions, extraction rates of 0.84 and 6.36 g/l/d for copper and zinc respectively (data not shown) were achieved. This suggests that with the developed inoculum it is possible to get the
considerable metal extraction even at such a high pulp density, which was almost equivalent to that achieved at 15% pulp density with fractional pulp addition [5].

Table 3. Bioleaching profiles during fractional addition of pulp in STR

<table>
<thead>
<tr>
<th>Leaching profile</th>
<th>Before inoc.</th>
<th>After inoc.</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.95</td>
<td>2.24</td>
<td>2.16</td>
<td>2.25</td>
<td>2.12</td>
<td>1.93</td>
<td>1.82</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>322</td>
<td>332</td>
<td>373</td>
<td>412</td>
<td>430</td>
<td>446</td>
<td>525</td>
</tr>
<tr>
<td>Soluble Fe(^{2+}) (g/l)</td>
<td>0.42</td>
<td>0.50</td>
<td>0.75</td>
<td>0.94</td>
<td>1.11</td>
<td>0.80</td>
<td>0.09</td>
</tr>
<tr>
<td>Acid consumption (g/Kg concentrate)</td>
<td>-</td>
<td>1.82</td>
<td>1.82</td>
<td>1.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper (%)</td>
<td>6.2</td>
<td>18.4</td>
<td>56.80</td>
<td>64.57</td>
<td>92.7 (140h)</td>
<td>71.34</td>
<td>83.62 (215h)</td>
</tr>
<tr>
<td>Zinc (%)</td>
<td>12.8</td>
<td>25.15</td>
<td>58.0</td>
<td>63.54</td>
<td>69.08</td>
<td>74.34</td>
<td>88.0 (215h)</td>
</tr>
<tr>
<td>Lead (%)</td>
<td>5.3</td>
<td>6.0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>83.0 (215h)</td>
</tr>
</tbody>
</table>

The better extraction results achieved during fractional addition of the pulp in STR study opened the way for semi continuous fed batch process. The semi continuous metal extraction was started with a stabilised leaching system which, reached to 75% metal extraction, thus even at initiation time the concentration of the extracted metals present in leachate was 33 ± 2.0%. At the time of calculation of the percent metal extraction this amount was substracted, and the results presented in Table 4 are of net average extraction of 15 cycles.

The highest metal extractions of 92, 87 and 80% were achieved with copper, zinc and lead respectively. The metal extraction time was reduced by 35 h for copper while for lead and zinc it was reduced by 65 h as compared to the bioleaching with fractional pulp addition STR process. The bioleaching time is the major factor affecting the economy of the process. The other cost factor in the process was the external acid addition or acid consumption, which was 3.99 fold less or 75% reduction compare to STR batch leaching process.

Table 4. Polymetallic bioleaching profile in semi continuous STR process based on average of 15 cycles

<table>
<thead>
<tr>
<th>Leaching profile</th>
<th>Before inoc.</th>
<th>After inoc.</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.06</td>
<td>2.23</td>
<td>2.15</td>
<td>2.06</td>
<td>1.96</td>
<td>1.80</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>321</td>
<td>338</td>
<td>397</td>
<td>448</td>
<td>502</td>
<td>521</td>
</tr>
<tr>
<td>Soluble Fe(^{2+}) (g/l)</td>
<td>0.79</td>
<td>1.06</td>
<td>1.23</td>
<td>0.94</td>
<td>0.88</td>
<td>0.07</td>
</tr>
<tr>
<td>Acid consumption (g/Kg concentrate)</td>
<td>-</td>
<td>1.73</td>
<td>0.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper (%)</td>
<td>31.59</td>
<td>37.4</td>
<td>71.86</td>
<td>92.0 (105h)</td>
<td>73.54</td>
<td>65.98</td>
</tr>
<tr>
<td>Zinc (%)</td>
<td>32.63</td>
<td>45.68</td>
<td>57.07</td>
<td>63.08</td>
<td>73.81</td>
<td>87.0 (150h)</td>
</tr>
<tr>
<td>Lead (%)</td>
<td>35.12</td>
<td>36.4</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>80.0 (150h)</td>
</tr>
</tbody>
</table>
The comparative metal extraction rates of all the four studied processes are depicted in Table 5. As can be seen from the data, a higher metal extraction rate was obtained in the STR semi continuous process as compared to any of the processes studied. This was 43.81 and 348.0 mg/l/h, which comes out to be 1.05 and 8.35 g/l/d copper and zinc solubilisation respectively. The total metal extracted from the poly metallic concentrate was 4.6 g/l copper, 52.5 g/l zinc and galena oxidised equivalent to 21.12 g/l or 105.60 g/Kg lead in leached residue in the form of lead sulphate.

Table 5. Comparative soluble metals in leachate, galena oxidised and the overall metal extraction rate

<table>
<thead>
<tr>
<th>Process</th>
<th>Metals</th>
<th>Copper</th>
<th>Zinc</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/l mg/l/h g/l mg/l/h g/l* g/Kg concentrate mg/l/h*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALGR</td>
<td>2.05 14.64 45.0 209.30 17.16 85.80 79.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STR (batch)</td>
<td>3.80 27.14 48.0 223.36 18.48 92.40 85.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STR (fraction)</td>
<td>4.64 33.14 52.8 245.58 21.91 109.55 101.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STR (semi continuous)</td>
<td>4.60 43.81 52.5 348.00 21.12 105.60 140.80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: equivalent lead due to oxidation of galena to lead sulphate

The selection and development of the efficient bioleaching consortium and modification of pulp addition resulted in reduction of leaching time from 30 d at shake flask level with wild type consortium [14] to as low as 5 to 6 d. The observed high metal extraction as well as high rate proved suitability of the semi continuous process over the other three processes studied. Moreover, the reduction in retention time as well as external acid consumption leads to economization of the process. On the basis of obtained data the bioextraction process was successfully scaled-up to 600 dm³ STR level in our laboratory and was efficiently operated for 17 cycles with more than 80% average metal extractions which is detailed elsewhere [13].

The noteworthy developments of this investigation are the room temperature operations, high pulp density as well as the higher zinc extraction rate obtained as compared to the reported values in literature [1, 7, 8, 19-21]. The zinc extraction data obtained in this investigation can be compared with those of Steemson et.al [21] who have reported 93.8% zinc extraction in the overall residence time of 3.7 days at 40-45°C temperature and 6-8% solids. The highest overall rate of zinc extraction achieved in this work is 348 mg/l/h. When the amount of zinc extraction is compared per day on one litre pulp volume basis, the extraction works out to be 8.35 g/l/d, which is comparable with the calculated value of 8.75 g/l/d from the Steemson et.al report [21]. What is interesting is, that the presented work was performed at ambient temperature compared to 40-45°C. Moreover, the pulp density is 3 times higher in this investigation compared to the above reference. When adopted on commercial basis the process may be more cost effective in terms of capital as well as operating costs.

4. CONCLUSION

To summarise, the achieved equivalent zinc extraction rate, higher than that reported by others, even at lower temperatures and higher pulp density using indigenously developed consortium, indicates comparatively higher cost effectiveness of the bioleaching process developed for GMDC poly metallic bulk concentrate. Interestingly, the
investigation was carried out with a Cu-Pb-Zn system, which allowed as much as 90% copper extraction and 80% oxidation of lead sulphide simultaneously.

**ACKNOWLEDGMENTS**

We are thankful to Gujarat Mineral Development Corporation for the project grant and research fellowship to D. R. Tipre.

**REFERENCES**


Effect of pH and temperature on the biooxidation of a refractory gold concentrate by *Sulfolobus metallicus*

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Abstract

The biooxidation of refractory gold concentrates using thermophilic microorganisms is considered an interesting alternative to current processes. Many operation conditions influence the process, pH and temperature being two important parameters among others. The objective of this work was to evaluate the influence of these two variables on the biooxidation of an enargite-pyrite gold concentrate containing 40 g Au/ton, using the thermophilic archaeon *Sulfolobus metallicus*. The experiments were run in shake flasks with 1% pulp density and particle size under 200 mesh. The pH was kept constant at 1.0, 1.5 and 2.0 by daily addition of 0.5 M NaOH. One experiment was run at non-controlled condition with initial pH at 2.5. Every pH condition was tested at 60, 65, 70 and 75ºC. The extent of biooxidation was measured through the iron solubilisation.

The best run at constant pH condition and at all different temperatures was 1.5, obtaining the highest percentage of iron extraction at 65ºC which amounted 75%. Iron extractions at pH 1.0 were between 10% and 24% of those obtained at pH 1.5, while at pH 2.0 they were in the range of 75% to 95%. All experiments run at 75ºC showed almost no iron solubilisation.

However, the non-controlled pH experiment with initial pH at 2.5 was shown to be a more suitable condition for biooxidation. The highest iron extraction was 83% at 65ºC which is 10% higher than the one obtained at pH controlled at 1.5.

It is concluded that, under the studied conditions, the best pH and temperature to perform the biooxidation are 65ºC and a non-controlled pH policy with initial pH of 2.5.

*Keywords*: enargite, biooxidation, thermophiles, bioleaching, archaea

1. INTRODUCTION

Biooxidation has become an attractive alternative as a pretreatment of refractory gold concentrates in order to facilitate gold extraction by cyanidation [1-4]. Biooxidation competes against roasting and pressure oxidation presenting several advantages such as low capital demand, low energy input, mild operation conditions and low environmental impact. Nowadays there are operating no less than ten large scale biooxidation plants located in South Africa, Brazil, Australia, Ghana, Peru and USA [5, 6].

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Most of these commercial gold processing plants use stirred tank bioreactors for the biooxidation of refractory gold concentrates, although a few of them use heap leaching to pre-treat low-grade ores and tailings [7]. The advantage of using stirred tank bioreactors is the control that can be exerted on important environmental variables like pH and temperature [6, 8, 9]. Microorganisms that are involved in these operations occur naturally at mineral sites and are mostly of the genus Acidithiobacillus preferentially Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Acidithiobacillus caldus together with Leptospirillum ferrooxidans [11-13]. All of them are acidophiles and mesophiles, being used at operating pH between 1.2 to 2.2 and temperature between 30 and 40°C [14]. Several attempts have been made in order to evaluate the performance of thermophilic bacteria and more recently hyperthermophilic archaea which grow at temperatures higher than 60°C. Some examples of these archaea are Sulfolobus metallicus, Sulfolobus acidocaldarius, Sulfolobus solfataricus, Acidianus brierleyi and Acidianus infernus [15-17]. Although these archaea have natural environments different from mineral sites, they have adapted very well to oxidize different mineral species like pyrite, arsenopyrite, enargite, chalcopyrite, chalcocite and covellite [18-21]. The bioleaching mechanisms used by archaea are less understood than those of Acidithiobacillus and much more basic information is needed. This work presents results that show the influence of pH and temperature on the solubilisation of a refractory gold concentrate with high content of enargite, a recalcitrant species present in several gold minerals in Chile using the archaeon Sulfolobus metallicus.

2. MATERIALS AND METHODS

2.1 Experimental conditions

A strain of Sulfolobus metallicus, kindly supplied by Dr. Antonio Ballester (Faculty of Chemical Science, Universidad Complutense, Madrid), was used throughout this study. The cells were cultured in a medium containing 0.4 g/L of (NH₄)₂SO₄, 0.5 g/L of MgSO₄.7H₂O, 0.2 g/L of KH₂PO₄, 0.1 g/L of KCl and 1% (w/v) of gold concentrate as energy source. The mineralogical composition of the gold concentrate (El Indio Mining Company, La Serena, Chile) was: 40.7% enargite, 42.8% pyrite, 3.9% chalcopyrite, 0.8% chalcosine and 0.3% covellite. Its elemental composition was: 42 g Au/ton, 21.1% Cu, 22.6% Fe, 37.8% S and 7.7% As. Particle size was lower than 200 mesh.

Experiments were carried on in 500 mL erlenmeyer flasks with 100 mL of medium, incubated in a rotary shaker at 200 rpm. The different culture pH were kept constant at 1.0, 1.5 and 2.0 by daily addition of 0.5 M NaOH. One experiment was conducted under non-controlled pH policy with an initial pH of 2.5. Each pH condition was tested at 60, 65, 70 and 75°C. The evaporated water was quantitatively replaced on a daily basis adding distilled water.

The inoculum was obtained from a fully adapted culture grown on a medium of the same composition as described above.

2.2 Analytical procedures

Total soluble iron, after reduction of ferric ion with hydroxylamine, was determined by the o-phenanthroline method [22]. Eh was measured with a Ag/AgCl probe.
3. RESULTS AND DISCUSSION

Figures 1, 2 and 3 depict the total soluble iron concentration profiles at all pH and temperature conditions studied, with the exception of the experiments run at 75°C, a temperature at which after eight days iron solubilisation was not observed. This temperature was too high as to allow this archaeon to grow, a result that is consistent with the culture temperature reported by different authors that use *Sulfolobus* species in biooxidation studies [23, 24].

Under the operating conditions used in this work, the biooxidation of the mineral stopped after eight to ten days. Also, it was observed that under the different tested conditions biooxidation started after three to four days of lag, a situation that is related to the difficulties that the cells face when they are transferred as inoculum to a fresh medium.

Experiments run at pH 1.0 showed almost no iron extraction independently of the cultivation temperature. It is thought that the initial iron solubilisation should be due mostly to a chemical action more than an initial biological activity.

As pyrite is the most abundant mineral component of the refractory gold concentrate, the iron obtained in solution as a consequence of biooxidation must come from pyrite and therefore constitutes a measurement of the extent of pyrite oxidation. In this respect, from the results it is clear that the highest iron extractions were obtained at a temperature of 65°C. In the case of the biooxidation experiments conducted at controlled pH, the highest extraction was obtained at a pH of 1.5, amounting to 75% of the iron present initially in the refractory gold concentrate.

Table 1 summarizes the percentages of Fe extraction obtained under the different operating pH and temperature considered in this work. The extraction values obtained at pH 1.0 are between one fifth to one tenth of those obtained at pH 1.5, which reveals the importance to keep biooxidation pH above 1.0 in order to avoid a marked reduction on the process yield.

![Iron solubilisation kinetics during the biooxidation of a refractory gold concentrate at 60°C and different pH (nc: non-controlled)](image)

**Figure 1.** Iron solubilisation kinetics during the biooxidation of a refractory gold concentrate at 60°C and different pH (nc: non-controlled)
Figure 2. Iron solubilisation kinetics during the biooxidation of a refractory gold concentrate at 65ºC and different pH (nc: non-controlled)

Figure 3. Iron solubilisation kinetics during the biooxidation of a refractory gold concentrate at 70ºC and different pH (nc: non-controlled)

Table 1. Iron extraction by biooxidation of a 1% pulp density refractory gold concentrate at different pH conditions and temperature (%)

<table>
<thead>
<tr>
<th>Culture pH</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-controlled</td>
<td>64</td>
<td>14</td>
<td>58</td>
</tr>
<tr>
<td>6.5</td>
<td>83</td>
<td>7</td>
<td>75</td>
</tr>
<tr>
<td>7.0</td>
<td>69</td>
<td>14</td>
<td>67</td>
</tr>
</tbody>
</table>

In the case of experiments conducted under pH controlled condition and as a way to predict the optimal pH and temperature to carry out the biooxidation of the refractory gold concentrate using *Sulfolobus metallicus*, the experimental data was fitted to a second order polynomial function to generate a surface response. This equation represents the
The following equation was drawn using a statistical software fed with the experimental results tabulated according to a central composite rotatable experimental design:

\[
[\text{Fe}] = -19.2326 + 7.4686 \cdot \text{pH} + 0.4244 \cdot T - 3.1629 \cdot \text{pH}^2 + 0.046 \cdot \text{pH} \cdot T - 0.0036 \cdot T^2
\]  

(1)

The values of the coefficient of determination \( (R^2) \) and the standard deviation are 0.975891 and 0.138646 respectively.

Calculating the first partial derivatives of equation (1) the optimum culture pH and temperature were quantified as 1.68 and 69ºC, respectively. These operating conditions predict a soluble iron concentration of 1.72 g/L, which represents a 76% of iron extraction. The optimal pH and temperature fit in the ranges reported in the literature for *Sulfolobus metallicus* [25, 26]. However, as can be seen in Table 1, this figure still is lower than the iron extraction obtained in the experiment run at 65ºC and under non-controlled pH condition. In this case the iron extraction was 83%, showing that the biooxidation of a 1% pulp density refractory gold concentrate by *Sulfolobus metallicus* was more efficient when conducted under non-controlled pH condition. Although there is not a precise explanation for this phenomenon, it has been noted that sometimes microbial activity is higher under a policy of initially adjusting the pH and letting it change freely during the time course of the oxidation. The pH varied moderately during the biooxidation as can be seen in Figure 4, leveling off at a lowest value of 1.7, very close to the one predicted by equation (1). This policy is thought to be worthwhile for the biooxidation of solid suspension of low pulp density as in this work, since the pH downfall is restricted by the total amount of iron that can be extracted. As pulp density goes up, the total iron solubilisation increases and consequently the pH drop should become more important, reaching values that would interfere with microbial activity and extent of the biooxidation process. Also in Figure 4 is observed a typical Eh behavior during biooxidation, starting from low values and increasing as solubilisation of different elements goes up, especially iron.

![Figure 4. pH and Eh evolution during the biooxidation of a refractory gold concentrate at 65ºC and under non-controlled policy with initial pH of 2.5](image)

From the data contained in Figures 1, 2 and 3, it is possible to calculate the global volumetric productivity of iron solubilisation at each experimental condition. According to Table 2, again this parameter is maximum for biooxidation conducted at 65ºC and pH
controlled at 1.5. This time the global volumetric productivity is slightly higher at the pH controlled condition compared to non-controlled operation. This situation is clearly confirmed in Figure 2 where it is possible to infer that the rate of iron solubilisation at pH controlled at 1.5 is faster than at the non-controlled pH condition.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Culture pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-controlled</td>
</tr>
<tr>
<td>60</td>
<td>0.132</td>
</tr>
<tr>
<td>65</td>
<td>0.208</td>
</tr>
<tr>
<td>70</td>
<td>0.171</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS
The results allowed the definition of the pH and temperature values that maximized the iron extraction from a refractory gold concentrate through biooxidation using *Sulfolobus metallicus*, a thermophilic archaeon. Under the experimental conditions studied in this work, the best temperature and pH to perform the biooxidation are 65°C and a non-controlled pH policy starting with initial pH of 2.5. On the other side, operating at a pH controlled condition, using surface response methodology it is shown that pH controlled at 1.68 and a temperature of 69°C maximize the extent of iron solubilisation. On the other hand, the highest value of the global volumetric productivity of iron solubilisation is found in the pH controlled operation mode and correspond to a biooxidation conducted at pH 1.5 and 65°C.

ACKNOWLEDGEMENTS
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REFERENCES
Effect of the pulp density and particle size on the biooxidation rate of a pyritic gold concentrate by *Sulfolobus metallicus*

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**Abstract**

It is recognized that increasing pulp densities and decreasing particle sizes have positive effects in the volumetric rate of biooxidation of refractory gold concentrates. It has also been noted that a variety of phenomena can limit this positive effect. The objective of this work was to determine the values of pulp density and particle size that maximize the volumetric rate of solubilisation of iron from a pyritic gold concentrate. The leaching was carried on in agitated flasks with the thermophilic archaeon *Sulfolobus metallicus*. The concentrate contained 66.7% pyrite, and the constant operation conditions were 220 rpm, 68ºC initial pH of 2.0. Pulp densities were 2.5, 5, 10 and 15% w/v and the size fractions were 150-106, 106-75, 75-38 and –38 µm.

Total solubilised iron concentrations were in the range of 8 to 25 g/L. In the 2.5 and 5% pulp density runs, iron extractions were in the range of 80 to 100%. After 15 to 25 days of leaching the rate declined to almost zero in the runs with 2.5 and 5% pulp densities, while the same occurred in the other two runs after 20 to 45 days.

A complete experimental design of 16 runs allowed the definition of response surfaces from which the optimal conditions that maximize the rate of iron solubilisation were determined. These optimal conditions are 7.8% pulp density and particle size of 35 µm.

**Keywords:** optimal conditions, response surface, iron solubilisation, thermophilic archaeon

1. **INTRODUCTION**

The rate of biooxidation of refractory gold concentrates is influenced by several operational factors. It is recognized that increasing pulp densities and decreasing particle sizes have a positive effect in the volumetric rate of biooxidation, as both situations result in an increase in surface area. Nevertheless, it has also been noted that the interaction among these factors together with a variety of associated phenomena such as mechanical effects, metabolic stress and inhibitory concentrations of ferric ion, can limit this positive effect and even result in declining leaching rates [1].

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The negative effects of high pulp densities and small particle sizes were early reported in bioleaching with mesophilic bacteria [2-3]. The detrimental effect of high pulp densities is likely to be larger in operations with archaea because of their weaker cell wall [4-5] that make them susceptible to mechanical damage and metabolic stress caused by the intense agitation needed for maintaining a homogeneous suspension [6-9].

On the other hand, decreasing particle size can reduce the leaching rate probably because of difficulties in cell attachment when the diameters of the particles and cells become of similar magnitude. It is also likely that the rate of collision between particles increases as particle size diminishes [9-10].

The objective of this work was to determine the optimal values of pulp density and particle size that maximize the volumetric rate of solubilisation of iron from a pyritic gold concentrate when using the thermophilic archaeon *Sulfolobus metallicus* in shake flasks.

2. MATERIALS AND METHODS

2.1 Microorganism and culture conditions

A strain of the thermophilic archaeon *Sulfolobus metallicus*, kindly supplied by Dr. Antonio Ballester from the Universidad Complutense, Madrid, was used throughout this work. The microorganism was maintained in Norris medium [11] (0.4 g/L (NH₄)₂SO₄, 0.5 g/L MgSO₄·7H₂O, 0.2 g/L K₂HPO₄, 0.1 g/L KCl, H₂SO₄ to pH 2.0) in the presence of the gold concentrate. All experiments were run in 1-L shake flasks with 90 mL of the same culture medium with the concentrate at pulp densities of 2.5, 5, 10 and 15% w/v. The flasks were inoculated with 10 mL of a culture of adapted cells with total soluble iron concentration of 10 g/L, so in all runs initial concentration was 1 g Fe/L. Uninoculated flasks were run for each pulp density.

Other culture conditions were 68ºC, initial pH of 2.0 and agitation in orbital incubator of 220 rpm.

2.2 Gold concentrate

The concentrate contained 15 g gold/tonne and was supplied by Las Ventanas Copper Refinery, Las Ventanas, Chile. Its mineralogical and elemental composition is presented in Table 1.

Table 1. Gold concentrate composition

<table>
<thead>
<tr>
<th>Component</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mineralogical</strong></td>
<td></td>
</tr>
<tr>
<td>Pyrite (FeS₂)</td>
<td>67.55</td>
</tr>
<tr>
<td>Chalcopyrite (CuFeS₂)</td>
<td>8.98</td>
</tr>
<tr>
<td>Sphalerite (ZnS)</td>
<td>0.56</td>
</tr>
<tr>
<td>Others</td>
<td>0.71</td>
</tr>
<tr>
<td>Gangue</td>
<td>22.20</td>
</tr>
<tr>
<td><strong>Elemental</strong></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>39.47</td>
</tr>
<tr>
<td>Cu</td>
<td>3.32</td>
</tr>
<tr>
<td>Fe</td>
<td>34.43</td>
</tr>
<tr>
<td>Zn</td>
<td>0.38</td>
</tr>
<tr>
<td>Others</td>
<td>22.40</td>
</tr>
</tbody>
</table>
Four fractions of the original concentrate were used in this work: 150-106, 106-75, 75-38 and –38 µm.

2.3 Analytical methods

Ferrous ion was measured colorimetrically by the modified o-phenanthroline method [12]. Total soluble iron was determined by the same method after reduction of the ferric iron with hydroxylamine. Ferric ion was calculated as the difference between total and ferrous iron.

Sulfate was determined volumetrically using the Cole-Parmer (Vernon Hills, IL) sulfate kit Nº 05542-23.

2.4 Experimental design

A 2^4 factorial design was used. The factors were particle size and pulp density. The complete set of experiments is shown in Table 2. The coded variables were generated by defining the highest value of each variable as 1 and the lowest as –1.

Table 2. Experimental design with the independent physical and coded variables

<table>
<thead>
<tr>
<th>Run</th>
<th>PS (µm)</th>
<th>PD (% w/v)</th>
<th>X₁</th>
<th>X₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-38</td>
<td>2.5</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>75-38</td>
<td>2.5</td>
<td>-0.312</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td>106-75</td>
<td>2.5</td>
<td>0.132</td>
<td>-1</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>2.5</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>5</td>
<td>-38</td>
<td>5</td>
<td>-1</td>
<td>-0.6</td>
</tr>
<tr>
<td>6</td>
<td>75-38</td>
<td>5</td>
<td>-0.312</td>
<td>-0.6</td>
</tr>
<tr>
<td>7</td>
<td>106-75</td>
<td>5</td>
<td>0.132</td>
<td>-0.6</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>5</td>
<td>1</td>
<td>-0.6</td>
</tr>
<tr>
<td>9</td>
<td>-38</td>
<td>10</td>
<td>-1</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>75-38</td>
<td>10</td>
<td>-0.312</td>
<td>0.2</td>
</tr>
<tr>
<td>11</td>
<td>106-75</td>
<td>10</td>
<td>0.132</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>150</td>
<td>10</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>-38</td>
<td>15</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>75-38</td>
<td>15</td>
<td>-0.312</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>106-75</td>
<td>15</td>
<td>0.132</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>150</td>
<td>15</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

PS: particle size; PD: pulp density, X₁: coded particle size; X₂: coded pulp density

Results were modelled by multiple regression analysis [13] based in the empiric model:

\[
P_0 = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5x_1x_2
\]  \( (1) \)

Equation (1) was used to generate response curves of the effect of particle size and pulp density on the maximum volumetric rate of production of soluble iron, \( Q_P \).

Maximum iron solubilisation rates were calculated as the slopes of the straight lines drawn from the iron concentration at time zero passing tangent to the solubilisation curve.
3. RESULTS AND DISCUSSION

3.1 Bioleaching kinetics

The results of the 16 runs are presented in Figure 1. At all particle size fractions, pulp density of 15% proved to be deleterious, a similar result than that obtained by Nemati and Harrison [14] with a similar *Sulfolobus* strain. This effect can be due to a number of factors, namely mechanical, metabolic stress and gas transfer limitations [7, 10, 14-16]. Attrition of the cells by the concentrate particles can result in mechanical damage due to the delicate nature of the archeal cell envelopes, while this same situation can cause metabolic stress. It is believed that stress can be overcome by an extended adaptation period. High pulp densities can cause oxygen and carbon dioxide demands higher than the actual supplies, as has been suggested by Gerike et al. [15], d’Hugues et al. [16] and Boogerd et al. [17].

High percent iron solubilisations were obtained for pulp densities of 2.5 and 5%, as can be seen in Table 3. Iron solubilisation was affected by increasing pulp densities and by coarser particles. In fact, most of the soluble iron obtained with 15% pulp density and the coarser fraction (run 16) came from chemical leaching as evidenced by the results of the uninoculated flasks (not shown). The contribution of chemical leaching became less important with decreasing pulp densities and particle sizes, and was not significant in the 2.5 and 10% pulp density runs.

Table 3. Maximum solubilisation rates and percent solubilisation at different particle sizes and pulp densities

<table>
<thead>
<tr>
<th>Run</th>
<th>PS* (µm)</th>
<th>PD* (% w/v)</th>
<th>Iron solubilisation (%)</th>
<th>Solubilisation rate (g/L·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-38</td>
<td>2.5</td>
<td>86.2</td>
<td>0.819</td>
</tr>
<tr>
<td>2</td>
<td>75-38</td>
<td>2.5</td>
<td>64.0</td>
<td>0.740</td>
</tr>
<tr>
<td>3</td>
<td>106-75</td>
<td>2.5</td>
<td>82.9</td>
<td>0.712</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>2.5</td>
<td>69.9</td>
<td>0.776</td>
</tr>
<tr>
<td>5</td>
<td>-38</td>
<td>5</td>
<td>81.4</td>
<td>0.948</td>
</tr>
<tr>
<td>6</td>
<td>75-38</td>
<td>5</td>
<td>73.7</td>
<td>1.042</td>
</tr>
<tr>
<td>7</td>
<td>106-75</td>
<td>5</td>
<td>68.1</td>
<td>0.985</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>5</td>
<td>59.0</td>
<td>0.646</td>
</tr>
<tr>
<td>9</td>
<td>-38</td>
<td>10</td>
<td>45.6</td>
<td>1.009</td>
</tr>
<tr>
<td>10</td>
<td>75-38</td>
<td>10</td>
<td>40.9</td>
<td>1.089</td>
</tr>
<tr>
<td>11</td>
<td>106-75</td>
<td>10</td>
<td>29.3</td>
<td>0.794</td>
</tr>
<tr>
<td>12</td>
<td>150</td>
<td>10</td>
<td>31.5</td>
<td>0.420</td>
</tr>
<tr>
<td>13</td>
<td>-38</td>
<td>15</td>
<td>32.9</td>
<td>0.618</td>
</tr>
<tr>
<td>14</td>
<td>75-38</td>
<td>15</td>
<td>21.5</td>
<td>0.582</td>
</tr>
<tr>
<td>15</td>
<td>106-75</td>
<td>15</td>
<td>29.3</td>
<td>0.318</td>
</tr>
<tr>
<td>16</td>
<td>150</td>
<td>15</td>
<td>6.4</td>
<td>0.084</td>
</tr>
</tbody>
</table>

* PS: particle size; PD: pulp density
Figure 1. Iron solubilisation kinetics from a pyritic gold concentrate by *Sulfolobus metallicus* in shake flasks at 68°C initial pH 2.0 and agitation of 220 rpm. Pulp densities: ■ 2.5%; ● 5.0%; ▲ 10%; ▼ 15%
3.2 Iron solubilisation rates

Maximum iron solubilisation rates are given in Table 3. Multiple regression analysis of these results allowed the evaluation of the coefficients of equation (1):

\[ Q_p = 0.9591 - 0.2032x_1 - 0.1862x_2 - 0.1304x_1^2 - 0.3039x_2^2 - 0.1305x_1x_2 \] (2)

All the coefficients of equation (2) have a significant effect on \( Q_p \) as their F values are much higher than the tabulated F at the 95% confidence limit (\( F_{5,10} = 3.3, p < 0.05 \)) [13]. F values also allow the conclusion that the interaction between particle size and pulp density had the weakest effect (\( F = 9.24 \)) and that pulp density (\( F = 81.24 \)) showed a stronger effect on \( Q_p \) than particle size (\( F = 14.15 \)).

The response surface generated by equation (2) is showed in Figure 2 and the corresponding contour plot is depicted in Figure 3. It can be seen that a maximum value of \( Q_p \) occurs at a pulp density of 7.8% and particle size of 35 µm, a value very near the fraction 75-38 µm. The -38 µm exhibits a negative effect on the biooxidation rate, but this effect is not as strong as that observed by Nemati et al. [10], who found a complete inhibition of microbial action at particle sizes under 25 µm.

![Figure 2. Response surface of the effect of particle size and pulp density on the rate of iron solubilisation from pyrite by Sulfolobus metallicus in shake flasks at 68°C initial pH 2.0 and agitation of 220 rpm](image-url)
Figure 3. Contour plot of the effect of particle size and pulp density on the rate of iron solubilisation from pyrite by *Sulfolobus metallicus* in shake flasks at 68°C initial pH 2.0 and agitation of 220 rpm

4. CONCLUSIONS

It is concluded that the operation variables particle size and pulp density have an effect on the rate of iron solubilisation from pyrite by *Sulfolobus metallicus* in shake flasks. Under the experimental conditions used in this work, the set of these variables that produce the highest rate is 35 µm particle size and 7.8% pulp density. It is also concluded that, in the range studied, the interaction between the variables is weak and that pulp density has a much stronger effect than particle size.

ACKNOWLEDGMENTS

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REFERENCES

Enhancement of chalcopyrite bioleaching capacity of an extremely thermophilic culture by addition of ferrous sulphate

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Abstract

One of the mainly problems of the bioleaching processes applied to copper concentrates and refractory ores are the low kinetics of the reactions, with high residence time that does not permit it to be an economic process. For this reason, current researches into this field are focused on how to increase this bioleaching rate. Apart from improving engineering design of bioreactors, the possibilities to increase bioleaching rates depend on the use of catalyst and isolation and adaptation of new microorganisms with high capacity to leach these ores.

Many researches have investigated the possibility of using thermophilic microorganisms to improve metal-leaching rates instead to mesophilic microorganisms.

In this sense, it was obtained a mixed natural thermophilic culture from a typical chalcopyritic copper concentrate of the Spanish Pyritic Belt. This culture has selectivity with respect to leaching chalcopyrite, when this is present together with other sulphides in ores and concentrates, and presents high copper leaching rates at high pulp density [1].

In this paper the study of enhancement of the bioleaching capacity of this culture on a chalcopyritic copper concentrate of the Spanish Pyritic Belt when ferrous sulphate is added is presented.

The results obtained show that an initial addition of 1.8 g/L of iron as ferrous sulphate increases the copper bioleaching rate in all pulp densities studied. These results are according with other investigations that suggest the enhancement in copper leaching of chalcopyrite obtained by addition of ferrous sulphate [2]. From these results authors suggest an indirect in situ mechanism of bioleaching of chalcopyrite for this culture in which ferrous ion have a significant role.

Keywords: extremely thermophilic culture, ferrous iron catalysis, chalcopyrite, bioleaching

1. INTRODUCTION

The resources of high ores in the world are becoming more and more scarce being necessary the processing of more complex ores. Conventional mineral processing on complex sulphide ores, carried out by differential flotation, often produces concentrates
not enough clean and difficult to commercialise. Therefore, plenty effort has been put into of hydrometallurgical process development for treatment of these ores, but the greatest number of proposed methods are complex and expensive [3].

Biohydrometallurgical processes appear as an alternative. These processes were first applied industrially to copper and uranium extractions using bio-assisted heap, dump and in-situ technologies, are today successfully used in extraction of gold from refractory sulphide-bearing ores and concentrates [4]. However, for other metal concentrates this technology isn’t still viable alternative to conventional pyrometallurgical extractions. In case of chalcopyritic concentrates there is a more complicated situation due to natural refractivity of chalcopyrite. The leaching of chalcopyrite is slow and incomplete in relation to other sulphides, and this is thought to be as a result of formation of a passivating layer.

The use of new microorganisms isolated and adapted and of catalyst could improve the bioleaching rates. Thermophilic microorganisms had been used in bioleaching process because can be to increase leaching rates due to high temperatures, tolerant capacity and metabolic characteristic [5, 6]. Moreover, utilisation of these microorganisms that naturally thrive on ore samples and in its aqueous environments could be good options, since that, those probably have chalcopyrite specificity and much higher capacity for adaptation.

In this sense, the authors have obtained a mixed natural thermophilic culture from a typical chalcopyritic copper concentrate of the Spanish Pyritic Belt. This culture has selectivity with respect to leaching chalcopyrite, when this is present together with other sulphides in ores and concentrates [7].

The use of catalytic ions like silver can increase bioleaching copper rate. But other ions can be accelerating this process too. Although ferrous iron is a reagent involved in the process, its role in bioleaching process is not still overall understanding.

In acidic solutions, chalcopyrite is oxidised by ferric ions and dissolved oxygen to release copper ions. Ferrous ions are rapidly oxidised to ferric ions in presence of iron oxidising bacteria such as *Acidithiobacillus ferrooxidans*. It appears that role of ferrous iron in leaching is only as a source of ferric ions.

The role of iron in bioleaching of sulphur ores is complex and depends of the interactions between these in its different oxidation states, bacteria and minerals particulate.

However, there are reports, which suggest that ferrous iron contributes to copper extraction from chalcopyrite. There is a critical potential at which the leaching rate is very great and the rate suddenly decreases above this potential. As the suspension potential increases with increasing ferric to ferrous ratio, these results indicate that the leaching rate is faster with an optimum concentration of ferrous ions that without ferrous ions [2].

Further, if addition of catalytic ions to bioleaching can effectively increase the oxidation rate, the combination of both thermophilic microorganisms and catalyses using ferrous iron could aid to increase the slow kinetic problem that exhibit this process.

This paper is presented in context of copper bioleaching, where it is assumed that the ferric ions and protons produced by microbial action, act as the leach agents in copper dissolution. The objective of this study is to establish the rate of chalcopyrite dissolution as a function of to added ferrous iron, determining the role of this in copper extraction with this thermophilic culture.
2. METHODS

2.1 Ore sample

A chalcopyritic copper concentrate obtained from conventional differential flotation was used to obtain the mixed thermophilic culture. This concentrate came from the Spanish Pyritic Belt and its composition is given in Table 1. The mineralogical composition of this sample shows chalcopyrite and pyrite as main mineralogical species and galena and sphalerite as secondary mineralogical phases. The particle size distribution presents a passing $d_{80}$ of 20µm.

Table 1. Chemical composition of copper concentrate

<table>
<thead>
<tr>
<th>Chemical analysis (wt %)</th>
<th>Cu</th>
<th>Zn</th>
<th>Pb</th>
<th>Fe</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23.37</td>
<td>2.58</td>
<td>2.52</td>
<td>32.63</td>
<td>38.00</td>
</tr>
</tbody>
</table>

2.2 Microbial inoculum

A mixed thermophilic culture (MTC) of native microorganisms, isolated on this chalcopyritic concentrate was used as inoculum.

The optimal condition of this culture is a 65°C temperature, with a pH of 1.30.

For all tests done, the inoculum was obtained by the following way. The final pulp of the bioleaching test was filtered, obtaining the leach liquor and the solid residue. The solid was intensively stirred during two hours with a solution at pH 4. This pulp was filtered again and the final liquid obtained, which contains most of the bacteria, which were attached to the solid residue, was filtered through a 0.22 µm Millipore filter where bacteria were retained. Finally, the bacteria were re-suspended in 50 mL of the leach liquor obtained in the first filtration in order to get the inoculum volume (5% v/v) for the next bioleaching test.

The cultures were successfully adapted to higher pulp density of copper concentrate until obtaining high copper extractions in short residence times.

2.3 Bioleaching experiments

Bioleaching experiments were carried out in 1 L glass cylindrical reactors provided with a cap with four holes to allow mechanical stirring (at 130 rpm), aeration (10-15 L/h) and sampling. These reactors were placed in a thermostatic bath to keep the temperature constant at 65°C.

During the experiments the pH was kept at 1.3 by the addition of 10N H$_2$SO$_4$ when necessary. This was made to avoid the precipitation of iron in form of jarosites, which damage the bioleaching process.

Redox potential and pH were measured daily, while the levels of copper, zinc, and iron in solution were analysed daily or every two days, depending on the test. Water was added to the reactors in order to compensate for evaporation losses. Once bioleaching tests were finished, solids were removed by filtration, and chemically characterised as well as the leachate.
Sterile bioleaching tests were carried out with ore sterilised by autoclaving at 121°C, 30 minutes and 1atm of pressure, and adding a solution of 10% ethanol to the leaching media.

To study the effect of the addition of ferrous iron on the bioleaching process, the test were carried out using ferrous sulphate solutions of varying concentration that were added initially to the leaching media. The ferrous iron concentration varied from 400 mg/L to 9000 mg/L depending of the tests.

2.4 Analysis

Soluble species of copper, zinc, lead, total iron and minor elements were analysed by ICP [8] using a spectrophotometer ICAP-61 Thermo Jarrel Ash. The ferrous iron was analysed by a volumetric method by titration with potassium dichromate [9]. Copper, zinc, lead and iron content samples and leaching solid residues were analysed by XRF [10] using a spectrophotometer Philips PW-1404, and minor elements by ICP. Total sulphur was gravimetrically determined and elemental sulphur was analysed by toluene extraction in a Soxhlet apparatus.

The pH was measured with a 704 pH-meter Metrohm. The redox potential (Eh) was measured with a platinum electrode with an Ag/AgCl reference electrode.

Mineralogical composition was determined by XRD using a diffractometer PW-1700 Philips.

3. RESULTS AND DISCUSSION

Initially the were carried out at 1% pulp density and using concentrations of ferrous iron of 450, 900, 1800, 3600 and 4500 mg/L.

Figure 1 shows copper extraction during the tests carried out with more representative ferrous iron concentration. Initial Fe^{2+} addition increase copper overall leaching, obtaining with 1800 mg/L more than 96% of extraction in only 69 hours. With 3600 mg/L of ferrous iron addition the catalyst effect is maintained, but with higher ferrous iron concentrations, 4500 mg/L, copper extractions was diminished. At early hours, this catalysed effect is not appreciable, having lower copper extractions when ferrous iron was added respecting to the control (no addition of ferrous sulphate).

**Figure 1. Copper extraction evolution with the different ferrous iron concentration added. (1% pulp density (w/v), MTC culture at pH 1.30 and 65°C)**
Relationship between both total iron and ferrous iron in solution are shows in Figure 2. In all tests during the first hours iron in solution was in ferrous form, without apparent oxidation of Fe$^{3+}$ added. This form of iron was increased in solution with all ferrous iron concentration added, but after 45 hours of assay the concentration was practically constant in solution. Only is observed a reduction in the solution ferrous iron concentration, with 900 and 1800 mg/L of ferrous iron added, when copper had been leached.

![Figure 2](image.png)

**Figure 2.** Relationship between iron forms present in solution. (Left) Total iron. (Right) Ferrous iron. (1% pulp density (w/v), MTC culture at pH 1.30 and 65°C)

In this sense, it seems that both ferrous iron, ferrous iron added and ferrous iron produced by chalcopyrite leaching reaction, only are being oxidised when the chalcopyrite was not available. Ferric iron in solution was not higher than 30% of total iron in all tests.

Figure 3 shows the zinc bioleaching of the copper concentrate during the tests. This present a typical kinetic observed in all tests, in which the zinc did not leached whilst copper was bioleaching. Thus, in 1800 mg/L of ferrous iron addition test, can be clearly observed zinc bioleaching after total copper bioleached (72 hours).

![Figure 3](image.png)

**Figure 3.** Zinc bioleaching evolution with the ferrous iron concentration added. (1% pulp density (w/v) (MTC culture at pH 1.30 and 65°C)
pH evolution was similar in all tests, increasing in the first hours but was enclose in 1.30 adding sulphuric acid (10N) to avoid iron precipitation.

XRD analyses of final solids of all test is showed in Table 2. The copper leaching produced elemental sulphur as principal species, increasing the presence of jarosites when ferrous addition was increased.

Table 2. XRD diffraction of final solids obtained in bioleaching tests

<table>
<thead>
<tr>
<th>Addition of Fe^{2+} (mg/L)</th>
<th>Main species</th>
<th>Secondary species</th>
<th>Minor species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Anglesite, Pyrite</td>
<td>-</td>
<td>Elemental sulphur</td>
</tr>
<tr>
<td>900</td>
<td>Pyrite, Elemental sulphur</td>
<td>Anglesite</td>
<td>Jarosite</td>
</tr>
<tr>
<td>1800</td>
<td>Pyrite, Elemental sulphur</td>
<td>-</td>
<td>Jarosite, anglesite</td>
</tr>
<tr>
<td>3600</td>
<td>Pyrite, jarosite, Elemental sulphur</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4500</td>
<td>Pyrite, Elemental sulphur, jarosite</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

At the same way as done with 1% of pulp density, bioleaching tests adding ferrous iron were carried out at 5% and 10% of pulp density using ferrous iron addition of 900, 1800, 2400, 3600 and 9000 mg/L. In Figure 4 can be observed that with 1800 mg/L of ferrous iron addition, also better copper extractions was obtained, with a copper extraction of 80% in only 165 hours.

![Figure 4. Copper extraction evolution with ferrous iron concentration added. (5% of pulp density (w/v), MTC culture at pH 1.30 and 65°C)](image)

At 10% of pulp density, copper extraction of 90% in 270 hours was obtained when 1800, 2700 and 3600 mg/L of ferrous iron were added (Fig. 5).

The evolution for the rest of parameters studied, such as ferrous and ferric iron in solution and zinc bleaching, were similar as obtained at 1% of pulp density.

In all bioleaching tests carried out, addition of ferrous iron increase copper extraction with this thermophilic culture. Addition of 1800 mg/L of ferrous iron seems to have the optimum catalysed effect in all pulp densities studied (1, 5 and 10% (w/v)). This optimum extraction could be related wit lesser presence of jarosites in solid residues.

Most of the soluble iron, was present in ferrous form and the ferric concentration was negligible. The ferrous iron was produced by chalcopyrite leaching, and only when chalcopyrite is almost oxidised, ferrous iron oxidation is observed. Ferrous oxidation by this thermophilic culture is very low in liquid medium (data confirmed in early tests in 9K medium).
Trying to explain leaching mechanisms with this thermophilic culture, authors think that, in acidic solution, chalcopyrite can be principally oxidised by dissolved oxygen according to the following reaction:

\[
\text{CuFeS}_2 + \text{O}_2 + 4\text{H}^+ = \text{Cu}^{2+} + \text{Fe}^{2+} + 2\text{SO}_4^- + 2\text{H}_2\text{O}
\]  

(1)

In addition to this, the ferric ion that can be produced by oxidation of ferrous iron in acidic medium through Eq. (2) was rapidly utilised to leach the chalcopyrite by Eq. (3).

\[
4\text{Fe}^{2+} + 4\text{H}^+ + \text{O}_2 = 4\text{Fe}^{3+} + 2\text{H}_2\text{O}
\]

(2)

\[
\text{CuFeS}_2 + 4\text{Fe}^{3+} = \text{Cu}^{2+} + 5\text{Fe}^{2+} + 2\text{SO}_4^-
\]

(3)

taking place the chalcopyrite oxidation.

Considering the final products obtained in our case, an indirect leaching mechanism could be postulated. However, ferric iron in solution was minimum and MCT possess very low capacity to oxidise ferrous iron in solution. Besides, according with rest potentials, sphalerite should be the most easily mineralogical species to leach, but this is not true, in our case. Because of that, the authors think according to postulated in (2), ferrous oxidation to ferric ions is produce onto chalcopyrite surface, where ferrous ions added are adsorbed on chalcopyrite surface. This ferrous iron is oxidised on surface by culture not in leaching media. This process that occur also with no addition of ferrous iron, have a catalyse effect when initial ferrous ions were added. These, provide of ferric ions that rapidly oxidises chalcopyrite producing more ferrous iron that can be adsorbed in other points of the chalcopyrite surface acting in the same way.

When the chalcopyrite leaching is almost exhausted, ferric ion concentration is high passing to the solution and could leach sphalerite by ferric leaching, as can be observed in Figs 2 and 3.

4. CONCLUSIONS

A natural mixed thermophilic culture was obtained from a chalcopyritic copper concentrate with an ability to preferentially leach chalcopyrite in concentrates and with high leaching rates at high pulp densities. In our case, the enhancement of chalcopyrite bioleaching capacity of the mixed thermophilic culture by addition of ferrous ions confirm the indirect in situ leaching mechanism, postulated in our previous work [1], for this culture respecting the chalcopyrite.
The results obtained in this study confirm the main role of iron forms in the bioleaching processes of chalcopyrite.

From these laboratory results this culture can be considered as a promising advance in the biohydrometallurgical treatment of chalcopyritic concentrates and its potential use on an industrial scale.

REFERENCES
Evaluation of microbial leaching of uranium from Sierra Pintada ore. Preliminary studies in laboratory scale

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Abstract

The feasibility of bacterial leaching was studied in our laboratory with mineral from Sierra Pintada mine (Province of Mendoza, Argentina). It is a low U grade ore with 6% of carbonates and low quantity of pyrite. The experiments were performed in shake flasks and scaled up to static flood tanks.

Shake cultured leaching studies were carried out with 0.5 inch crushed ore and a pulp density of 10%. Acidithiobacillus ferrooxidans (ATCC 33020) was grown in 9K medium and used as inoculum. Ferrous sulphate (FeSO₄) was added as energy source. Percent of U leached and acid demand to maintain acidity values of pH=2 were measured during the experiment. Three conditions were tested: ore, ferrous sulphate and inoculum; ore and FeSO₄ and a control with ore but without inoculum or FeSO₄. After 90 days, the acid added in the flask with bacteria and FeSO₄ was 20 times less than the control, and the U leached yielded 90.5%, that is 83% more than the control.

Scale up experiments were performed in static flood tank system. Sixteen kg of 0.5 inch crushed ore were placed in a static tank reactor and flooded with 32 liters of 9K medium without FeSO₄. Ferrous sulphide (SFe) was added as energy source. Gentle fine bubble aeration was accomplished by passing air through diffusers, and liquid recirculation was forced by a pump. At. ferrooxidans was grown in 9K medium and then used as inoculum. Three different conditions were tested: ore, inoculum and SFe; ore and inoculum, and a control without inoculum (ore only). Daily pH was measured and sulphuric acid added to maintain pH=2.0 was recorded. Weekly U leached was measured. The assay was carried on for five months. An initial 7 weeks lag phase was observed for all three conditions. This was associated to pH adjustments required for neutralization of acid demand due to the high carbonate presence in the ore. After five months, 72.8% of U was extracted in the At. ferrooxidans and SFe tank, with a total of 43.0 g of sulphuric acid added per g of U leached. An uranium extraction of 32.3% and 73.2 g acid/g U leached was observed in the ore and At. ferrooxidans (no energy source added) tank, while the control showed 3.6% of U leached and 73.4 g acid added /g U leached.

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The slowness of the process may be due to gypsum and jarosite precipitation on the mineral, impairing bacterial action and liquid recirculation as well. Both, shake flasks and static flood systems results, showed that the addition of an energy source is needed to obtain a reasonable rate of uranium leaching.

A second static flood system experiment was performed, with an acid cure treatment before bacteria addition and counter current media flow. Different energy sources were also tested. The four conditions were: ore, inoculum and carbon steel scrap as energy source; a blank without inoculum; ore, inoculum and exogenous pyrite as energy source, and its corresponding blank without bacteria.

After a month, 90.7% of U was extracted from the inoculated and carbon steel scrap tank with an acid consumption of 60.9 g/g U leached. In the tank with pyrite and inoculum an 89.6% of U leached was observed with 54.9g acid /g U.

The second static flood experiment was notoriously effective in diminishing the leaching time, and avoiding precipitation that cause blockages in tubing. Both scrap and pyrite have shown to be suitable and economic energy sources.

The experiments describe here are a promising first step in the evaluation of a possible pilot scale application.

1. INTRODUCTION

In Argentina the uranium industry is controlled by the federal government through the Atomic Energy National Commission (CNEA).

In the fifties, the geological group found several uranium deposits around the country. In the sixties, the mining exploitation began to supply the fuel to the nuclear power plants Atucha and Embalse de Río Tercero.

Atucha (RWU type) works with low enriched uranium and Embalse de Río Tercero (Candu type) only with natural uranium, consuming nearly 120 tU/year. From 1979 to 1995 CNEA produced, by heap leaching, 1,000 tU in yellow cake form in Sierra Pintada plant (Mendoza province), to supply the fuel to the two nuclear power plants.

During the nineties, due to the lower price in the spot market and overvaluation of the currency, the Sierra Pintada mine and the yellow cake production plant were shut down and Argentina begun to buy yellow cake in the spot market.

Nowadays both activities have been started again and, the engineering group started to work on some other methods to reduce the cost and improve leaching and waste treatment.

The mineral of this mine is sandstone with high concentration of calcium carbonate. This situation demands huge amounts of sulfuric acid to extract the uranium from the rock. The sulfuric acid, the mining operation and the manpower, are the most significant costs in yellow cake production.

The main tasks implemented were: new design in the open pit, changing heap leaching by flooded leaching, and studies on bioloeaching. In 1999, combined work began between two groups from CNEA, the Engineering and the Microbiology groups, to investigate bioloaching, as reported in this paper.

Microbial leaching is a simple and effective technology used for metal extraction from low-grade ore. Metal recovery is based on the activity of chemolithotrophic bacteria, mainly Acidithiobacillus ferrooxidans, At. thiooxidans and Leptospirillum ferrooxidans (1-3).
Most of the uranium ores occur as a mixture of mineral containing uranium in either the tetravalent or the hexavalent state. Uranium is soluble only in its most oxidized hexavalent state (4).

Tetravalent uranium can be oxidized to the hexavalent state by ferric iron, but oxidation occurs much more rapidly in the presence of the iron-oxidizing *At. ferrooxidans*(5). Bacterial leaching of uranium occurs via an indirect mechanism in which the bacteria oxidize the pyrite in the ore, generating an acidic ferric sulfate solution which carries out the chemical oxidation of the tetravalent uranium to the soluble hexavalent state.

The studies reported here were initiated in order to determine the feasibility of microbial leaching of Sierra Pintada ore and whether bioleaching could contribute in the costs reduction by increasing the uranium recovery rate, reducing the acid consumption and / or diminishing the leaching time.

Bioleaching of uranium from Sierra Pintada ore was carried out using *Acidithiobacillus ferrooxidans* at bench scale; exogenous ferrous compound were added for laboratory tests due to the low content of pyrite in the ore.

2. MATERIALS AND METHODS

2.1 Bacterial culture preparation

A pure strain of *Acidithiobacillus ferrooxidans* (ATCC 33020) was used in this study.

The bacteria were cultured in 2L Erlenmeyer flasks with 1L of basal medium containing 0.1g KCl, 3.0g/l (NH₄)₂SO₄, 0.5g/l MgSO₄·7H₂O, 0.5g/l K₂HPO₄·3H₂O, 44.22 g/l FeSO₄·7H₂O, 0.01 g Ca(NO₃)₂ (9K medium) (6).

The pH of the medium was adjusted to 1.80 with sulfuric acid. The culture was kept at 30°C in a rotary shaker at 100 r.p.m.

The culture in exponential growth was used directly as inoculum in each experiment.

2.2 Analytical methods

The majority of the uranium analyses were performed by LivestockGroup laboratory. The uranium content of the ore and pulp residues was determined by the Laboratory Section of Complejo Minero Fabril San Rafael, Province of Mendoza. Uranium in leach liquor and pulp residues was determined spectrophotometrically by using dibenzoylmethane method (8). Daily pH was measured and the volume of sulfuric acid added to maintain pH 2.0 was recorded.

The bacterial viability in both shake flasks and static tanks was checked by subculturing 1 ml of supernatants in 9ml of 9K medium twice a month.

2.3 Uranium ore

The Sierra Pintada ore consisted of moderately well-sorted grains of quartz, feldspar and rock fragments cemented by calcite with minor clay replacement. This mineral is a sandstone with high quantity of carbonates. It is a low-grade ore, with an average of U₃O₈ content of 0.15%, 3.8% of carbonates and a low quantity of pyrite. The radioactive mineralization occurs mainly as uraninite, brannerite and coffinite. Analysis of the ore gave average values of 0.26% magnesium, 0.14% phosphorous, 2.6% calcium and 1.72% total iron (9).
2.4 Pyrite
Pyrite from an outcrop near Sierra Pintada mine was characterized and used in leaching experiments. Sulfur and iron contents were 30% and 38% respectively.

2.5 Shake culture leaching
Four Erlenmeyer flasks with two liters of medium containing 0.5g/l $K_2HPO_4$, 0.5g/l $(NH_3)_2SO_4$, 0.5g/l $MgSO_4\cdot7H_2O$ at pH: 2.0 (TyK iron-free medium) were inoculated with 100 ml of a 96 hour culture of *Acidithiobacillus ferrooxidans* (7). Two hundred grams of Sierra Pintada ore (>1.0, <1.3cm) was added to the medium (10% pulp density) in each flask.

Ferrous sulfate ($FeSO_4\cdot7H_2O$) 2% w/w was added as energy source when it was necessary. The initial pH was 2.0 in all flasks.

2.6 Static flood tank system leaching
The system consisted of two plastic tanks (38cm x 30 cm) connected in series. Gentle fine bubble aeration was supplied by an aquarium air pump (fig. 1).

One tank contained TyK medium and the other was packed with 16 kg of uranium ore crushed and screened to > 1.0 <1.3 cm. The iron free TyK medium was continuously cycled through the ore by means of a submerged centrifugal water pump.

Different energy sources were tried: 2% w/w of ferrous sulphide (1.5 cm); 2% w/w exogenous pyrite (100 Mesh) and 0.5% w/w of carbon steel scrap.

![Figure 1. Scheme of static flood tank system (A: Aquarium air pump; B: TyK medium; C: Centrifugal water pump; D: Valve; E: Ore and medium flooding; F: Filter bed; --- Fluid circulation direction) ](image)

3. RESULTS

3.1 Shake-culture leaching
Four flasks containing 10% w/w uranium ore were incubated, three of them with bacterial inoculum and one abiotic control.

Ore with neither inoculum nor FeSO$_4$ (Flask A) worked as control. The other had ore and inoculum (Flask B); ore, FeSO$_4$ and inoculum (Flask C) and ore with FeSO$_4$ (Flask D). The shake cultures were in TyK iron-free medium at room temperature and shaken at 100 rpm. The uranium grade of the ore used in the assay was 1600 µg U/g ore. Bioleaching was monitored by following the uranium content in the supernatants. Acid demand to maintain acidity values of pH 2.0 were measured and recorded. The assay was
carried out for 90 days. At this stage the uranium in the pulp residues was analyzed. The data are presented in Table 1 and Fig. 2.

**Table 1. Shake culture leaching of Sierra Pintada ore with *At. ferrooxidans* (*At. fe*) for 90 days**

<table>
<thead>
<tr>
<th>Flask</th>
<th>% Uranium in leached liquor</th>
<th>% Uranium in pulp residue</th>
<th>Acid Consumed g/ Kg ore</th>
<th>g/ g U leached</th>
</tr>
</thead>
<tbody>
<tr>
<td>A- Control</td>
<td>6.9</td>
<td>85.0</td>
<td>312</td>
<td>2227</td>
</tr>
<tr>
<td>B- Ore+<em>At. fe</em></td>
<td>74.0</td>
<td>22.5</td>
<td>193</td>
<td>132</td>
</tr>
<tr>
<td>C- Ore +Fe²⁺ + <em>At. fe</em></td>
<td>90.5</td>
<td>6.2</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>D- Ore + Fe²⁺</td>
<td>80.7</td>
<td>10.7</td>
<td>124</td>
<td>71</td>
</tr>
</tbody>
</table>

The yield of leaching was about 90% after 90 days in the flask C with bacteria and FeS0₄ with the lowest acid consumption. In contrast, only about 7% yield of uranium was achieved in Flask A, the control (Table 1).

Flask B containing only *At. ferrooxidans* extracted 74% of the uranium from the ore. The reason for this high percentage of uranium recovery respect to the control could be due to the iron present in the ore which was used by the bacteria.

The time course of leaching is seen in Fig. 2.

![Figure 2. Shake flasks microbial leaching of uranium by *At. ferrooxidans* using ferrous sulfate as energy source](image)

At the end of the assay, flask D showed 80% of U leached. This result could be explained by confirmation of *At. ferrooxidans* presence by sampling flask D supernatant and subculturing in 9K medium. It could have been contaminated during manipulation of samples.

This first experiment showed that bioleaching process is possible in Sierra Pintada ore and that an energy source is necessary for a faster uranium extraction.

**3.2 Static flood tank leaching system**

According to the results obtained in shake flasks leaching, a static flood leaching system was tested and scaled up 80 times respect to the flasks (Fig. 1). Sixteen kg of >1.0 <1.3 cm crushed ore were placed in a static tank reactor and flooded with 32 liters of TyK iron-free medium by using industrial grade reagents and tap water. Three different
conditions were tested: tank 1: ore, inoculum and ferrous sulphide (FeS); tank 2: ore and inoculum without external energy source and control tank without inoculum nor FeS. The assay was carried out at room temperature. The pH was measured daily and the sulfuric acid added to maintain pH 2.0 was recorded. The assay was monitored by measuring the uranium content in leach liquor. Periodically aquarium pump valve and tubing were checked and the blockages removed. After five months of incubation, the experiment was finished because of the increase of uranium leached in the liquor stopped.

The uranium mineral in Sierra Pintada is hosted in sandstone deposits mostly containing 3.8% carbonates and minor amounts of pyrite (9). The leaching from this carbonate bearing sandstone uranium ore requires sulfuric acid addition to neutralize the carbonates. As a consequence during the first seven weeks of the assay, important pH adjustments were made (Fig. 3). It is obvious that carbonates buffer the acid bioleaching solution and may adversely affect the metabolic activity of acidophilic *At. ferrooxidans* (10,11).

![Figure 3. Acid consumption in the static flood tank system during bioleaching assays](image)

Table 2 shows the performance of the static flood bioleaching system.

**Table 2. Bacterial static flood leaching of Sierra Pintada ore at room temperature with *At. ferrooxidans* for five months. Ferrous sulphide (Fe²⁺) added as energy source**

<table>
<thead>
<tr>
<th>Tank</th>
<th>% Uranium in leached liquor</th>
<th>% Uranium in pulp residue</th>
<th>Acid Consumed g/Kg ore</th>
<th>Acid Consumed g/g U leached</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Ore +Fe²⁺+At. fe</td>
<td>72.8</td>
<td>33.5</td>
<td>45.3</td>
<td>43.0</td>
</tr>
<tr>
<td>2 - Ore + At. fe</td>
<td>32.3</td>
<td>50.2</td>
<td>34.3</td>
<td>73.2</td>
</tr>
<tr>
<td>Control - Ore</td>
<td>3.6</td>
<td>86.0</td>
<td>38.1</td>
<td>734.6</td>
</tr>
</tbody>
</table>

After five months, 73% of uranium was extracted in tank 1 with bacteria and FeS, with a total of 45 g of sulfuric acid added per kg of ore during the experiment.

Thirty three percent of uranium recovery and 34 g of acid/kg ore was recorded for tank 2 without energy source addition, while the blank without inoculum or FeS (Control tank) showed 3.6% of uranium leached and 38 g acid/kg ore (Fig. 4).

It can be seen in Fig. 4 that the rate of uranium leaching becomes reasonable after 7 weeks. In that initial period, acid additions were necessary to maintain a pH value in *At. ferrooxidans* at optimal range.
Figure 4. Microbial leaching of uranium by *At. ferrooxidans* using ferrous sulphide as energy source: static flood tank system

The percentage of uranium recovery reached 72.8% in tank 1 after fourteen weeks and it becomes almost constant. In later stages of the experiment, bioleaching rates decreased probably because during the process gypsum (CaSO₄) was formed and precipitated on the ore particles in the same way as jarosite did, diminishing the available surface for bacterial attack. Moreover, recirculation and aeration systems were frequently blocked by gypsum, jarosites and other inorganic precipitates. This blockage altered the optimal conditions for *At. ferrooxidans* activity and periodic maintenance of tubing became necessary.

At the end of the experiment the efficiency of *At. ferrooxidans* activity in uranium extraction was evident. Both, uranium leaching rate and acid consumed to leach one gram of uranium were notoriously better in tank 1 with inoculum and FeS, than in the other two conditions (Table 2). The addition of an energy source was necessary to obtain a reasonable rate of uranium leaching as it can be seen when comparing tank 1 and tank 2 performances (Fig. 4). This response to addition of Fe²⁺ was much more evident in static tank system than in shake flasks (Fig. 2).

Based on the results and conclusions of the static flood system, a new experiment was carried out in order to improve the performance of the system. Fourteen kg of >1.0 <1.3 cm crushed ore were placed in a tank reactor and flooded with 28 liters of TyK iron free medium. The media recirculation was in a counter current direction. An acid cure treatment was performed before leaching to diminish the leaching time and to avoid the gypsum precipitation which causes blockages in pump valves and tubing. A better media recirculation through the ore and aeration were expected. At the same time a better bacteria performance should be observed.

The cure acid treatment was carried out for a week. A total 55.0g of concentrated sulfuric acid was added per liter of TyK media and recirculated through the ore to permit carbonates dissolution and to maintain a pH value between 2.0 and 3.0. Four different conditions were tested: tank 1: ore, inoculum and carbon steel scrap; control 1: ore and carbon steel scrap without bacteria; tank 2: ore, inoculum and exogenous pyrite, and control 2: with ore and pyrite but without inoculum.

After the acid cure treatment, 0.5% w/w of carbon steel scrap (tank 1 and control 1) and 2.0% of pyrite (tank 2 and control 2) were added. The next day, tanks 1 and 2 were inoculated with *At. ferrooxidans* and was considered the day one of the experiment. As it
was described before, the assay was monitored by measuring U content in the liquor weekly. The assay was carried out at room temperature, and acid demand for pH 2.0 maintenance was measured and recorded for each tank. After 33 days, the assay was finished because most of the uranium contained in the ore was leached. The remain uranium in the pulp residues was determined.

The final data are summarized in Table 3 and the time course of process is showed in Figure 5.

**Table 3. Static flood bioleaching system of Sierra Pintada ore using carbon steel scrap and pyrite as exogenous sources of energy. Results obtained after 33 days of incubation**

<table>
<thead>
<tr>
<th>Tank</th>
<th>% Uranium in leached liquor</th>
<th>% Uranium in pulp residue</th>
<th>Acid Consumed g/ Kg ore</th>
<th>g/ g U leached</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Ore + scrap + <em>At. fe</em></td>
<td>90.7</td>
<td>9.3</td>
<td>74.6</td>
<td>60.9</td>
</tr>
<tr>
<td>Control 1- Ore + scrap</td>
<td>91.1</td>
<td>8.9</td>
<td>74.3</td>
<td>60.4</td>
</tr>
<tr>
<td>2- Ore + pyrite + <em>At. fe</em></td>
<td>89.6</td>
<td>10.4</td>
<td>66.5</td>
<td>54.9</td>
</tr>
<tr>
<td>Control 2- Ore + pyrite</td>
<td>48.5</td>
<td>51.5</td>
<td>61.3</td>
<td>93.6</td>
</tr>
</tbody>
</table>

**Figure 5. Static flood tank system: microbial leaching of uranium using *At. Ferrooxidans*. Carbon steel scrap and pyrite were used as energy source**

After 33 days of incubation the tank with bacteria and carbon steel scrap extracted 90.7% of uranium with 60.9 g of acid added per gram of uranium leached. Tank 2 with pyrite as energy source showed 89.6% of U extracted and 54.9 g acid/g U leached (table 3). Carbon steel scrap is a waste product of carbon steel pieces turned in a turning lathe. In this experiment, both carbon steel scrap and pyrite were used to tested weather it could be an alternative of commercial FeS and the results obtained showed that it is possible (table 3, Fig. 5).

Comparison of acid consumed in tank 1 and 2 showed that it was slightly minor (9.0%) in tank 2 (containing pyrite (S₂Fe)) than in tank 1 (scrap). This difference could be attributed to *At. ferrooxidans* acid production by sulphur oxidation from pyrite. However that difference was lower than we had expected. The absence of adaptation of the bacteria to pyrite as energy source could be a reason. In future experiments a major advantage of pyrite as substrate will be evaluated.
As can be seen in Fig. 5 initially control 1 performance was according to a blank. Afterwards the U leached was increasing and at the end of the experiment it was as in tank 1 with bacteria. The *At. ferrooxidans* presence in control 1 was confirmed by microscope observation and supernant subculturing in 9K medium.

The gypsum formation was not evident and tubing clogging did not occur.

The performance of the assay was notoriously improved by diminishing the leaching time approximately five times respect to the first static flood assay. The uranium recovery was also increased in almost 20%.

4. COMMENTS AND CONCLUSIONS

Laboratory bench scale approaches to bioleaching evaluation usually involves shaken flask techniques. It is a suitable methodology for screening and preliminary testing because complete metal recovery is reached in a few days. However it has some limitations. The steady state cannot be reached due to continuous change of conditions and the ore sample homogeneity is difficult to achieve because the amount of ore quantity employed is usually small.

The ore property and characteristics should be considered when a biological leaching process is being evaluated on a bench scale. No two ores are identical and within each ore deposit the mineralogical composition and the concentration of metals show heterogeneity.

These variations demand experimental evaluation of multiple samples even from a single ore body, because they have a major effect on the microbial leaching of the material (12).

After a few approaches to bioleaching using shake flasks, a static tank reactor technique was attempted. This technique represented a scale up of 80 times over the shake flask method. The ore material homogeneity had less influence in the results than in the reduced scale. Moreover the ore material could be more representative of the material that will be used in large-scale commercial application.

The tank leaching studies have longer duration and develop different zones within the ore that differ in redox potential, iron precipitation, chemical and physical gradients. These characteristics are similar to large-scale applications, which is an advantage when the objective is a pilot plant scale.

The presented results showed different performance of bioleaching between shake flask and the static flood techniques tested. We concluded that static flood tank system gave better results through being a larger scale system.

The first static flood system experiment ran with some difficulties, mainly design variables, that were solved by introducing counter current flow leaching and acid cured treatment. This impaired the undesirable precipitates that cause tubing blockages. Counter current flow leaching improved recirculation and reduced ore compaction. As a consequence, aeration and pH value improved, and a more optimal *At. ferrooxidans* activity condition was reached. An improved kinetics of the leaching process and considerably shorter leaching period were attained.

Biological leaching profiles of mineral samples from every ore are unique. In this paper the ore tested was poor in pyrite content so different ferrous sources were tested that permitted a reasonable leaching rate. Commercial ferrous sulphide, carbon steel scrap and pyrite were attempted. Both, carbon steel scrap and pyrite were effectively used as energy
substrates by *At. ferrooxidans* and can also be considered as suitable iron sources for bioleaching.

The Sierra Pintada ore is alkaline in nature; as a consequence the bioleaching process using acidophilic bacteria becomes a slow process and has a high acid demand to neutralize the carbonates. In future experiments, an *At. ferrooxidans* culture adapted to pyrite will be used as inoculum in order to become an acid producer reaction diminishing the acid consumption.

The presented preliminary results indicate that it is possible to use bioleaching for uranium recovery from Sierra Pintada ore; that is necessary to add an exogenous iron source and the results also are a promising first step in the evaluation of a possible pilot scale application in Sierra Pintada ore.

REFERENCES

Extraction of copper from mining residues and sediments by addition of rhamnolipids

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Abstract

Rhamnolipids are anionic biosurfactants produced by the bacteria Pseudomonas aeruginosa. They are less toxic and more biodegradable than many synthetic surfactants. They were evaluated as agents to enhance removal of heavy metals from mining tailings and sediments. Mining tailings were obtained from a copper oxide mine. Another media for extraction of metals was also examined, contaminated sediments from a canal. A series of washings was performed on the mining tailings and sediments using 0.5%, 1% and 2% rhamnolipid solutions with and without 1% NaOH. The mining residue contained 8,950 mg/kg of copper and the sediments contained 140 mg/kg copper and 4,850 mg/kg zinc. Biosurfactants were added to slurry of either the mining residues or sediments with 10% mining solids. For the mining residues, with a 2% concentration of rhamnolipid, 28% of the copper could be removed. Adding 1% NaOH with the 2% rhamnolipid increased extraction up to 42% over a 6 day period. Lower concentrations of biosurfactant (0.5 and 1.0%) removed lower amounts of copper. Addition of 1% NaOH always showed higher removal rates than without the NaOH. Copper was also analyzed in the supernatants after washing the sediments. Maximal removal was 15.6% copper with 1% NaOH. For example, for a 2% rhamnolipid concentration, approximately 250 mg/kg of zinc were removed compared to 20 mg/kg of copper (0.5% rhamnolipid with NaOH). Therefore, copper can be removed from two different metal containing media by rhamnolipids.

Keywords: rhamnolipids, mining tailings, sediments, copper

1. INTRODUCTION

Cadmium, copper, lead, mercury, nickel and zinc are considered the most hazardous heavy metals and are included on the EPA's list of priority pollutants [1]. Recently, the EPA has announced that the decontamination of sediments will receive the highest priority. Sources of metals include domestic and industrial effluents, the atmosphere, runoff and lithosphere. Once heavy metals are allowed to pass through the municipal waste treatment facility, they return to the environment where they are persistent, cannot be biodegraded and can thus follow a number of different pathways. The metals can adsorb onto the soil, runoff into rivers or lakes or leach in the groundwater, an important source of drinking water. Exposure to the heavy metals through ingestion or uptake of drinking water (particularly where water is reused) and foods can lead to accumulation in animals, plants and humans.
Between 1850 and 1990, production of these three metals increased nearly 10-fold, with emissions rising in tandem [2]. The world mining industry has made some remarkable improvements in worker safety and cleaner production but yet it remains one of the most hazardous and environmentally damaging industries. Mining of heavy metals and of coal and other minerals is a major route of exposure since during the extraction process, a part of the heavy metals remains in the ore after discarding.

Treatment methods for contaminated sediments are similar to those used for soil and include pretreatment, physical separation, thermal processes, biological decontamination, stabilization/solidification and washing [3]. However, compared to soil treatment, few remediation techniques have been commercially used for sediments. Solidification/stabilization techniques are successful but significant monitoring is required since the solidification process can be reversible. In addition, the presence of organics can reduce treatment efficiency. Vitrification is applicable for sediments but expensive. Only if a useful glass product can be sold will this process be economically viable. Thermal processes are only applicable for removal of volatile metals such as mercury and costs are high. Biological processes are under development and have the potential to be low cost. Since few low cost metal treatment processes for sediments are available, there exists significant demand for further development. Pretreatment may be one of the methods that can reduce costs by reducing the volumes of sediments that need to be treated. Methods to determine the requirements for remediation are necessary for optimal design.

Biological removal of heavy metals has been the subject of several studies. Mulligan and Galvez-Cloutier [4] presented the result that heavy metals could be removed from mining residues by growth of the fungus \textit{Aspergillus niger} through organic acid production. They report that up to 65% of the copper can be removed from the sediments by rhamnolipids. Chartier et al. [5] could wash 51% of copper from the same sediment by biological treatment using \textit{Thiobacillus ferroxidans}. Mulligan et al. [6] also used biosurfactants to remove heavy metals from oil-contaminated soil. In their study, they found that a combination of 2% rhamnolipids with 1% NaOH provided maximum removal of 25% of the copper from the soil.

1.1 Surfactants

A surfactant is a "surface active agents". They are also called surface-active substances and surface-active compounds [7]. These materials are able to lower the surface tension of a solvent. Meanwhile they form aggregates, micelles, in aqueous media [8]. This property is very important, as their effectiveness depends on their ability to reduce surface tension [9]. An effective surfactant is able to reduce the air-water interface to 35 mN/m and the oil-water interfacial tension to 1 mN/m [10]. An important characteristic of surfactants is that the hydrophobic portion has little affinity for the bulk medium while the hydrophilic portion is attracted to the bulk medium. Surfactants have three important characteristics: surface tension lowering, hydrophobic-lipophilic balance or HLB, and critical micelle concentration or CMC. Low CMC values represent a more efficient surfactant since less surfactant is needed to decrease the surface tension [11].

1.2 Biosurfactants

Biosurfactants are a group of surfactants naturally produced by certain types of microorganisms. Although the low level of toxicity is a big advantage of biosurfactants over synthetic surfactants, it is not the only one. Other remarkable advantages of biosurfactants are: small molecular size, increased biodegradability, effectiveness, and ease of synthesis. Biosurfactants are made by microorganisms and this increases the
possibility of in-situ production [12, 13] since they are synthesized as metabolic by-products. The composition and yields depend on the fermentation conditions including, pH, nutrient composition, substrate, and temperature used [10].

The biosurfactant that is used in this study, a rhamnolipid, is from the glycolipids group and is made by *Pseudomonas aeruginosa* [7]. There are four types of rhamnolipids. Rhamnolipid type I and type II are suitable for soil washing and heavy metal removal while type III is for metal processing, leather processing, lubricants, pulp and paper processing. Type IV is usually used in textiles, cleaners, foods, inks, paints, adhesives, personal care products, agricultural adjuvants, and water treatment [14].

In the present study, the feasibility of using a biosurfactant (rhamnolipids) for the extraction of copper from a low-grade oxide mining residue and sediment were investigated. The effect of various parameters was investigated to enhance copper removal.

2. MATERIALS AND METHODS

2.1 Ore

The ore is obtained from a copper mine in the Gaspé region, Quebec, Canada. The oxide ore was already crushed into smaller particles that remained larger than 2.54 cm in nominal diameter. The ore was crushed in the lab into smaller size and sieved to find the best size for the ore particles in the extraction process. The maximum sieve size chosen was number 5 and the minimum was number 200. To remove colloidal materials from the particles, the ore was washed with tap water until no more colloids were suspended in the water. Using method ASTM D422 [15], the washed materials were placed in an oven and dried at 100°C for 24 hours. After 15 minutes in shaking sieving, the collected ores from the sieves were kept separately in a dry keeper. The method recommended by Environment Canada [16], for digestion of ores containing copper, was used to digest the prepared ore. The copper in the ore was measured by an Atomic Absorption Spectrophotometer (Perkin Elmer Aanalyst 100). The results are shown in Table 1.

2.2 Characterization of the sediment sample

The metal-contaminated sediment sample was obtained from a canal area which was surrounded by metal and steel industries. The sample was air-dried. The grain size distribution of the sediment indicated 10% sand, 70% silt and 20% clay. X-ray analysis indicated the presence of quartz (30%), feldspar (36%), illite (2%), kaolinite (27%), chlorite (3%) and carbonate (0.5%) as performed by Mulligan et al. [4]. Total organic matter was 20% (w/w). The sediments were digested by the method recommended by Environment Canada [16] and then analysed by a Perkin Elmer Atomic Absorption Aanalyst 100 Spectrophotometer for heavy metal content. These results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration (mg/kg) in mining residues</th>
<th>Concentration (mg/kg) in sediments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>ND</td>
<td>145</td>
</tr>
<tr>
<td>Copper</td>
<td>8,950</td>
<td>140</td>
</tr>
<tr>
<td>Nickel</td>
<td>27</td>
<td>76</td>
</tr>
<tr>
<td>Lead</td>
<td>ND</td>
<td>572</td>
</tr>
<tr>
<td>Zinc</td>
<td>201</td>
<td>4,854</td>
</tr>
</tbody>
</table>
2.3 Biosurfactants

The rhamnolipids, used in this study, were biosurfactants type I and type II from the glycolipid group made by *Pseudomonas aeruginosa* with the trademark JBR215 from Jeneil Biosurfactant Co. JBR215 is an aqueous solution of rhamnolipid at 15% concentration. It is produced from a sterilized and centrifuged fermentation broth. Two major types of rhamnolipids, RLL (R1) and RRLL (R2), are present in the solution. Several tests done by the manufacturer and independent laboratories show the degree of biodegradability and toxicity of JBR215 match the EPA requirements. The CMC was found to be 0.035 g/L through conductivity measurement at various dilutions. This value is equivalent to 0.003% rhamnolipid. Therefore, for all experiments, a concentration above the CMC was used to ensure the formation of micelles.

2.4 Washing of mining residue

To evaluate the effect of various parameters on the extraction of copper from the low-grade mining residue as well as to optimize extraction, several tests were performed. All tests were duplicated and the difference between tests never exceeded more than 5%. Parameters were optimized step by step. Since the results of each step were used for the next parameter, tests were repeated between 2 to 8 times. All of them showed reasonable agreement.

The ore was placed in batch reactors for copper extraction. In some cases samples were placed on a rotary shaker for a desired period of time. For analysis, the ore particles were allowed to settle. The supernatant solution was then decanted from the ore and digested according to the procedure of APHA [17], Method 3030E. To release the biosurfactant from the copper, 30% solution of hydrogen peroxide was added until no reaction was observed. The following steps were then followed:

- 50 mL of the sample was transferred to a 125 mL beaker
- 25 mL of concentrated nitric acid and a few boiling chips were added to the beaker
- The beaker was then heated on a hot plate and brought to a slow boil.
- Once the solution became a clear light-colored liquid, the digestion was considered complete
- The solution was cooled to room temperature, filtered through filter #40 ashless paper, and the volume was readjusted to 50 mL with distilled water.

The quantity of copper was measured by an Atomic Absorption Spectrophotometer.

2.5 Washing of the sediment with the biosurfactants

The biosurfactant solution was diluted with distilled water or 1% NaOH as required. A quantity of 1.5g from the sediment of each particle size was placed in individual 50mL vials. 15.0mL of surfactant solution was added to each vial. The samples were kept in the temperature incubator at constant temperature (25°C) for 1 day. Blank samples including 15.0mL distilled water and 1.5g sediment were provided. Vials were placed on the side in order to achieve the maximum contact surface between the ore particles and the biosurfactant. The pH of the rhamnolipid with NaOH was 13 and without NaOH was 6.0. Blanks included the same additives as for the biosurfactant studies without the presence of the biosurfactant. Washing of the supernatants was performed for 6 days, and then removing the supernatants (3,000 x g, 30 min). All experiments were performed in triplicate. Results were reproducible to ±10%. The solutions from each sample were collected. The samples were digested and the concentrations of copper and other metals in each sample were measured by the Atomic Absorption Spectrophotometer. In order to
release the heavy metals trapped in the biosurfactant micelles, the organic copper colloids were oxidized by adding 30% H₂O₂ slowly until no reaction was observed. The method chosen for biosurfactant digestion was 3030E, which is recommended by APHA [17] and approved by the EPA.

3. RESULTS AND DISCUSSION

3.1 Mining residue studies

3.1.1 Effect of particle size

Seven ore samples with different sizes were collected from sieves and 1g of each was placed in individual vials and 10mL of the rhamnolipid solution of 1% (pH 6.5) was added. The control consisted of the same ore with distilled water. The test was repeated while the samples were placed on a shaker. The shaker was set at 100 rpm and after 5 days, the solutions were digested and analysed. A comparative diagram in Fig. 1 is presented. Controls were not shown since they were not significant.

Since shaking did not remarkably improve the extraction, the experiments were continued without shaking. The results show that a maximum of 17.7% copper extraction occurs in the unshaken samples containing ore particles between 0.15mm and 0.3mm. This particle size was chosen for the rest of the study.

![Figure 1. Comparative diagram of copper extraction for the chosen particle sizes for shaken and unshaken samples](image)

3.1.2 Effect of pH on copper extraction

Using the data from the first test, one gram of ore with particle sizes between 0.15mm and 0.3mm was placed in the vials with 10mL of 1% rhamnolipid for 5 days at various pH values. The pH was adjusted to between 6 and 9.5 and the extraction efficiencies were found by the measurement of the copper concentration in the solutions. Fig. 2 shows how the pH affects extraction of copper from ore. The minimum extraction of copper occurred
when the pH was around 8.8. This is similar to information in the solubility curve of copper hydroxide of Radhakrishnan [18] which show that minimum solubility is at pH 8.7. This can explain the occurrence of minimum extraction at the same pH.

![Figure 2. Variation of copper extraction with pH. Particles were between 0.15 to 0.3 mm in diameter. Each sample contains 1 g ore in 10 mL solution of 1% rhamnolipid. The samples were kept at 25°C for 5 days and left without shaking.](image)

3.1.3 Effect of the concentration of biosurfactant and NaOH

The effect of the concentration of rhamnolipid on copper extraction was determined by using 0.05% to 5.0% of rhamnolipid. Higher concentrations were not tested since the solution would be considerably viscous and hard to work with. According to previous studies [6], adding 1% NaOH to the solution will improve the copper extraction process. To verify the effect of NaOH, another test was designed in which the same concentrations were used but with co-addition of 1% NaOH.

A comparative graph is presented in Fig. 3. Controls are not shown since the controls did not demonstrate significant extraction. As the figure shows, the effect of NaOH is quite remarkable on copper extraction for concentrations of 2% rhamnolipid or less while it has a negative effect on the extraction for more concentrated rhamnolipid. In the later case, the extraction of copper in the solution of 1% NaOH and 2% rhamnolipid has almost the same value as of 4% rhamnolipid without NaOH. The added NaOH increases the pH up to 13.5.

3.2 Sediment washing studies

A series of washings was performed on the sediments using 0.5%, 1% and 2% rhamnolipid solutions with and without 1% NaOH. The control was 1% NaOH. Copper was analyzed in the supernatants and is plotted in Fig. 4. Removal rates for copper were highest for 0.5% rhamnolipid with 1% NaOH. Increasing the biosurfactant concentration when NaOH was added did not enhance removal rates. Maximal removals were 15.6% copper. For example, for a 2% rhamnolipid concentration, approximately 20 mg/kg of copper (0.5% rhamnolipid with NaOH). Zinc removal on the other hand was higher without 1% NaOH and was maximal at 2% rhamnolipid. Although zinc removal at 5% does not seem significant, the actual amount of zinc is higher than for copper at 250 mg/kg of zinc removed. This could be one of the reasons why the removal for copper by the rhamnolipid is lower for the sediments than for the mining residues. In other words, there
seems to be some preference for the certain heavy metals by the biosurfactant in the sediment. Another reason may also be that the organic content in the sediment is high and may lead to some adsorption of the biosurfactant.

![Figure 3. The effect of 1% NaOH on the extraction process for various concentrations of rhamnolipid. Particles were between 0.15 to 0.3 mm in diameters. Each sample contains 1 g ore in 10 mL of rhamnolipid solution while the pH was adjusted to 6. The samples were kept at 25°C through the test and left without shaking for 6 days.]

![Figure 4. Percentage of copper and zinc removed from sediments by various washing agents]

4. CONCLUSIONS

This research was performed to evaluate the application of rhamnolipid on extraction of copper from mining residue. The ore initially has a low concentration of copper that cannot be economically removed. From an environmental point of view, the existing copper in the ore is a heavy metal contaminant and should be removed so that it will not impact the environment.
This study dealt with several chemical and physical factors involved in the extraction to find the best conditions for the extraction. These parameters were ore particle size, pH, concentration of biosurfactant, effect of NaOH addition, and shaking. The importance of particle size was in the contact surface area between the ore particle and the biosurfactant. Rhamnolipids were also evaluated for removing contaminants from sediments. Results were not as good as for the mining residues possibly due to competition from the elevated levels of zinc in the sediments or the high levels of organic matter in the sediments.

ACKNOWLEDGEMENTS

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REFERENCES

Improving of film coating bioleaching using biorotor process

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Abstract

A sample of gold bearing pyrite concentrate from Mouteh plant, Iran was partially leached in a biorotor at a level of ten percent, then it was used for coating the crushed ore. Coated particles were subjected to thirty days column bioleaching with a culture of \textit{Thiobacillus ferrooxidans} named Tsho. This study showed that pyrite biooxidation could be increased about two times. Furthermore by using the pretreated concentrates the lag phase period decreased dramatically. This enhanced biooxidation caused the gold recovery by cyanidation to be increased of about seven percent.

Keywords: biorotor, GeoBiotics process, pyrite, \textit{Thiobacillus ferrooxidans}

1. INTRODUCTION

Film coating bioleaching (developed by GeoBiotics, Inc.) is an innovative and economic process that has been designed to sum the advantages of biooxidation in stirred tank reactors and heap leaching. At the same time this system is aimed at overcoming several drawbacks of both processes. This process is economical with low-grade concentrates and with mineral sulfides such as pyrite, whose biooxidation is slow [1]. This work presents results of column testing of the Mouteh gold concentrate coated on a support-crushed rock. Furthermore it was found that by using a pretreated concentrate the efficiency of film coating bioleaching could be considerably increased. The rotating drum was selected for preliminary biooxidation of the concentrate because this type of bioreactor can be operated at much higher pulp densities than conventional stirred tank reactors [2].

2. MATERIALS AND METHODS

2.1 Mineral sample

A representative sample of pyritic gold concentrate from Mouteh gold plant, Isfahan, Iran, was used in the bioleaching experiments. Table 1 shows the primary mineralogical species and the chemical composition of the concentrate. Particle size analysis revealed that 85 percent of the sample was finer than 150 microns.

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Table 1. Mineral and chemical composition of the Mouteh pyrite gold concentrate used in the bioleaching tests

<table>
<thead>
<tr>
<th>Minerals Identified</th>
<th>Wt. %</th>
<th>Chemical Composition</th>
<th>Wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrite</td>
<td>68.4</td>
<td>Sulfide sulfur</td>
<td>30.09</td>
</tr>
<tr>
<td>Chalcopyrite</td>
<td>-</td>
<td>Silicate</td>
<td>24.46</td>
</tr>
<tr>
<td>Covellite</td>
<td>-</td>
<td>Aluminum</td>
<td>3.32</td>
</tr>
<tr>
<td>Tennantite</td>
<td>-</td>
<td>Magnesium</td>
<td>0.55</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>Trace</td>
<td>Iron</td>
<td>32.08</td>
</tr>
<tr>
<td>Galena</td>
<td>Trace</td>
<td>Gold</td>
<td>30ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcium</td>
<td>1.19</td>
</tr>
</tbody>
</table>

2.2 Basal medium

HP medium with the following composition was used for the bioleaching experiments: 0.4 g/L (NH₄)₂SO₄, 0.4 g/L MgSO₄·7H₂O and 0.1g /L K₂HPO₄. The solution pH was adjusted with H₂SO₄ [3].

2.3 Microorganism

The strain used in the experiments was *Thiobacillus ferrooxidans* Tsho [4]. The strain was previously adapted to HP medium containing 3% (by weight) concentrate at 30°C.

2.4 Biological pretreatment of concentrate

The mineral sample was partially oxidized at a level of ten percent in a Plexiglas biorotor [2]. For monitoring the process in the biorotor, the pulp was sampled every 24 hours, and the samples were analyzed for pH, ferrous iron, total soluble iron, total iron. The samples were decanted and the solids were returned to the reactor. The volume of the pulp was maintained constant by periodical additions of acidified distilled water. During the leaching process, some portion of the released iron was precipitated. Therefore, total iron was measured after acid digestion with 6N HCl for 30 min at 65°C. The metal content was analyzed by titrimetric method using 0.06 N K₂Cr₂O₇. Also the total number of cells in solution was analyzed by Neubauer improved counting chamber.

2.5 Column leaching of coated support rocks

The concentrate was mixed with the bacterial inocula so as to form thick slurry, which was then sprayed onto a sized supporting rock 1 x 2.5 cm diameter (20% by weight of the support rock). The support rock was a refractory sulfide ore that had been crushed in a jaw crusher. The thickness of concentrate coating layer was 0.5 to 1 mm. The tests were performed at 20°C in a column 9 cm in diameter by 40 cm in height. The feed solution at pH 1.7 was applied to the column through glass wool pad at various flow rates. Sampling of the effluent solutions was performed every 4 days, and the samples were analyzed for pH, Eh, ferrous iron and total iron. Also, measurements of residual nutrients were performed periodically. Gold recovery, before and after biooxidation, was determined by conventional cyanide roll bottle tests.

3. RESULTS AND DISCUSSION

Figure 1 shows the effect of the different flow rates on the biooxidation of coated material. The experiment was carried out at room temperature and pH 1.7. Flow rate is an important factor in column leaching of coated particles, since it can affect the
characteristics of the feed materials. The results showed that a flow rate of 4 dm$^3$ m$^{-2}$ h$^{-1}$ was not sufficient, compared to the higher flow rates, to oxidize the sample. Increasing the flow rate to 8 dm$^3$ m$^{-2}$ h$^{-1}$ significantly increased the oxidation rate. Higher flow rate increments did not improve the oxidation rate. Figure 2 shows the comparison of the biooxidation rate of the pre-treated sulfide concentrate with that of the untreated concentrate in column leaching. Pre-treatment of the concentrate resulted in increasing the oxidation of the concentrate more than twice after 28 days. The lag phase of bacterial growth has also been considerably decreased. This improvement of biooxidation produced an increase of about 7 per cent in recovery of gold by cyanidation.

![Figure 1. Column bioleaching operation of pyrite coated particles at various flow rates](image1)

![Figure 2. The effect of biologically pretreatment of concentrate on the efficiency of column bioleaching](image2)

REFERENCES

Isolation and evaluation of indigenous iron- and sulphur-oxidising bacteria for heavy metal removal from sewage sludge

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Abstract

The bioleaching of copper and cadmium from sewage sludge contaminated with heavy metals (obtained from a wastewater treatment plant in a large agglomeration) was optimised using indigenous bacteria. The enrichment and adaptation of naturally existing microflora was achieved after sludge supplementation with elemental sulphur (ferrous sulphate was added as a flocculent to crude wastewater), intensive aeration and four successive transfers. The application of enriched and adapted bacteria resulted in sludge acidification to pH 1.9 after 10 days of cultivation without the use of inorganic acid. The maximal extraction of copper and cadmium achieved in a laboratory-scale experiment was 70% and 100%, respectively.

Two groups of sulphur and iron-oxidising bacteria were isolated from the sewage sludge using Starkey and 9K media. The morphology and physiology of the isolated bacteria grown in mineral medium as well as in sterilised sewage sludge were investigated.

Examination of the isolates by scanning electron microscopy indicated the presence of rod–shaped bacteria and revealed a characteristic leaching pattern on sections of copper sulphide ore. The morphological and ultrastructural differences between cells grown in mineral medium and in the sewage sludge were clearly visible.

Keywords: sewage sludge, iron- and sulphur-oxidising bacteria, TEM, SEM

1. INTRODUCTION

The growth of many species of chemolithotrophic bacteria is inhibited by some organic compounds. According to Tutle and Dugan [1] the acidophilic leaching bacteria Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans are the most strongly affected by the presence of organic substances in a mineral medium. The iron- and sulphur-oxidising bacteria, however, are very often isolated from environments containing organic matter. According to Zagury et. al. [2] iron-oxidising bacteria are present in contaminated soil and can be easily enriched and adapted by supplementing the soil with ferrous sulphate. Additionally, the high pH of soil, high organic content, oil and grease does not inhibit the growth of those microorganisms [2]. Iron- and sulphur-oxidising bacteria identified as A. ferrooxidans and A. thiooxidans, were also successfully enriched by Gomez and Bosecker [3] from various soil samples contaminated with heavy metals.
and organic matter. The adapted bacteria were used for heavy metal removal from soil and sediments.

Similarly, iron-oxidising bacteria were detected by Tyagi et al. [4] in different types of sewage sludge and can be also adapted by supplementing the sludge with ferrous sulphate. Blaise et al. [5] characterised the naturally occurring microorganisms responsible for metal leaching activity in different sewage sludges. According to them the initial acidification of the sludge in the bioleaching process is brought about by the growth of indigenous less-acidophilic thiobacilli, followed by the growth of acidophilic thiobacilli, resulting in pH reduction to approximately 2.0. The successive growth of less-acidophilic and acidophilic bacteria was observed in different types of sludge and with varying sludge solid concentration.

Taking into account the chemical composition, the existence of chemolithotrophs in sewage sludge seems to be a very interesting phenomenon. The sewage sludge as an end product generated in a wastewater treatment plant besides heavy metals, accumulates many chemical substances that are not fully degraded during this treatment. The spectrum of xenobiotic organic compounds in sewage sludge is extremely wide and constantly changing. Typical representatives of those substances detected in sewage sludge are polycyclic aromatic hydrocarbons, polychlorinated biphenyls and chlorinated pesticides [6].

The influence of organic matter on speciation of heavy metals in sewage sludge is well known. However, the effect of these substances on microorganisms used in the bioleaching process is still unclear.

The scope of our paper was the evaluation of indigenous bacteria for copper and cadmium bioleaching from sewage sludge. The possibility of enrichment and adaptation of naturally existing microflora was estimated and the growth, protein profile, morphology and ultrastructure of isolated bacterial cells grown in mineral medium and in sewage sludge were compared.

2. MATERIALS AND METHODS

2.1 Samples

Anaerobically digested and dehydrated sewage sludge was obtained from a wastewater treatment plant in Warsaw.

2.2 Enrichment and adaptation

The process was carried out in 5.0-litre flask containing 500g of unsterilised, dehydrated, anaerobically digested sludge suspended in 2000 ml of mineral medium (9K medium without energy source). The sludge was supplemented with 10 g of elemental sulphur per litre. The cultures were aerated with pressurised air and were maintained at room temperature. 200 ml of culture was transferred to fresh sewage sludge after 10 days and cultivated under the same conditions. The experiment was repeated successively four times (series I, II, III, IV).

2.3 Evaluation for heavy metal bioleaching

The pH of sludge in series I, II, III and IV was estimated every day. The cadmium and copper concentration in sewage sludge was determined in the beginning and at the end of every series. The metal was analysed with flame atomic absorption spectrometry according to FAAS protocol after acid digestion.
2.4 Isolation
The sludge obtained in series IV was used for the isolation of two groups of iron- and sulphur-oxidising bacteria. Media 9K [7] and Starkey [8] were used. 125 ml of sterile medium was inoculated with 25 ml of sewage sludge. The culture was cultivated on a rotary shaker at room temperature.

2.5 Growth characterisation
The isolated iron- and sulphur-oxidising bacteria were cultivated in 9K mineral medium and in Starkey medium, respectively. Additionally, after six months of cultivation in mineral medium, the growth of isolates was tested in sterilised sewage sludge supplemented with 9K or Starkey medium without an energy source. The sludge was preacidified to pH of 2.5 (9K) or 4.0 (Starkey). The cultures were performed in 500 ml flask containing 50 mg of sludge and 200 ml of mineral medium. The cultures were incubated on rotary shaker at room temperature. The number of bacterial cells in a population was assessed by counting the cells in a sample using a counting chamber under a microscope.

2.6 Preparation of samples for SEM and TEM
Thin polished sections of naturally occurring copper sulphide ore were introduced into actively growing bacterial cultures according to the method described by Ostrowski and Sklodowska [9]. Samples were incubated at room temperature without stirring for two months. After the bioleaching process thin sections were carefully rinsed with water, allowed to dry at room temperature and fixed with 3% glutaraldehyde. They were dehydrated in the increasing concentration of ethyl alcohol and propylene oxide. Samples were coated with gold and examined under a scanning electron microscope.

For TEM cells were fixed with 3% glutaraldehyde in sodium cacodylate buffer and then treated with osmium tetroxide for 4h. An increasing concentration of the ethanol to 100% was used for dehydration. Ultrathin sections of epon-embedded cells were treated with uranium acetate and lead citrate.

2.7 Extraction of protein fraction
The method of Neu and Heppel [10] was used for isolation of periplasmic proteins. Cytoplasmic and membrane proteins were isolated according to method of Witholt [11]. Proteins were isolated from bacterial cells cultivated in mineral medium and sewage sludge according to the description in section 2.5.

2.8 Protein analysis
SDS-PAGE electrophoresis was performed on 8-25% polyacrylamide gradient gel using Phast System equipment (Amersham Pharmacia Biotech). Gels were stained with silver and analysed with ImageMaster 1D Elite (NonLinear Dynamics). The molecular mass of proteins was determined using DP-Soft (analySIS) software (Soft Imaging Systems for Olympus) according to a high (not presented) and low molecular weight calibration kit (Amersham Pharmacia Biotech).

3. RESULTS AND DISCUSSION
A mixture of indigenous bacteria, containing autotrophic as well as heterotrophic acidophili, was used for bioleaching of copper and cadmium from anaerobically digested and dehydrated sludge. The enrichment and adaptation of natural microflora was achieved
after the sludge supplementation with elemental sulphur as the energy source and after four successive subcultures (series I, II, III, IV). The sludge acidification in the course of bioleaching is presented in Fig. 1. The enrichment and adaptation of bacteria contributed to gradual sludge acidification. Finally, a decrease in pH to about 1.9 after 10 days of cultivation was observed in series IV.

Figure 1. The sludge acidification in the course of bioleaching process

The efficiency of copper and cadmium bioleaching in the successive series is presented in Table 1. The concentration of removed copper and cadmium was the lowest in series I and gradually increased in the following subcultures. The maximal extraction of cadmium was 100% and was obtained in series III and IV. Copper bioleaching was the highest in series III (70%).

Table 1. Cadmium and copper bioleaching efficiency (%)

<table>
<thead>
<tr>
<th>Metal</th>
<th>Series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Cadmium</td>
<td>36</td>
</tr>
<tr>
<td>Copper</td>
<td>8</td>
</tr>
</tbody>
</table>

In the next part of the experiment a mixture of enriched and adapted indigenous bacteria (obtained in series IV) was used for isolation of autotrophic bacteria. The two groups of microorganisms were separated depending on the energy source. The iron-oxidising bacteria were isolated in 9K medium with ferrous sulphate as the energy source. The Starkey medium with elemental sulphur was used for the isolation of sulphur-oxidising bacteria. The existence of iron-oxidising bacteria in sewage sludge was explained by a high concentration of ferrous sulphate added as a flocculent to crude wastewater. As indicated later the isolated iron-oxidising bacteria were also able to oxidize sulphur.

The growth of isolates was screened by acid production and cell number. Growth curves of bacteria (Fig. 2A, B) and pH decrease (data not presented) confirmed that the isolates were capable of lithotrophical growth with ferrous iron or sulphur as the energy source under laboratory conditions. In the culture of iron-oxidising bacteria in 9K medium the pH decreased to about 1.9 during 6 days.

Liquid medium with ferrous sulphate changed from green to red-brown with ferric sulphate. At pH close to 2.0 precipitation and encrustation of jarosites was observed.
In Starkey medium the elemental sulphur was oxidised by isolated strains resulting in pH decrease to 1.25 after 14 days of cultivation. This process was also confirmed by the appearance of colloidal sulphur.

The ability of the isolated bacteria to re-growth (after six months of cultivation in mineral medium) in sterilised sewage sludge without any additional energy sources was also proved (Fig. 2A, B).

![Graph A](image1.png)

![Graph B](image2.png)

**Figure 2. Growth of indigenous iron- (A) and sulphur-oxidising (B) bacteria in mineral medium and sewage sludge**

The comparison of growth curves of two groups of bacteria grown in mineral medium or in sewage sludge reveals differences, especially in the beginning of cultivation. A lag phase of the culture of iron- as well as sulphur-oxidising bacteria grown in the sewage sludge was observed which lasted about 4-5 days. Exponential growth of bacteria cultivated in the mineral medium begins immediately after inoculation. In all cases an exponentially growing culture was inoculated into the same medium under the same growth conditions. As seen on electron micrographs of ultrathin sections of bacteria grown in sewage sludge (Fig. 3) many cells are completely destroyed, membranes and leaky spheroplasts are also observed. The lag phase could be also connected with the inhibitory effect of chemical substances present in the sewage sludge.

According to Tutle and Dugan [1] the chemolithotrophic growth of some strains of *A. ferrooxidans* is inhibited by a wide variety of organic substances. The growth and particularly iron or sulphur oxidation is inhibited by yeast extracts, peptones, amino acids, carbohydrates, carboxylic acids, anionic detergents, cationic surfactants and ammonium compounds. Some of these compounds (carboxylic acids) were completely inhibitory at
the concentration used, others (malate, succinate, fumarate) caused an extended lag phase [1, 12].

Finally, after 10 days the number of bacterial cells in the sewage sludge was the same (iron-oxidising bacteria) or even higher (sulphur-oxidising bacteria) than in the mineral medium.

The chemical analysis of sewage sludge used with GC-MS showed the presence of such components as organic acids esters (benzodicarboxylic, hexanoic, propenoic), alcohols, benzene and derivatives, polycyclic aromatic hydrocarbons (phenanthrene, anthracene, naphthalene), phenols, aliphatic saturated and unsaturated hydrocarbons and terpenes (data unpublished). The concentration of Cd, Cu and Pb in the sewage sludge was 12, 476 and 117 mg/kg d.w., respectively.

Microscopic observation of isolated bacteria revealed the presence of single, rod-shaped, Gram-negative bacteria. No evidence for *Leptospirillum ferrooxidans* in the culture of iron-oxidising bacteria was showed on microscopic figures. Preliminary identification of isolates with FISH method using probe Thio820 specific for *A. ferrooxidans* and *A. thiooxidans* [13] revealed that isolated bacteria belong to the genus *Acidithiobacillus* (unpublished results).

The bacterial cells immobilised on the surface of the ore section introduced into growing bacterial culture were observed under a scanning electron microscope.

Indirectly, the ability of isolates to oxidize copper sulphide in ores was shown. Deep corrosion sponge-like pits after two months of the bioleaching process were visible (Fig. 4 A, B, C). The destruction of the ore was more visible in the case of the culture in the mineral medium than in the sewage sludge. The bacterial cells attached to the surface of the section and in the pits were visible at large magnifications (Fig. 5A, B, C). The surface of ore and bacterial cells was covered by a mucosal biofilm (Fig. 5B). However more colloidal and crystal-like particles were visible in sections from the mineral medium (Fig. 5A, C).
Figure 4 A, B, C. Corrosion of ore after two months of bioleaching with sulphur-oxidising bacteria in Starkey medium (A) and sewage sludge (B, C).

Figure 5 A, B, C. Iron-oxidising bacteria attached to surface of ore during bioleaching in 9K medium (A, C) and sewage sludge (B).
Figure 6. Transmission electron micrographs of ultrathin sections of iron-oxidising bacteria cultivated in 9K medium (A, B, C) and in sewage sludge (D, E, F). Bar, 0.5 µm.
Cross and longitudinal ultrathin sections of isolated bacteria were examined using a transmission electron microscope. The morphological and ultrastructural differences between cells grown in mineral medium and in the sewage sludge were clearly visible. Cells of iron- and sulphur-oxidising bacteria grown in mineral media showed typical shape and ultrastructure. Autotrophically-grown cells possessed a multilayered cell envelope with a cytoplasm containing a dispersed nucleus, ribosomes and the inclusion of polygonal profiles (carboxysomes) (Fig. 6A, B, C, 7A, B). In the thin section the carboxysomes have a granular structure of medium electron density and there are several (3-5) bodies per cell, which is shown in Fig. 6B and 7B.

Figure 7. Transmission electron micrographs of ultrathin sections of sulphur-oxidising bacteria cultivated in Starkey medium (A, B) and in sewage sludge (C, D). Bar, 0.5 µm

Sewage sludge grown cells were embedded with slime, slightly deformed and contained reserve material (Fig. 6D, E, F, 7C, D). It is likely that the electron transparent areas seen in the thin section are deposits of poly-β-hydroxybutyrate (PHB) (Fig. 6E, F). It is known that the polymer accumulates when carbon and energy sources are accessible and according to Shively [14] PHB is considered to be a cellular reserve of energy or carbon and energy. Tabita and Lungren [15] indicated that the growth of *A. ferrooxidans* in mineral medium with glucose resulted in poly-β-hydroxybutyrate accumulation. The section of iron- as well as sulphur-oxidising bacterial cells grown in sewage sludge also showed more peripherally localised electron dense bodies than cells grown in mineral
Bioleaching Applications

medium (Fig. 6E, 7C]. Regular hexagons, with a solid, granular interior may be regarded as polyphosphate granules. Additionally, in either case, the nucleoplasm of cells cultivated in sewage sludge had a characteristic consistence. It seemed to be very dense and tightly arranged. Carboxysomes did not occur.

As mentioned earlier cell envelope disruption and partial leakage of cellular material in samples of bacteria cultivated in sewage sludge were visible (Fig. 3). The comparison of the size of undistorted cells grown under two conditions revealed that the cells grown under autotrophic conditions (9K or Starkey) were slightly smaller.

The deformation of cells of *A. ferrooxidans* after treatment with organic acids was reported earlier. Electron micrographs showed blebbing and waviness of the cell envelope and void spaces inside the cell. According to Tutle et al. [16] the cell envelope is the site of the toxic action of organic acids. It was postulated that organic acids disrupt the cell envelope by dissolving in it and by complexing with cations that maintain its integrity [16].

Organic compounds in the growth medium had also a detectable effect on the ultrastructure of another chemolithotrophic bacterium, *Nitrobacter agilis* [17]. Most cells grown under heterotrophic conditions were irregular in shape, distorted and filled with PHB. Pope et al. [17] reported that the cells were larger than cells grown under strictly autotrophic conditions.

The comparative analysis of proteins extracted from iron- and sulphur-oxidising bacteria grown in mineral medium and in sewage sludge was performed.

Preliminary protein profile characterisation showed visible differences. Fractions of iron-, as well as sulphur-oxidising bacteria, grown in sewage sludge were characterised by small number of protein bands. This phenomenon was observed in all analysed fractions. Additionally, in the case of cells grown in the sewage sludge difficulties in isolating and separating the proteins on the gel appeared. Unseparated bands were obtained in a few cases although the same methods of isolation and separation were used.

Analysis of proteins synthesised by bacteria grown in sewage sludge revealed that only a few of them remained unchanged in comparison with proteins synthesised by bacteria cultivated in mineral medium. Generally, many proteins were completely absent or expressed in different amount. Only few new proteins appeared in sewage sludge-cultivated bacteria.

Iron-oxidising bacteria grown in sewage sludge (Fig. 8A) synthesised two proteins present only in the membrane fraction (36, 32.2 kDa) and three (15.2, 29.9, 68.3 kDa) in the cytoplasmic fraction. Two other proteins (56.6, 69.2 kDa) isolated from the periplasmic fraction were expressed at a higher concentration than in bacteria cultivated in mineral medium.

In the case of sulphur-oxidising bacteria (Fig. 8B) grown in sewage sludge specific results were obtained. Only one new protein of molecular weight of approximately 81 kDa was identified in the periplasmic fraction and one (19.4 kDa) in the cytoplasmic fraction. There was also only one unseparated protein band in the membrane fraction detected.

In the literature, many examples of adaptation of Gram-negative microorganisms to different environmental conditions can be found. For example, *A. ferrooxidans* responds and adapts to changes in its external mining environment by the synthesis of an outer membrane protein [18, 19]. The 40-kDa protein (omp40) was found, whose synthesis is regulated by external pH and concentration of phosphorus in medium.
Figure 8. Patterns of protein synthesis by iron- (A) and sulphur-oxidising bacteria (B) cultivated in mineral medium (9K or Starkey, St) and in sewage sludge (SS). Periplasmic (PP), membrane (MP) and cytoplasmic (CP) protein were analysed using SDS-PAGE. LMW – low molecular weight protein standards.

Taira et. al. [20] suggested that the enhanced resistance to cadmium in *A. ferrooxidans* is connected with the synthesis of some protein. The presence of cadmium in the growth medium also indicated several changes in the cytoplasmic and membrane fraction protein profile. The small amount of proteins bands isolated from cells cultivated in sewage sludge seems to be connected with the fact that many cells of indigenous bacteria were destroyed. Proteins, especially, of the cytoplasmic and periplasmic compartments could be released to supernatant. Earlier, Tabita and Lungren [15] reported that treatment of cell suspension of *A. ferrooxidans* with organic acids, such as oxaloacetic or hexanoic, led to cell envelope disruption and the release of various cell components, including proteins, DNA, RNA and reducing sugars.

4. CONCLUSIONS

The cultivation of chemolithotrophic bacteria under nutrient conditions prevailing in sewage sludge results in changes in physiology (growth), morphology, ultrastructure and protein synthesis of iron- and sulphur-oxidising bacteria. The organic compounds may directly inhibit the iron and sulphur oxidation resulting in slower decrease of the pH of the sludge and slower growth rate. The disruption of cells cultivated in the sewage sludge was observed, but many bacterial cells were able to survive in the presence of organic contaminants and heavy metals. The morphological and ultrastructural changes observed under the electron microscope were evidently connected with adaptation to those conditions. Moreover, the application of the mixture of naturally existing acidophilic
microflora, containing among other the described strains, in the biotechnology of heavy metal removal from sewage sludge is possible and very effective.

ACKNOWLEDGEMENTS

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REFERENCES

Kinetics of ferrous iron oxidation with *Sulfolobus metallicus* at 70°C

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Abstract

The kinetics of ferrous ion oxidation under the catalytic action of *Sulfolobus metallicus* at pH = 1.5 and 70°C was characterized. Measurements were conducted using a two-chamber electrochemical cell, which enabled to obtain accurate measurements of the oxidative activity of *Sulfolobus metallicus* on ferrous iron at controlled solution redox potential, preventing the influence of ferric iron precipitates formation.

The kinetic oxidation of ferrous iron with *Sulfolobus metallicus* was well described with a Monod expression with a ferric inhibition term (product competitive inhibition) given by the equation:

\[ V_{Fe^{2+}} = \frac{V_{\text{Max}} \times [Fe^{2+}]}{[Fe^{2+}] + K_s (1 + K_i * [Fe^{3+}])} \]

with \( V_{\text{Max}} = 1.8 \times 10^{-6} \, \text{[µg Fe/(hr cell)]}, \) \( K_s = 203.6 \, \text{[mg/l]} \) and \( K_i = 3.83 \, [-] \). The maximum specific oxidation rate \( (V_{\text{Max}}) \) for this archaea is about 50% the value of those previously reported for mesophilic and moderate thermophilic microorganisms. On the other hand, the ferrous iron affinity constant \( (K_s) \) and the inhibition constant \( (K_i) \) are about 6 times larger than the respective values reported for those microorganisms. According to the present kinetic characterization the catalytic influence of *Sulfolobus metallicus* on ferrous iron oxidation can be optimized when operating with a solution with high ferrous iron concentration while keeping simultaneously a low ferric iron concentration.

*Keywords*: bacterial activity; biolaching; ferrous iron oxidation; indirect action; *Sulfolobus metallicus*

1. **INTRODUCTION**

Use of thermophile microorganism in the biolaching of mineral sulphides is particularly attractive for treating sulphides such as chalcopyrite and molybdenite, which are quite refractory to dissolution in the temperature range where mesophiles operate (10-40°C). The mechanism of catalysis in the biolaching of sulfide minerals with thermophiles is partially等各种催化的1基础上的间接机制，该机制基于在硫化物矿物氧化中催化影响的微生物在铁氧化的反应：
\[ Fe^{2+} + H^+ + \frac{1}{4} O_2 \xrightarrow{\text{microorganism}} Fe^{3+} + \frac{1}{2} H_2O \]  

The ferric ion produced then chemically attacks the sulfide according to the general reaction:

\[ 2Fe^{3+} + MS \rightarrow M^{2+} + S^0 + 2Fe^{2+} \]

where M correspond to a divalent metal cation. Thermophiles can effectively catalyze the oxidation of ferrous ion (reaction 1) up to temperatures between 60 – 90 °C which enables to bioleach refractory sulfides in a temperature range where its dissolution with ferric ion (reaction 2) is much faster than at room temperature.

The high temperature biooxidation of ferrous ion with thermophiles has been studied by several authors. Nemati and Harrison [1] determined the ferrous ion oxidation activity of *Acidianus brierleyi* and concluded that was significantly smaller than that of mesophiles like *Acidithiobacillus ferrooxidans*. Marsh et al. [2], Norris and Barr [3] and Mier et al. [4] reported studies on ferrous ion oxidation by *Sulfolobus metallicus*, an acidophilic chemolithothrophic archaea that can grow autotrophically at temperatures between 65 and 80°C. All this studies, however, report qualitative data which precludes the activity of this microorganism to be compared with other microorganisms or to model its behaviour in bioreactors.

In the present work the kinetics parameters of ferrous ion oxidation under the catalytic action of *Sulfolobus metallicus* were determined pH = 1.5 and 70°C. Measurements were conducted using a two-chamber electrochemical cell which enabled to obtain accurate measurements of the oxidative activity of *Sulfolobus metallicus* on ferrous iron at controlled solution redox potential, preventing the influence of ferric iron precipitates formation.

### 2. MATERIALS AND METHODS

#### 2.1 Archaea culture

*Sulfolobus metallicus* was cultured in 250 ml Erlenmeyer flasks containing basal medium with the following composition: (NH₄)₂SO₄ (0.4 g/l), MgSO₄·7H₂O (0.5 g/l), KH₂PO₄ (0.2 g/l). The culture was supplemented with a source of reduced sulfur, K₂S₂O₆ (1.5 mmol), necessary for this microorganism to maintain an efficient ferrous iron oxidation activity [2, 3, 4]. The pH of the medium was adjusted to 1.8 with sulfuric acid. The culture was maintained at 70°C in a rotary shaker, with periodically subculturing.

The inoculum for the bioelectrochemical cell was prepared from 80 ml of culture sample that was filtered through a 0.22µm Millipore® membrane. Cells were washed three times with 20 ml of pH 1.5 sulfuric acid solution to remove iron and then resuspended in 20 ml of iron-free basal medium. The cell population in this inoculum, determined by direct counting using a Petroff-Hausser chamber, was typically in the range: 2-6 x 10⁸ cells per ml.

#### 2.2 Electrochemical cell

The scheme of the experimental set up is shown in Figure 1. The electrochemical cell, made of Pyrex®, has two compartments separated by a 1 cm² cation exchange membrane (Nafion® 90209). The working electrode is a platinum wire (diameter 0.5 mm, length 2 m), with a 16 cm² total area. This electrode together with the reference electrode (Radiometer Analytical Model REF601 Hg/HgSO₄ electrode) were placed in the cathodic
compartment with 30 ml of basal medium containing the microorganisms to be characterized. In this compartment there was also a Cole-Palmer 5990-57 combination platinum electrode for Eh determination. The counter electrode, a platinum foil of 3 cm² total area, was in the anodic compartment.

The catholyte was stirred with a magnetic impeller and sparged with air. The electrochemical cell was placed in a thermostatic water bath maintained at 70.0 ± 0.1 ºC. The working, reference and counter electrodes were connected to a Model 363 Electrochemical Interface from EG&G (Princeton Applied Research). A 7150 Solartron Schlumberger digital voltmeter was used for current measurement.

2.3 Procedure

The cathodic chamber was filled with 30 ml of basal medium which contained an initial concentration of ferrous iron varying in the range 50-200 mg/l and an initial archaea population varying in the range 2-20 x 10⁷ cells / ml. The microorganisms were added as aliquots of the inoculum described above. A constant potential was then applied to the working electrode with values ranging between 0.2 and 0.6 V (SHE), which induced a cathodic current related to the reduction of ferric iron. The potential was maintained until a final steady-state cathodic current was reached which indicated that the rate of ferrous generation at the cathode equaled the rate of biological ferrous iron oxidation in the solution.

![Experimental Apparatus Diagram](image)

**Figure 1. Schematic diagram of the experimental apparatus**

Successive amounts of ferrous iron were added into the cathodic chamber of the cell, each one inducing a further increase in the steady-state cathodic current related to the increase in the rate of biological ferrous iron oxidation. Each time that a steady – state current was established, the value of this current and the Eh were recorded and the solution was sampled to determine the microorganisms and total iron concentrations. Total iron was determined by the o-phenantroline method [5]. Ferrous iron was determined from total iron concentration and Eh determinations, using a Nernst type equation obtained experimentally [6 - 9]. The Nernst equation specifically determined, at 70ºC in the same basal medium for a total iron range 0.05 – 1 g/l and the [Fe³⁺]/[Fe²⁺] ratio range 0.01-100, was:
\[ Eh = 0.689 + 0.0675 \times \log \left( \frac{[Fe^{3+}]}{[Fe^{2+}]} \right) \]  
(3)

where \( Eh \) is the solution potential in Volts versus the Standard Hydrogen Electrode (SHE). From each steady-state cathodic current the specific rate of bacterial ferrous iron oxidation, \( V_{Fe^{2+}} \), was determined from the expression:

\[ V_{Fe^{2+}} = \frac{I}{z \times F \times N} \]  
(4)

where \( V_{Fe^{2+}} \) is expressed in (mol Fe\(^{2+}\) / (s x cell)), \( I \) is the steady-state cathodic current (A), \( z \) is the number of electrons in the ferric reduction (\( z=1 \)), \( F \) is the Faraday constant (96496 C/eq) and \( N \) is the number of bacteria in the cathodic compartment.

3. RESULTS AND DISCUSSION

Figure 2 shows the specific rate of ferrous iron oxidation by *Sulfolobus metallicus* as a function of ferrous iron concentration determined at different applied potentials (\( Va \) vs SHE). Plotted values correspond to the average of two duplicate experiments. Ferrous iron concentration in this figure is the one established in the catholyte when the cathodic current reaches a steady value at each applied potential.

It can be observed that rate of ferrous iron oxidation increases with the concentration of ferrous iron, but for a given ferrous iron concentration the rate of oxidation decreases when the applied potential becomes less cathodic. The decrease of \( Va \) was paralleled by an increase in the solution \( Eh \), which evidenced an increase of the ferric/ferrous ratio established in solution. Therefore, the decrease in bacterial oxidative activity obtained when \( Va \) was less cathodic can be associated to the inhibiting influence of ferric iron, which increases its concentration when \( Va \) decreases. The inhibiting influence of ferric iron can be more clearly visualized in Figure 3 which show now measured ferrous iron oxidation rates as a function of the ferric/ferrous ratio established in solution at each applied potential \( Va \).

![Figure 2. Effect of the ferrous iron concentration and the applied potential at the cathode on the biooxidation rate. \( Va \) represents the working electrode potential](image-url)
Figure 3. Effect of solution Eh on the ferrous iron biooxidation rate

Considering the previous experimental results the kinetics of ferrous iron oxidation with *Sulfolobus metallicus* was described in terms of a Monod equation with ferrous iron as limiting substrate with a ferric inhibition term (product competitive inhibition), of the following form:

\[
V_{\text{Fe}^{2+}} = \frac{V_{\text{Max}} \times [\text{Fe}^{2+}]}{[\text{Fe}^{2+}] + K_s \times (1 + K_i \times [\text{Fe}^{3+}])}
\]  

(5)

This equation has been found to describe well the kinetics of ferrous iron oxidation with mesophilic iron oxidizers such as *Acidithiobacillus ferrooxidans* [6, 10, 11, 12]. Equation (5) was applied to experimental data shown in Figures 2 and 3 using a nonlinear least square regression and a good correlation was found (92%) with the values of parameters shown in Table 1. Kinetics parameters for equation 5 previously reported for the oxidation of ferrous iron with *Acidithiobacillus ferrooxidans* [7] are also included in Table 1, for comparing purposes.

Values in Table 1 show that the maximum specific oxidation rate \(V_{\text{Max}}\) for *Sulfolobus metallicus*, the most determinat kinetic parameter, is only about about a half the value of that reported for mesophilic microorganisms. On the other hand, the ferrous iron affinity constant \(K_s\) of *S. metallicus* is about 6 times smaller than the respective value for *A. ferrooxidans* which indicates that ferrous iron oxidation with this thermophile will be much more effective at high ferrous iron concentration. Finally, the inhibition constant \(K_i\) of *S. metallicus* is about 6 times larger than the respective value of *A. ferrooxidans* which indicates a strong inhibiting influence of ferric iron on the activity of this thermophile.

The influence of the parameters shown in Table 1 on the kinetics of ferrous iron oxidation with *S. metallicus* and *A. ferrooxidans* can be visualized in Figure 4. Curves in that figure show ferrous iron oxidation rate as a function of ferrous iron and ferric iron concentration for both types of microorganisms, calculated from equation 5 with parameters in Table 1, at 2 different ferric iron concentrations. According to this figure the oxidative activity of *S. metallicus* can be comparable to that of *A. ferrooxidans* if the ferrous iron concentrations is, for a similar ferric iron concentration, six times higher than the one used with the mesophile. Similarly, the oxidative activity of *S. metallicus* can be comparable to that of *A. ferrooxidans* in the whole range of ferrous iron concentration, if
the ferric iron concentration used by the thermophile is 1/15 of the ferric iron concentration used by the mesophile. According to the present kinetic characterization one can conclude then that the catalytic influence of *Sulfolobus metallicus* on ferrous iron oxidation can be optimized when operating with a solution with high ferrous iron concentration while keeping simultaneously a low ferric iron concentration.

**Table 1. Adjusted parameters values for Sulfolobus metallicus and reported values for Acidithiobacillus ferrooxidans**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for <em>Sulfolobus metallicus</em></th>
<th>Value for <em>Acidithiobacillus ferrooxidans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{Max}}$ [µg Fe/(hr cell)]</td>
<td>$1.836 \times 10^{-6}$</td>
<td>$2.817 \times 10^{-6}$</td>
</tr>
<tr>
<td>$K_S$ [mg/L]</td>
<td>203.55</td>
<td>73.128</td>
</tr>
<tr>
<td>$K_I$ [-]</td>
<td>3.831</td>
<td>0.641</td>
</tr>
</tbody>
</table>

![Figure 4. Ferrous iron oxidation rates with Acidithiobacillus ferrooxidans and Sulfolobus metallicus calculated according to equation (5)](image)

The strong inhibiting effect of ferric iron on the oxidative activity of *S. metallicus* is probably linked to the formation jarosite-type precipitates which is usually triggered in the presence of this ion. In fact, the formation of jarosites has been proved to play an inhibiting effect on the ferrous iron oxidative activity of *A. ferrooxidans* [13]. The inhibiting influence of these precipitates is expected to be much important in the case of thermophiles because the solubility of jarosites decreases and the kinetics of jarosites formation increases in the temperature range where these microorganisms operate (70-90°C). Bioleaching of sulfides with thermophiles usually operate with solutions containing large concentrations of ferric iron (5-17 g/l) in the presence of important concentrations of suspended jarosite-like precipitates. As in this type of reactors normally operate with a consortia of thermophilic microorganisms it very likely than the demand for ferrous iron oxidation would be relying on the oxidative activity of microorganisms different than *S. metallicus*.

**4. CONCLUSIONS**

The kinetics of ferrous ion oxidation under the catalytic action of *Sulfolobus metallicus* at pH = 1.5 and 70°C can be well described with a Monod expression with a ferric inhibition term (product competitive inhibition) given by the equation:
The values of the parameters in this equation are: \( V_{\text{max}} = 1.8 \times 10^{-6} \) [\( \mu g \text{ Fe/(hr cell)} \)], \( K_s = 203.6 \) [mg/l] and \( K_I = 3.83 \) [-]. According to this kinetic characterization, in order to optimize the catalytic influence of *Sulfolobus metallicus* on ferrous iron oxidation it is necessary to operate in solutions with high ferrous iron concentration while simultaneously minimizing the concentration of ferric iron.

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**REFERENCES**

Kinetics of sulphur oxidation: pH and temperature influence on bioleaching

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\textsuperscript{b} Metallurgie et Traitement des Minerais, Geomac-Faculty of Applied Sciences, Université de Liège-Belgium

Abstract

The oxidation of sulphur constitutes a key process in mineral biotechnology from the point of view chemical and biological. The sulphur oxidation rate was determined in one strain of \textit{Acidithiobacillus ferrooxidans} at 35°C and in one strain of \textit{Sulfolobus metallicus} at 70°C, grown in different media. Because of the eventual liberation of protons upon sulphate formation, the sulphur oxidation was monitored by following the pH variations.

1. INTRODUCTION

Bioleaching is playing an increasingly important role in the extraction of the metals from low-grade ores and refractory sulphide minerals. Two mechanisms have been proposed for bioleaching of sulphide minerals, the direct and the indirect. The direct dissolution of minerals is caused by the attack on sulphide by the enzymatic system of the microorganism situated at the mineral surface. In the indirect mechanism the primary attack on the sulphide mineral is assumed to be a ferric iron chemical leaching with the role of micro-organism, whether at the mineral surface or not, being to oxidise ferrous iron to ferric iron, maintain a high redox potential and also to oxidise the sulphur produced to sulphate. The chemical oxidation can be complete, in which case ferrous iron and sulphate are produced. The role of the bacteria is to regenerate ferric iron by oxidizing the ferrous iron. This does not require that the bacteria are closely associated with the mineral surface. The chemical oxidation reaction can also be incomplete, in which case ferrous iron and elemental sulphur are produced. The role of the bacteria is to oxidize the sulphur to sulphate and so preventing the mass transfer limitation caused by sulphur layer [1].

\textit{Acidithiobacillus ferrooxidans}, \textit{Acidithiobacillus thiooxidans} and \textit{Leptospirillum ferrooxidans} are conventionally implemented in bioleaching processes at temperatures ranging from 30 to 45°C and as such the mesophilic biooxidation of ferrous iron has been studied extensively [2,3]. In recent years, however, there has been interest in application of high temperature processes (65 to 80°C) utilising thermophilic archaea such as \textit{Sulfolobus acidocaldarius}, \textit{Sulfolobus metallicus}, \textit{Acidianus brierleyi} and \textit{Metallosphaera sedula} [4-6]. These earlier studies focused mainly on the improvement of bioleaching rate through verification of optimum particle size and pulp density mineral, as well as the bioreactor configuration.
In Table 1 the simplified stoichiometric equations of the incomplete and complete chemical oxidation and direct bacterial oxidation of sphalerite, pyrite, covellite, chalcocite and chalcopyrite are listed.

Table 1. Stoichiometric equations for the direct and indirect bacterial oxidation of different minerals [3]

<table>
<thead>
<tr>
<th>Stoichiometric Mechanism</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnS + 2 Fe^{3+} → Zn^{2+} 2 Fe^{2+} + S^0</td>
<td>Incomplete</td>
</tr>
<tr>
<td>ZnS + 8 Fe^{3+} + 4 H_2O → ZnSO_4 + 8 Fe^{2+} + 8 H^+</td>
<td>Complete</td>
</tr>
<tr>
<td>ZnS + 2 O_2 → ZnSO_4</td>
<td>Biological</td>
</tr>
<tr>
<td>FeS_2 + 2 Fe^{3+} → 3 Fe^{2+} + 2 S^0</td>
<td>Incomplete</td>
</tr>
<tr>
<td>FeS_2 + 14 Fe^{3+} + 8 H_2O → 15 Fe^{2+} + 2 SO_4^{2-} + 16 H^+</td>
<td>Complete</td>
</tr>
<tr>
<td>FeS_2 + \frac{15}{4} O_2 + \frac{1}{2} H_2O → Fe^{3+} + 2 SO_4^{2-} + H^+</td>
<td>Biological</td>
</tr>
<tr>
<td>CuS + 2 Fe^{3+} + Cu^{2+} 2 Fe^{2+} + S^0</td>
<td>Incomplete</td>
</tr>
<tr>
<td>CuS + 8 Fe^{3+} + 4 H_2O → CuSO_4 + 8 Fe^{2+} + 8 H^+</td>
<td>Complete</td>
</tr>
<tr>
<td>CuS + 2 O_2 → CuSO_4</td>
<td>Biological</td>
</tr>
<tr>
<td>Cu_2S + 2 Fe^{3+} → CuS + Cu^{2+} + 2 Fe^{2+}</td>
<td>Incomplete</td>
</tr>
<tr>
<td>Cu_2S + 10 Fe^{3+} + 4 H_2O → 2 Cu^{2+} + SO_4^{2-} + 10 Fe^{2+} + 8 H^+</td>
<td>Complete</td>
</tr>
<tr>
<td>Cu_2S + \frac{5}{2} O_2 + 2 H^+ → 2 Cu^{2+} + SO_4^{2-} + H_2O</td>
<td>Biological</td>
</tr>
<tr>
<td>CuFeS_2 + 4 Fe^{3+} → Cu^{2+} + 5 Fe^{2+} + 2 S^0</td>
<td>Incomplete</td>
</tr>
<tr>
<td>CuFeS_2 + 16 Fe^{3+} + 8 H_2O → Cu^{2+} + 17 Fe^{2+} + 2 SO_4^{2-} + 16 H^+</td>
<td>Complete</td>
</tr>
<tr>
<td>CuFeS_2 + \frac{17}{4} O_2 + H^+ → Cu^{2+} + Fe^{3+} + 2 SO_4^{2-} + \frac{1}{2} H_2O</td>
<td>Biological</td>
</tr>
</tbody>
</table>

As can see from Table 1, the ferrous iron and sulphur formation constitutes two key processes in mineral biotechnology due at their further required chemical and/or biological oxidation. Biological oxidation of ferrous iron by mesophile have been demonstrated to be 10^6 times faster than chemical and has received great attention [2,3].

As thermophilic bioleaching is applied increasing in the mining industry, there is a growing need for an understanding of the principles governing the high temperature bioleaching of the sulphide mineral. Fundamental kinetics studies indicate that the rate of chemical reactions approximately doubles with every 10°C rise in temperature. Not surprisingly, biological oxidation of elemental sulphur has received as much attention. This paper shows the comparative kinetics of the biological sulphur oxidation, by mesophilic and thermophilic biooxidation and a biotechnology strategy is recommended.

2. EXPERIMENTAL, MATERIALS AND METHODS

Both mesophilic and thermophilic bacteria have been described which can utilize inorganic compounds of Fe and S as electron donor [7,8]. At present, although thermophiles are characterized by higher oxidation rates, only mesophilic iron-and sulphur-oxidizing acidophiles are used in large-scale leaching processes for metal recovery from sulphides ores.
The chemical oxidation of sulphur can be expressed by the following reaction:

\[ S^0 + 6 Fe^{3+} + 2 H_2O \rightarrow H_2SO_4 + 6 Fe^{2+} + 6 H^+ \]  

(1)

Previous studies have showed that chemical oxidation of sulphur by ferric iron is very low compared with that mesophile biooxidation. However, at higher temperature, i.e. 70°C, chemical oxidation contribution is very important reaching up to 20% of the global oxidation.

The ultimate end product of the bacterial sulphur oxidation is the sulphate ion. Therefore, the elemental sulphur oxidation can be presented with the following net equations:

\[ S^0 + 3 O_2 + 2 H_2O \rightarrow 2 SO_4^{2-} + 4 H^+ \]  

(2)

\[ S^0 + 3 O_2 + 2 H_2O \rightarrow 2 HSO_4^{2-} + 2 H^+ \]  

(3)

Because of the eventual liberation of protons upon sulphate formation, the sulphur oxidation can be monitored by following the increase in hydrogen ion activity. Under normal culture conditions, bacterial sulphur oxidation is coupled with growth and the measurement of oxidation based on pH changes \((d\text{pH}/d\text{t})\) can therefore also be used to monitor bacterial growth. Exponential growth coupled with sulphur oxidation hence should yield a linear decrease in pH values. Measurement of pH was the method of choice in the present work because homogeneous sampling and direct quantitative measurement of growth by determination of biomass concentration by either cell counts or chemical methods have not been developed for cultures growing with flowers of sulphur.

*Acidithiobacillus ferrooxidans* from Cerro Colorado Mining, Iquique-Chile, was grown in MC medium at pH 2.6 and temperature 35°C and *Sulfolobus metallicus* obtained from the German Collection of Microorganism and Cell Cultures-Germany, was growing in Norris medium at pH 2.6 and temperature 70°C. Composition of utilized medium are described in Table 2. Shake flasks cultures consisted in 100-ml of nutrient medium in 250-ml shake flask at 180 rpm and supplemented with 1 g of elemental sulphur. Enriched experiments under shake flask cultures were incubated at 35 and 70°C in mineral salts media. Growth of the cultures with elemental sulphur was monitored by pH measurements.

**Table 2. Nutrient medium utilized in cultures**

<table>
<thead>
<tr>
<th></th>
<th>MC medium (g/l)</th>
<th>Norris medium (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂SO₄</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>0.056</td>
<td>--</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>--</td>
<td>0.2</td>
</tr>
</tbody>
</table>

3. **RESULTS AND DISCUSSION**

Figure 1 shows the measurement of variation pH during biological sulphur oxidation at 35 and 70°C. Results can be adjusted by the following equations:

\[ \text{pH} = 2.6938 - 0.0775\text{t} \] at 35°C  

(4)

\[ \text{pH} = 2.7 - 0.12\text{t} \] at 70°C  

(5)

where \(t\) is time in hours.
Extreme thermoacidophiles, mostly members of the archaea order *Sulfolobus*, represent a unique type of extremophile in that they must deal with high temperature and low pH simultaneously. In agreement with this, the pH variation rate in thermophilic culture is 1.5 times greater than mesophilic culture, as shows the results presented in Figure 1. From this, the variation of molar concentration and the hydrogen production rate can be calculated, as is shown by Figure 2 and Figure 3.

From Fig 3, it can see that at the beginning of the culture, acid generation rate is almost near to zero, due principally to the period of bacterial attachment on the sulphur surface. After this, extreme culture reachs faster acid production rate compared to mesophile as result of both, the required acidic environment by thermophilic microorganism and thermodynamical considerations.

On the other hand, a stoichiometric equation for bacterial growth on elemental sulphur can be derived from the elemental balances on C, H, O, N, S and the charge balance:

\[
\text{CO}_2 + 0.2\text{NH}_4^+ + \left(\frac{1.5-1.05Y_{sx}}{1.05Y_{sx}}\right)\text{O}_2 + \frac{1}{Y_{sx}}S + \left(\frac{0.6Y_{sx}+1}{Y_{sx}}\right)\text{H}_2\text{O} \rightarrow \text{CH}_{1.3}\text{O}_{0.5}N_{0.2} + \frac{1}{Y_{sx}}\text{SO}_4^{2-} + \left(\frac{0.2Y_{sx}+2}{Y_{sx}}\right)\text{H}^+
\]

As cells grow there is, as a general approximation, a linear relationship between the amount of biomass produced and the amount of substrate consumed (in this case elemtntal sulphur). This relationship is expressed quantitatively using the biomass yield, \( Y_{sx} \), which must be experimentally determined. From stoichiometric relationships, it’s possible to
evaluate the sulphur and oxygen consumption rates, \( r_{S^v} \) and \( r_{O_2} \) respectively, in function of the biomass production rate, \( r_x \), as:

\[
-r_{S^v} = \frac{r_x}{Y_{sx}} \quad (6)
\]

\[
-r_{O_2} = \left( \frac{1.5 - 1.05Y_{sx}}{1.5Y_{sx}} \right) r_x \quad (7)
\]

Eliminating \( Y_{sx} \) from equations (6) and (7) yields the degree of reduction balance (relationship between the oxidation rate of substrate and oxygen and carbon dioxide consumption rates):

\[
-1.5r_{S^v} = -1.05r_{O_2} - 1.05r_{CO_2} \quad (8)
\]

Due that at higher temperatures, the stress conditions (chemical and biological) are more important, and the microorganism uses a major amount of substrate to gain energy for maintenance. As strategy, this biotechnological tool can be used to evaluate the biokinetic and bioenergetic parameters of sulphur biooxidation. Further studies will be carried out, to continuously measurement of oxygen and carbon dioxide consumption rates.

4. CONCLUSIONS

The following conclusions could be made:

- It is possible to adapt the mesophile and thermophile bacterial culture using elemental sulphur as substrate.
- Production of acid is faster in thermophilic culture than mesophilic
- From measurement of pH evolution, it is possible to calculate the sulphur consumption rate using biomass yield.

REFERENCES

Leaching of iron from China clay with oxalic acid: effect of acid concentration, pH, temperature, solids concentration and shaking

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Abstract

China clay is an important mineral, which is used in the manufacture of ceramics and refractory, as also in other industries. Mined China clay contains iron oxides and silicates as impurity; if present in excess of a threshold level, the impurities affect the commercial value of the products. The currently available processes for lowering iron content in China clay to the desired level (< 0.8%) are energy and cost intensive, not sufficiently flexible, and may cause environmental pollution. An alternative approach for iron removal consists in the development of a biotechnological process which is expected to be cost-effective, less complex and eco-friendly. We reported earlier that several fungi, especially *Aspergillus niger*, and their culture filtrates could leach sufficient amount of iron from a China clay sample; oxalic acid was found to be the most active component of the culture filtrate. We now report the rates of iron leaching from another China clay sample by oxalic acid and by the culture filtrate of *A. niger* NCIM 548 that was found to be the most active strain in our earlier study. The rates increased with temperature (T) and followed biphasic kinetics. The effect of oxalic acid concentration (C), pH (H), solids concentration or pulp density (P), time, as also rate and mode of agitation on the rate of iron leaching is described. The rate of leaching with oxalic acid (Ro) can be calculated theoretically from the following relationship: Ro ~ (C)^0.76 (T)^1.76 (H)^0.80 (P)^0.20 under the specified set of conditions. Using the same concentration of oxalic acid in *A. niger* culture filtrate, the relationship of the rate differed; this may be due to the influence of other metabolites present in the culture filtrate on the rate.

Keywords: iron leaching, oxalic acid, reaction rate, activation energy, *Aspergillus niger*

1. INTRODUCTION

The importance of China clay in the manufacture of pottery, ceramics and refractory, as also in other industries is well known [1]. The common impurities of the natural mineral are iron oxides and silicates, which impart poor quality to the finished products and cause other problems, if present in excess. For the production of high quality materials, the iron content in China clay should be lower than 0.8% (w/w). Many methods,
such as froth floatation, gravity separation, acid treatment, reductive roasting and magnetic separation are used for the beneficiation of China clay [2, 3]. Based on these physico-chemical methods, several industrial processes (and related patents) have been developed [4-7]. But these operations are expensive, energy-intensive and not sufficiently flexible, and give rise to environmental pollution. They also are unable to lower the iron content to the desired level. On the other hand, suitable biotechnological methods are expected to produce low-iron clay at lower cost under environmentally safe and relatively less complex conditions [8, 9].

Iron is an essential element for growth. Though it is abundant in nature, it remains mostly in the insoluble state. Microorganisms have therefore evolved special mechanisms for extracting it from nature. One of them is the production of metabolites like organic acids and siderophores [10-12]. Compared to abiotic processes, these mechanisms help microorganisms in extracting more iron from several minerals [13]. Commercial feasibility of iron removal by bioleaching was first studied in 1980's [8, 14]. A process was developed for the removal of iron from quartz sands, kaolins and clays using the culture filtrates as leaching solution of acid producing fungi, mainly *Aspergillus niger*, at high temperature (90°C) [15]. Recently, a bacterial consortium occurring in kaolins and kaolin-containing rocks was utilized for removing iron from such materials. Microbial treatment followed by iron removal through subsequent magnetic separation resulted enrichment of kaolins and other minerals [16].

The percentage of iron in most of the China clay deposits in India is high and this cannot be lowered by conventional processes to the level required for the production of high quality materials [3, 17]. We observed that several fungi and their culture filtrates could leach iron from an iron-rich China clay sample [18]. At ambient condition, the highest iron-leaching activity was observed with the culture filtrate of an oxalic acid producing *A. niger* strain. It is well established that oxalic acid is a potential leaching agent for dissolving heavy metals from various minerals including clay and kaolin, and biohydrometallurgy groups are now considering processes for removal of heavy metals depending on this property of oxalic acid [4, 12, 19-22]. It is therefore worth while to study the effects of different environmental parameters on the rate of iron leaching from iron-rich China clay. The use of culture filtrate of an oxalic acid producing *A. niger* strain for removing iron from a China clay containing only 0.11% iron has been reported previously [23]. But the rate equations derived for a very low iron containing China clay may not be applicable for one having high content of iron, particularly because the limits of percentage of iron, within which the rate remains linear, is not known. Moreover, the rates calculated with culture filtrates containing other components besides oxalic acid, which might have influenced the iron-leaching rate, are expected to be different from those for pure oxalic acid solution. Therefore, it is necessary to evaluate the rate equations of iron dissolution from clay using oxalic acid or *A. niger* culture filtrate with respect to variables like temperature, pH, solids concentration and oxalic acid concentration. This report describes the results of such a study.

2. MATERIALS AND METHODS

2.1 Fungal strain and growth conditions

The *Aspergillus niger* NCIM 548 strain was obtained from Prof. A. K. Guha, Indian Association for the Cultivation of Science, Kolkata. The strain was grown for 7 days at 30°C on a rotary shaker (215 rpm) in a modified medium [9] of the following composition (in g/l): glucose, 105.5; NaNO₃, 1.5; KH₂PO₄, 0.5; MgSO₄ 7H₂O, 0.025; KCl, 0.025; yeast
extract, 1.6; and universal indicator solution, 2% (v/v). The pH of the medium was adjusted to 6 initially and maintained within 5.5-6.0 throughout the culture period with addition of 4M NaOH at regular intervals. The strain was maintained in Czapek-Dox medium [18].

2.2 China clay

The China clay sample (particle size ~300 mesh BSS) used was mined from a deposit near Mukdumnagar, Birbhum district, West Bengal, India. It had the following elemental composition (w/w as oxide): SiO₂, 45.72%; TiO₂, 1.52%; Al₂O₃, 35.96%; Fe₂O₃, 1.87%; CaO, 0.33%; MgO, trace; Na₂O, 0.18%; and K₂O, 0.19%; and the loss on ignition (LOI) was 13.79%. The clay was reddish white in colour suggesting that the iron was partly present as free iron oxyhydroxide.

2.3 Treatment of China clay with oxalic acid and culture filtrate

The culture filtrate was made 100 mM with respect to oxalic acid by adding the requisite amount of the acid. The clay sample was taken either in screw-capped bottles (30-ml capacity) or in Erlenmeyer flasks (100-ml capacity). The bottles containing 5 ml slurry of oxalic acid solution or A. niger culture filtrate with clay were rotated (cyclic) in a hybridization oven (Stuart, UK) at 60 rpm, and the flasks containing 25 ml of the slurry were shaken (orbital or reciprocating) at 215 rpm in an environmental incubator (Rosi 1000; Thermolyne, USA). Oxalic acid concentration (C), pH (H), solids concentration (P) and temperature (T) were varied from 10–300 mM, 0.75-4.0, 5-50% (w/v) and 40-80°C, respectively. After a specified time, either an aliquot from the containers or the whole content of a vessel was centrifuged, and the supernatant was collected for iron estimation.

2.4 Analytical methods

Iron content was measured following a modified method of May and Fish [24, 25] described in detail previously [15]. In practice, a small volume of sample solution containing less than 5 µg of iron was diluted to 1ml with 0.5 ml of 0.02 N HCl and water. This solution was incubated at 60°C for 2 h after adding 0.5 ml of freshly prepared Reagent A (0.6 N in HCl and 0.142 M in KMnO₄). The temperature was brought down to ambient, and 0.1 ml of Reagent B (5M in ammonium acetate, 2 M in ascorbic acid, 6.5 mM in ferrozine, and 13.1 mM in neocuproine) was added to the mixture. Absorbance at 562 nm was measured after 20 min but before 20 hr. Oxalate in the culture filtrate was precipitated with CaCl₂ and estimated by KMnO₄ titration.

2.5 Scanning electron microscopy

A scanning electron microscope (Model No. S440) of Leo Electron Microscopy Limited (UK) was used for this purpose.

2.6 Determination of iron-leaching rates

Plots were drawn with the values of log (rate of iron dissolution) on the y-axis versus log of the variable parameter X (viz. concentration, pH, temperature or solids concentration) on the x-axis. Rates (R) were determined from the slope (m) in each case and the relation between the leaching rate and the variable parameter was expressed as R \( \alpha X^m \).
3. RESULTS AND DISCUSSIONS

The leaching experiments were normally conducted under the following conditions: concentration of oxalic acid, 100 mM; temperature, 60°C; pH, 1.5; pulp density, 10% (w/v); and time, 4 h. The rates of iron leaching were calculated with change in oxalic acid concentration, temperature, pH, pulp density, reaction time and shaking conditions and are described in the respective sections. All the experiments and analyses were replicated. The rate of iron dissolution was observed to be very fast during the first hour. As this might be due to the dissolution of freely available iron oxyhydroxide, the rates were calculated from the data obtained after the first hour of reaction.

3.1 Effect of oxalic acid concentration on the iron dissolution rate

The experiment was carried out in 100-ml Erlenmeyer flask on an orbital shaker (16-mm throw) at 215 rpm using concentrations of oxalic acid varying from 10 to 300 mM. Iron dissolution rate (R) was slow up to 40 mM, but increased above this concentration. From the plot of log (rate of iron dissolution) versus log (concentration), the slope was calculated as 0.76 above 40 mM (Fig. 1). Therefore, the rate equation for oxalic acid, i. e. amount (%) of iron leached/minute can be written as R~ (C)\(^{0.76}\), where C is expressed in mM.

![Figure 1. Correlation between oxalic acid concentration and iron leaching rate](image)

The culture filtrate of *A. niger* was estimated to be 89 mM in oxalic acid. Since the rates for pure oxalic acid were determined at 100 mM concentration, the culture filtrate was enriched with additional oxalic acid to the same concentration (100 mM). It was noted that at the same oxalate concentration, much less iron was leached with culture filtrate than oxalic acid (5.4% vs. 24.9%). The result suggests that the culture filtrate of *A. niger* strain contains such materials (other than the medium components) that strongly inhibit the iron dissolution process under the conditions (short period leaching at temperature higher than ambient). It may be mentioned that on prolonged incubation for 15 days at ambient temperature (37°C), more iron was leached by the culture filtrate compared to oxalic acid [18].

3.2 Temperature effect on the iron dissolution rate

The experiments were conducted in screw-capped bottles in a hybridisation oven (cyclic rotation) at 40 to 80°C. After the first hour, iron dissolution occurred at a constant rate, which increased with temperature (Fig 2). During short-period leaching, little iron dissolution occurred with culture filtrate even at 50°C.

The rates of iron dissolution for different temperatures were calculated after the first hour of leaching. From the plot of log (rate of iron dissolution) versus log of temperature
(in absolute scale as T), the slope was derived as 1.76 for oxalic acid and 3.72 for culture filtrate (Fig. 3A). Therefore, the rate equations can be written as \( R_o \sim (T)^{1.76} \) and \( R_{cf} \sim (T)^{3.72} \) for oxalic acid and culture filtrate, respectively. When log values of the reaction rates at different temperatures were drawn against \( 1/T \), the slope was derived as \(-1.76 \) and \(-3.72 \) for oxalic acid and culture filtrate, respectively. With the help of Arrhenius rate equation, the activation energy of iron dissolution was calculated as 8.1 and 17.2 kcal / T / mol for oxalic acid and culture filtrate, respectively (Fig. 3B) suggesting a faster reaction rate for oxalic acid than the culture filtrate. The result further indicated that the rate of oxalic acid reaction with iron was inhibited by one or more compounds present in the culture filtrate; these may be either medium components or products of the fungus. In a previous report [23], where culture filtrate of another \( A. \text{niger} \) strain and a low-iron China clay sample were used, the rate equation was derived as \( R \sim (T)^{1.25} \) and the activation energy was calculated as 2.31 kcal / T / mol. Low activation energy in this case might be due to the low iron content of the mineral.

Figure 2. Leaching of iron from China clay at (■) 80°C, (×) 70°C, (○) 60°C, () 50°C and (▲) 40°C by 100 mM oxalic acid (A) and \( A. \text{niger} \) culture filtrate (B)

Figure 3. Correlation between iron leaching rate and temperature (°C) [A], and evaluation of activation energy [B] by 100 mM oxalic acid ( ) and \( A. \text{niger} \) culture filtrate (▲)

3.3 Effect of pH on the iron dissolution rate

Initial pH of the oxalic acid solution was allowed to vary from 0.75 to 4.0 in this experiment that was carried out in Erlenmeyer flasks placed in a reciprocal shaker (25.5-mm throw). It was observed that above pH 2.0, the amount of dissolved iron in the leached solution decreased rapidly; this was probably due to precipitation of iron at pH >2 (Fig. 4A). The highest rate of iron leaching was noted at pH 1.75, which is higher than the pK₁ of oxalic acid. From the plot of log (rate of iron dissolution) versus log (pH), the slope was calculated to be 0.8 (Fig. 4B). Therefore, the rate equation can be written as \( R \sim (H)^{0.8} \).
being the initial pH of oxalic acid solution. With culture filtrate as the leaching solution, iron leaching was highest at pH 1.25 and dropped above this pH. An almost similar observation was reported previously with culture filtrate where iron dissolution was highest at the lowest pH (0.5) tested; the rate equation was presented as $R \sim (H)^{0.4}$ [23].

![Figure 4](image4.png)

**Figure 4.** Effect of pH on the iron leaching rate by oxalic acid () and *A. niger* culture filtrate (▲) [A], and correlation of iron dissolution rate with initial pH of the oxalic acid solution [B]

### 3.4 Effect of solids concentration on the iron dissolution rate

This experiment was conducted by varying the pulp density from 5 to 50% (w/v), and was performed in the hybridisation oven. The rate of iron dissolution was observed to increase with increase in pulp density, reaching a maximum at 15%, and then declining slowly (Fig. 5). With culture filtrate, the rate of iron dissolution also increased up to 15% (w/v) solids concentration. At higher concentrations (>15%, w/v), the suspension probably becomes sufficiently thick, restricting free mixing of clay particles with the leaching solution, and thus decreasing the rate. From the plot of log (rate of iron dissolution) versus log (solids concentration) (Fig. 6), the slope was calculated as 0.2 for oxalic acid and 0.9 for culture filtrate; therefore, the rate equations are $R_o \sim (P)^{0.2}$ and $R_{cf} \sim (P)^{0.9}$, respectively. It was derived as $R_{cf} \sim (P)^{0.27}$ in a previous report [23].

![Figure 5](image5.png)

**Figure 5.** Effect of solids concentration variation on the rate of iron leaching with oxalic acid

![Figure 6](image6.png)

**Figure 6.** Relationship on rate of iron removal and solids concentration by 100 mM of oxalic acid solution (◊) and *A. niger* culture filtrate (▲)
3.5 Effect of shaking condition on the iron dissolution rate

Leaching of iron was slightly better when China clay suspension was agitated in a reciprocal rather than an orbital shaker. For example, under a set of conditions, 26% and 27.5% of iron was leached under orbital and reciprocal motion, respectively. But, from the various results obtained so far, it may be suggested that leaching must be much better under cyclic rotation if other parameters are kept constant. Variation of the shaking speed within the range of 60-250 rpm did not have much effect on iron dissolution.

4. CONCLUSION

This study was conducted with a China clay sample from which at least 40% (w/w) of total iron had to be removed to make it suitable for the production of quality materials. It was observed that the reddish colour of the clay was almost completely removed after treatment on a rotary shaker (60 rpm) with 100 mM oxalic acid (pH 1.5) for 6 h at 80°C (Fig. 7). Electron micrographs of the materials indicated reduction in the number of clumps in the treated sample (Fig. 8A) compared to the untreated one (Fig. 8B). It may therefore be concluded that the desired beneficiation of China clay could be achieved using oxalic acid. The leaching rate can be calculated theoretically from the relationship: 

\[ R_0 \sim (C)^{0.76} (T)^{1.76} (H)^{0.8} (P)^{0.2} \] 

under a set of conditions. The rate equations were derived at pH 1.5 where the iron-leaching rate was higher in case of culture filtrate compared to oxalic acid (Fig. 3A). This is reflected in rate equations for temperature (Rcf \( \sim T^{3.72} \)) and pulp density (Rcf \( \sim P^{0.9} \)). Thus, in spite of the higher activation energy, iron leaching from China clay continued at pH >1.25 when culture filtrate containing other component(s) was used.

![Figure 7. Photograph of China clay before (right) and after (left) leaching with oxalic acid](image-url)
Figure 8. Scanning electron micrograph of China clay after (A) and before (B) oxalic acid treatment

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REFERENCES

Mathematical modeling of the chemical and bacterial leaching of copper ores in stack

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Abstract

The objective of the work is to develop a system of mathematical models to allow improving the control of the process of leaching of ores through the simulation of this in the industrial level. For this purpose, were used the continuity equation in the materials balance, the kinetic model of the shrinking-core for the dissolution of the solid, the equation of Michaelis-Menten for the enzymatic activity of the bacteria and some values of variables showed in the bibliography with empiric models to relate variables of the ones that doesn’t have experimental data or specific operational.

It joins part of the work were developed in a Peruvian mining company using the database of the industrial leaching operations.

Keywords: bioleaching, modeling, copper sulphides, ferrooxidans, shrinking-core

1. INTRODUCTION

In the present work, a mathematical model was developed starting from the continuity equation supposing a plug-flow of the liquid phase inside the heap. The model of the shrinking-core it was applied to represent the reaction of the ore with the acid solution. In the production of the ferric sulfate it was used the model of Michaelis-Menten.

It is supposed that the predominant controlling mechanism in the leaching of the copper ores is the transport of mass, and for the oxides doesn’t exist any restriction in the application of this concept. However, in the case of the leaching of the sulfides the intermediate phenomenon of the biochemical reactions does exist (which have chemical controlling mechanism).

Due to that the data of the sequential analysis of copper of a database of industrial operations of leaching it was not achieved to determine experimentally some variables (i.e. difusivity), for the ones which or it was taken available values in the literature or it was applied empiric relationships giving as result a hybrid model empiric-fenomenological.
2. OVERVIEW

The solubility of the copper minerals in sulfuric acid solution is variable, being almost complete in the case of the oxidized mineral (70% for the cuprite, 100% for the azurite, the chrysocolla and the malachite) [1, 2, 3], very low in the secondary sulfides and almost null in the case of the primary sulfides.

In the leaching in moderate temperature of ores that contain a mixture of oxides and secondary and primary sulfides is necessary the application of an acid solution containing ferric ions and/or quimiolitotrofic bacteria of the gender *Thiobacillus* or *Leptospirillum* among others. The most usual (temperature between 10 and 40°C) are *Acidithiobacillus Ferrooxidans* and *Leptospirillum Ferrooxidans*. During the leaching is formed a layer of bacteria cells under the sulfide particle, these interact with the surface of the sulfide modifying her and provoking the dissolution of particle [4].

In the leaching systems, the relationship among the current generated by the flow of electrons and the electrode potential various in function of the transformations that happens in the structure and in the composition of the surface of the mineral, caused by reactions of dissolution and precipitation [4]. Thus, the ratio $\text{Fe}^{2+}/\text{Fe}^{3+}$ place should be maintained in a maximum that results in a great potential redox. The potential maximized redox provides a better use of the chemical force resulting in maxims oxidation ratios [4, 5, 6].

The presence of bacteria in the system of leaching of sulfides improvement the galvanic interaction, optimizing the selective leaching of some sulfidic minerals. The improvement is attributed to the bacterial oxidation of $\text{Fe}^{2+}$ and of $\text{S}^0$ produced in the anodic reaction [5].

In the primary minerals, the leaching can be so much for direct mechanism as indirect, however in the secondary sulfides, the leaching is accomplished by an oxidation that follows the indirect mechanism [4, 7, 9]:

$$2\text{Cu}_2\text{S} + 2\text{Fe}_3(\text{SO}_4)_3 \rightarrow 2\text{CuSO}_4 + 4\text{FeSO}_4 + 2\text{CuS} \quad (1)$$

$$2\text{CuS} + 2\text{Fe}_3(\text{SO}_4)_3 \rightarrow 2\text{CuSO}_4 + 4\text{FeSO}_4 + 2\text{S}^0 \quad (2)$$

3. DESCRIPTION OF THE PROCESS

The ore is extracted of the mine in a flow of 26.000 tons per day with a total grade of copper of 0.89%. Following, the ore is reduced to -3/8 of inch after passing for three crushing and screening combined circuits. Once reduced of size, the ore is agglomerated using 9.02 kg on average of sulfuric acid for ton of ore, being transported later to stacking. The ore in the heap is submitted a cure process for later to be leached by an average period of 256 days.

Each layer of the heap is divided in cells of 85 meters of width, with variable length according to the topography of the heap and an average height of 5 meters.

After the leaching the pregnant liquor solution is processed in the plant of solvent extraction and electrowinning (SX-EW) where is obtained as final product 188 tons of cathodes of copper degree A - LME.
4. DATA ANALYSIS

4.1 Sequential analysis

It is a laboratorial procedure of chemical analysis applied to the ore to know the lixiviability of this in function of mineralogy. This procedure is also used during the industrial process to determine the metallurgy losses, what leads to an auditing efficient metal works [1, 2, 3, 10].

![Graphs showing solubility in acid and cyanid]

**Figure 1. Maximum solubility of the copper minerals in sulfuric acid and cyanide of sodium solution**

The solubility of the copper minerals in the sulfuric acid and in the cyanide of sodium it is showed in the Figure 1 [1, 2].

The result is presented in three groups: the total copper (CuT) obtained by analysis of a complete sample, the soluble copper in sulfuric acid solution (CuSAc) and the soluble copper in solution of cyanide of sodium (CuSCN). Of these values, it can be deduced the insoluble copper, Cu_{insoluble} = CuT – (CuSAc + CuSCN), presumably composed for calcopyrite mainly.

The soluble copper in sulfuric acid (constituted mainly by oxided mineral of copper) it will be the easiest copper percentage to leach, needing a leaching strictly acid. Already the soluble copper in cyanide (composed by secondary sulfides of copper, mainly chalcocite, cc and covelita, cv) it requests of larger times of leaching and the presence of an effective oxidizer, as the ion Fe^{3+}. The leaching of the calcopyrite (cpy) represented by the insolubes in the sequential analysis is still more complex.

The values of the sequential analysis of the first layer of the heap* are presented in the Table 1.

The sequential analysis is used as base for the modelling, once this procedure supplies implicit information of the behavior of the copper minerals in function of the time and of the leaching solution.

* The sequencial analysis was applied to a sample of the ore to grinding to -100 mesh Tyler, leached in a beaker with sulfuric acid solution to 5% during one hour to 25ºC. Following the washed solid is cyanided with solution of NaCN to 10% for 30 minutes to 25ºC. The residual solid is taken to digestion and then do the analyzes of the solutions for atomic absorption.
4.2 Statistical analysis

Once clear the concept of the sequential analysis as medium to make the mathematical modelling, the data of the Table 1 are analyzed through statistical tools to know the homogeneity, the existent correlation with the operational variables and the relationship with the ore of deeper layers of the mine in the leaching.

The confidence interval was calculated for a degree of confidence of 99%, it was done a dispersion analysis for all the values of the Table 1, was determined the correlation between the copper grade and the process variables, besides the significant difference among the layers composed of ore of deeper zones in the mine. After that can be comment the following:

Table 1. Applied sequential analysis to the ore loaded in the first layer of the heap and made use by cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Ore, ton</th>
<th>CuT, %</th>
<th>CuSAc, %</th>
<th>CuSCN, %</th>
<th>CuInsoluble, %</th>
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</thead>
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<td>0.76</td>
<td>0.26</td>
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</table>

a. The values obtained in the calculus of the confidence interval, CuT = 0.07; CuSAc = 0.03; CuSCN = 0.07 e CuInsoluble = 0.02; are sufficiently small indicating that exists a distribution uniform of the copper grade more or less in the industrial heap;

b. It can be said that the data of the cell 103 are not valid for the modelling, they are values very dispersed respect to the general average;

c. Analyzing the values obtained in the determination of the significant differences among the layers, it can be said that the model obtained in function of the data of the first layer can be applied with good certainty degree the superior layers or future layers overlapped in the heap that have physical and mineralogical similar characteristics.
5. DEVELOPMENT OF THE MODEL

They are determined two mathematical models separated, being supposed that the leaching in the heap it carries through three phases formed by the minerals of oxided copper, secondary sulfides and primary sulfides. These phases can happen in parallel inside of the heap, but with kinetics and leaching mechanisms differentiated.

5.1 Strategy of the mathematical development

Will be taken in consideration the following hypotheses:

a. The growth of the microorganisms is controlled by the oxidation of $Fe^{2+}$ (limiting nutrient);

b. The enzymatic activity behaves according to the model of Michaelis-Menten;

c. The production of bacteria for unit of consumed substrate is constant;

d. The drainage of the solution at the bed of the heap happens without appreciable axial dispersion (plug-flow);

e. The half-time of residence of the solution inside the layer doesn't vary with the time of leaching;

f. The heap presents a granulometric and grade copper species distributions homogeneous;

g. The reactions of the minerals of oxided copper are controlled by diffusion of the solution inside the particles very little porous of the ore;

h. The leaching of the calcopryite is insignificantly.

A leaching differentiated is produced among the copper species in the heap, or either, exist the action separate from the ions $H^+$ as leaching agent of $CuSAc$ and the ion $Fe^{3+}$ as leaching agent of $CuSCN$.

5.2 Mathematical model for the soluble copper in acid

The diffusion of the solution in the pores of the ore is a process usually slow, much more than the diffusion that feels in the surrounding liquid to the particle. The process of chemical diffusion is composed for five successive stages:

a. Diffusion of the ion $H^+$ in the surrounding solution to the particle until the interface liquid-solid;

b. Migration of the ion $H^+$ of the interface liquido-sólido to the particles of copper mineral for the pores to the particle of the ore;

c. Chemical reaction of the ion $H^+$ with the copper inside of the ore particle;

d. Migration of the ion $Cu^{2+}$ among the pores of the particle to the interface solid-liquid;

e. Diffusion of the ion $Cu^{2+}$ of the interface solid-liquid to the surrounding solution of the ore particle.

For the calculus of the copper it is used the equation (3), it describes the diffusion in state of pseudo-stationary and the fast dissolution of the oxidized mineral of copper [11]:

$$1 - \frac{2}{3} F_{r_{0}} - \left(1 - F_{r_{0}}\right)^{\frac{1}{3}} = \left[\frac{2V_{Cu} D_{o} A_{0}}{B_{r_{0}^{2}}}\right] t$$

(3)
The effective coefficient of diffusion \( \left( D_{ef} \right) \) it can be made calculations of: \( D_{ef} = \frac{D_{ef}}{\tau} \).

The diffusivity for the species in water doesn't vary a lot and a reasonable approach to room temperature \((25^\circ C)\) is \( D = 1.5 \, \text{cm}^3\text{day}^{-1} \) \([11]\). Comparing the experimental data with the results calculated for the diffusion in porous media, the tortuosity factor is usually \( \tau = 2 \) \([11]\) and, for a volumetric fraction of pores in the heap, \( \varepsilon_s = 0.1 \); results in a value of \( D_{ef} = 0.075 \, \text{cm}^2\text{day}^{-1} \).

With the equation (4), can be calculated the molar volume of copper in the ore:

\[
V_{Cu} = \frac{M_{Cu}}{\rho_{ore} G}
\]

Being \( M_{Cu} \) the molecular weight of the copper and, with a specific weight of the ore of \( \rho_{ore} = 1.65 \, \text{g/cm}^3 \), and the fraction average of the grade of oxidized copper \((CuSAc)\) except for the cell 103 (very dispersed value), \( G = 1.79 \times 10^{-3} \); then: \( V_{Cu} = 22.65 \, \text{l/mol} \).

The initial concentration of sulfuric acid in the leaching solution is: \( A_o = 4.21 \times 10^{-2} \, \text{mol/l} \).

Therefore, with a initial radius average of particle, \( r_o = 0.0922 \, \text{cm} \) and supposing that all the \( CuSAc \) reacts with the sulfuric acid and making the correction corresponding to the consumption of acid for the gangue mineral (factor \( \approx 1.3 \)), the consumption of the acid for each kilogram of extracted copper is: \( B = 1.30 \, \text{mol/kg} \).

Relating graphically the factor \( F \) and \( \left[ 1 - \frac{2}{3} F - (1 - F)^{\frac{2}{3}} \right] \) to make easier the calculus of \( F_{\gamma_o} \) (fraction of leached copper), can be determined the value of the extraction of copper of the ore oxidized in function of the time \((t)\).

Making: \( X = \left[ 1 - \frac{2}{3} F_{\gamma_o} - (1 - F_{\gamma_o})^{\frac{2}{3}} \right] \), can be represented the relationship for an equation no linear of the type:

\[
F_{\gamma_o} = -3.44 \times 10^{-5} + 4.31 \left[ 1 - \exp(-0.001X) - 0.0099 \left( \frac{0.0119 + 0.001 \exp(-0.0129X)}{15.34 \times 10^{-5}} \right) \right] \]

With: \( r^2 = 0.999 \)

Calculating the value of \( X \) in Mathcad 2000 Professional®, MathSoft, Inc. for a period of 300 days (being the leaching period average of the first layer of the heap, 256 days), it can be said that the leaching of the soluble copper in acid is very fast and efficient in this stage, becoming theoretically according to the model of the shrinking-core in a 99% of reduction of the volume of the nucleus in the particle and 98.50% of the copper extraction in 300 days.
5.3 Mathematical model for the soluble copper in cyanide

The chalcocite is the predominant mineralogical specie in the processed ore, 81% of content of the sulfides of secondary copper. The general mineralogical distribution of the copper sulfides is the following: chalcopyrite, 24.19%; chalcocite, 60.99%; covellite, 14.04 and other sulfides, 0.78%.

It can be assumed as global reactions of this process the following estequimetric relationships:

\[
\begin{align*}
Cu_2S + \frac{5}{2} O_2 + H_2SO_4 & \rightarrow 2CuSO_4 + H_2O \quad (6) \\
FeS_2 + \frac{7}{2} O_2 + H_2O & \rightarrow FeSO_4 + H_2SO_4 \quad (7)
\end{align*}
\]

If the controlling factor the leaching reaction of the secondary sulfide is the bacterial activity in function of the production or generation of \( Fe^{3+}, Fe^{2+} \) should be maintained in excess and the limiting factor it starts to be the necessary oxygen for the oxidation reactions, as well as, for the metabolism of the bacteria.

Once the indirect mechanism of biochemical reaction in the phase hard-liquid is much faster than the direct mechanism, is considered that the leaching of the sulfides is strictly ferric being the restricted bacterial action to the conversion of the ion \( Fe^{2+} \).

Theoretically, it is known that for a maximum population of \( 10^{11} \) bacteria \( m^{-2} \) \( \approx 10^{10} \) bacteria \( 1 \text{ kg ore}^{-1} \), the dissolution of the mineral is controlled by the bacterial activity for particles of diameter less than 2 cm [12]. Therefore, considering an effective diffusion coefficient for the \( Fe^{3+} \) of \( 5 \times 10^{-11} \) m² s⁻¹ and supposing that the enzymatic behavior can be represented by the equation of Michaelis-Menten, the following relationship for the rate of copper dissolution in the stationary state is used [12]:

\[
\frac{dF_{ss}}{dt} = X V_m \left( \frac{B_{ss}}{G_{ss}} \left( \frac{C_L}{K_m + C_L} \right) \right)
\]

In function of the equations (6) and (7) the estequimetric factor can be calculated the following way:

\[
B_{ss} = \frac{M_{cc} M_{py}}{\frac{5}{2} M_{py} + \frac{7}{2} \text{ RPC} M_o M_{cc}}
\]

where, \( \text{ RPC } \) it is the ratio among the pyrite \( (py) \) and chalcocite leaching amounts: \( \text{ RPC } = 0.80 \text{ kg}_{py}. \text{kg}_{cc}^{-1} \), \( M_{cc}, M_{py}, M_o \), they are the molecular weight of the chalcocite, pyrite and oxygen respectively, \( B_{ss} = 0.844 \text{ kg}_{cc}. \text{kg}_{o}^{-1} \).

The relationship among the specific maximum ratio of breathing of the bacteria, \( V_m \), and the temperature was obtained starting from the data of oxidation of iron by Acidithiobacillus Ferrooxidans:

\[
V_m = \frac{6.8 \times 10^{-13} T \exp\left(-\frac{7000}{T}\right)}{1 + \exp\left(236 - \frac{74000}{T}\right)}
\]

(10)
For an average temperature \( T \) of the heap to 2.500 meters on the level of the sea is approximately 293 K, there are: \( V_m = 8.389 \times 10^{-21} \text{kg}_\text{O}_2 \cdot \text{bacteria}^{-1} \cdot \text{s}^{-1} \).

According to the equation (10) the largest consumption of oxygen and, consequently the maxim capacity of oxidation of the substrate (\( Fe^{2+} \)), is produced around 35°C. At this temperature, the maximum activity of *Acidithiobacillus Ferrooxidans* takes place, at \( \text{pH} = 2 \), \( k_m = 1 \times 10^{-3} \text{kg.m}^{-3} \), and considering a concentration of oxygen dissolved in the solution of 6.5\( \times 10^{-3} \text{g.l}^{-1} \).

The concentration of dissolved oxygen is calculated using Henry's law, supposing its equilibrium between the liquid and the gaseous phase. Henry's constant was obtained in function of the temperature using the solubility of the oxygen in the leaching solution for different temperatures, \( C_L = C_g H_e \), \( H_e \) express in function of the temperature:
\[
H_e = 21.312 + 0.784T - 0.00383T^2 - 35.46
\]

The concentration of oxygen in the solution to 298 K and 101 kPa in equilibrium with the concentration of oxygen in the air, \( C_g = 0.24 \text{kg.m}^{-3} \), is: 0.0084 \text{kg.m}^{-3}.

Being adopted an apparent density or density of the bed of the layer of \( \rho_{\text{bed}} = 1800 \text{kg.m}^{-3} \) and a average bacterial population, \( X = 5 \times 10^{13} \text{bacteria.m}^{-3} \), in the expression (8), the average fraction of soluble copper in cyanide for the heap is \( G_{ss} = 5.8 \times 10^{-3} \).

Integrating the equation (8) for a period of 300 days of leaching it could be calculated the extraction of secondary copper.

### 5.4 General model

Adding the results obtained so much for the soluble copper in acid as for the soluble copper in cyanide of sodium according to the previous models, the percentage of extracted copper referred to the copper contained in the oxides and secondary sulfides is calculated. The respective curve of extraction of the copper in function of the time and the comparison with the industrial curve of copper extraction is presented in the Figure 2.

![Figure 2. Graphic comparison of the calculated and industrial data of the copper extraction for chemical and bacterial leaching in mixed ores of copper](image)

6. VALIDATION

Using the program EMPV®, Effective Management of Process Variability - version 1.06, it was calculated the mistake in all of the points of the curve being obtained an average mistake of 7.77%.
It is also made a sensibility analysis for two process variables in order to visualize the performance of these variables in the model. In the Figure 3A the effect of the volumetric fraction of pores in the heap, $\varepsilon_s$, can be observed. The model simulations indicate that, for the range of $\varepsilon_s$ values typically found in the industrial practice, there is no appreciable variation in the total recovery of copper by leaching. The same happens with regard to the sulfuric acid concentration in the leaching solution, $A_o$.

On the other hand, Figure 3B shows that the most influential variable in the model is the ratio of the pyrite mass leached in relation to the mass of leached chalcocite, $RPC$. It can be said that, while the potential of the leaching solution doesn't reach very high values, the selective leaching of the secondary copper minerals will be comparatively more efficient.

The ratio of the pyrite mass leached in relation to the mass of leached chalcocite cannot be obtained directly, but it can be dear and used as parameter of calibration of the model and as parameter of the process for the control of the electrochemical potential and the concentration of $Fe^{3+}$ in the solution. Like this, this variable can be correlated empirically with another varied as the concentration of $Fe^{3+}$, the time of residence of the solution in the heap and the electrochemical potential of the leaching solution.

Figure 3. Effect of the volumetric pore fraction in the heap, $\varepsilon_s$ (A) and of the ratio of the pyrite mass leached in relation to the mass of leached chalcocite, $RPC$, (B)

7. CONCLUSIONS

In industrial practice, the sequential analysis of the ore is very useful not only to support decisions regarding the crushing degree, sulfuric acid addition in the agglomeration and irrigation rate and time in the heap leaching, but also to supply valuable data to be used in the estimation of copper extraction rates through phenomenological mathematical models, with a good degree of accuracy, 92.23% in the current case.

The copper oxides present a very fast leaching kinetics and the metal extraction is completed in the first leaching cycle (240 days). After this time, a sampling of the leaching residues still shows a reasonable amount of copper which would be dissolved in acid; however, this copper should belong to the secondary covellite formed during the oxidation of the chalcocite. This covellite is much more porous than the natural covellite and its leaching kinetics is very much faster. The factor of major importance in the leaching of copper sulfides is the concentration of $Fe^{3+}$.
REFERENCES

Model for bacterial leaching of copper sulphides by forced aeration

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Abstract

A two-dimensional model for bacterial leaching of copper minerals was developed, which can handle aeration by natural and forced convection with and without aeration channels. The use of forced aeration increases the reaction rate in the pile and may significantly decrease the operation time, however the costs are increased. When only natural convection is used, the leaching rate is commonly limited by the oxygen availability, and factors such as bacteria population and particle size have less significance. If forced aeration is used, the oxygen availability may be arbitrarily varied.

Two aspects are studied; the distance between aeration channels and the adequate aeration rate. Regarding the distance between aeration channels, it was found that for a large separation between channels the aeration is deficient in zones located between the channels. When the distance between channels is decreased, the aeration is improved, however, this improvement is less significant by further decrease of the distance between channels. The most advantageous aeration rate is a function of several factors such as particle size, type of minerals, number of bacteria, and dimensions of the pile. For example, if the pile consists of small particles, the consumption of oxygen increases and a greater aeration is required. The number of channels and the aeration rate are important when costs are considered.

Keywords: copper leaching, modelling, forced aeration, bacterial leaching, numerical modelling

1. INTRODUCTION

Bacterial leaching of copper minerals has become an important process in the mining industry. At the beginning, bacterial leaching was applied to low-grade ores, however, at present, due to exigencies of a better environment, the process is also applied to high-grade ores. Since the main reaction occurring in the heap is the oxidation of the sulphide minerals, aeration, which may take place either by natural or forced convection, is a critical issue. When natural convection is used, the leaching rate is commonly limited by the oxygen availability, and factors such as bacteria population, particle size and intrinsic reaction rate have less significance. The use of forced aeration, through channels in the bottom of the heap, may secure the adequate supply of oxygen to the greater part of the

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heap with increasing reaction rate as a result. This may significantly decrease the leaching time, but the costs are increased.

The bio-leaching process is complex and involves several steps: a) oxygen is introduced into the heap by natural or forced aeration, b) oxygen oxidises the ferrous ions by means of a reaction mediated by bacteria, c) ferric ions diffuse into the ore particles to reach the sulphides, d) the ferric ions oxidise the sulphides and e) the ferrous ions emigrate to the surface of the particle to re-initiate the cycle. The bio-leaching rate is controlled by the slowest of these steps and they may coexist in different zones in the ore bed (Ritchie, 1994; Casas et al., 1998).

Modelling of the leaching operation for sulphide ore beds has received considerable attention in the last years (Ritchie, 1994; Casas et al., 1998; Bartlett, 1997, Coderre, F., Dixon, D.G., 1999; Dixon, D.G., 2000; Orr, 2002; Orr and Vesselinov, 2002). The macroscopic models developed by Cathles (1994), Ritchie (1994), Casas et al., (1998), and Sidborn et al., (2003) showed that in the case of natural convection, the aeration is commonly not sufficient and in large zones of the heap the oxygen is almost totally depleted. Under these circumstances, bacterial leaching of the sulphide minerals is too low. Today, the tendency is to use forced ventilation through channels at the bottom of the heap in order to improve the air supply and to obtain high bio-oxidation rates. Lizama (2001) studied the impact of different rates of forced aeration on copper recovery. This mode of operation involves higher capital and operating costs, but they are compensated for by the faster metal recovery (Bartlett, 1997).

The rate of sulphide mineral dissolution is modelled according to the unreacted core model. The transport of ferric ions from the particle surface to the reaction zone is calculated considering film diffusion, diffusion within the particle and reaction kinetics. The rate of oxidation of the ferrous ion by bacteria attached to the ore surface is modelled using the Michaelis-Menten relationship. The influences of temperature, dissolved ferric iron and dissolved oxygen in the leaching solution are considered in the kinetic formulation. In order to show the capabilities of the model, the impact on the copper recovery of the distance used between the aeration channels and the aeration rate are studied. Modelling of the process is a useful tool to aid the design and optimisation of industrial operations.

2. THEORY

2.1 Leaching of copper ores

The copper-oxide mineral dissolution kinetics is rapid and oxide minerals are readily dissolved by sulphuric acid applied on top of the heap. Copper sulphide minerals are however much more stable and can only be dissolved under oxidising conditions. In copper leaching, the oxidising agent present may be the ferric ion that diffuses into the ore particle and reacts with the metal sulphide (MS) according to

$$\text{MS} + 2\text{Fe}^{3+} \rightarrow \text{M}^{2+} + 2\text{Fe}^{2+} + \text{S}$$  \hspace{1cm} (1)

Microorganisms catalyse the reverse oxidation of ferrous ions to ferric ions. The most important bacteria for this purpose is *Acidithiobacillus ferrooxidans*. The bacterial oxidation takes place according to

$$4\text{Fe}^{2+} + \text{O}_2(aq) + 4\text{H}^+ \xrightarrow{\text{bacteria}} 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$$  \hspace{1cm} (2)
This reaction consumes oxygen that has to be transferred to the leaching solution from air in the ore pile. The rate of consumption of oxygen by bacteria can be described in terms of the Michaelis-Menten equation:

\[
R_{O_2} = X V_m \left( \frac{[O_2]_L}{K_m + [O_2]_L} \right)
\]

(3)

where \( R_{O_2} \) is the rate of consumption of oxygen by bacteria, \( X \) is the number of bacteria per volume of bed, \( V_m \) is the maximum specific respiration rate of bacteria, \( [O_2]_L \) is the oxygen concentration in the liquid solution and \( K_m \) is the Michaelis constant for the system. For *Acidithiobacillus ferrooxidans*, the maximum specific respiration rate, \( V_m \), is dependent on the temperature according to (Casas et al., 1998)

\[
V_m = \frac{6.8 \times 10^{-13} T e^{\frac{7000}{T}}}{1 + e^{\frac{236 - 7400}{T}}}
\]

(4)

where \( T \) is the temperature in Kelvin. The rate-determining step in the bioleaching of small sulphide mineral particles is generally the slow intrinsic dissolution of sulphide minerals, provided that oxygen is available in the bed. For larger ore particles, however, a mixed-kinetics model has to be used to describe the leaching rate. Such a mixed kinetics model is the shrinking core model that includes resistances due to the intrinsic dissolution kinetics of the mineral, the diffusion resistance of ferric iron through an inert porous layer of reacted material, and the diffusion of ferric iron through the liquid film around the ore particle surface. The rate of decrease of the unreacted core radius for a given mineral species can be written as:

\[
\frac{-dr_c}{dt} = \frac{M_S}{\rho G \phi} \left[ \frac{[Fe^{3+}]}{1 + \left( \frac{\sigma}{D_{eff}} \right) \left( \frac{r_c}{R} \right) \left( R - r_c \right) + \left( \frac{1}{K_C} \right) \left( \frac{r_c}{R} \right)^2} \right]
\]

(5)

where \( r_c \) is the unreacted core radius, \( M_S \) is the ore molecular weight, \( \rho \) is the mineral particle density, \( \phi \) is the particle shape factor, \( G \) is the copper ore grade, \( \beta \) is the global specific kinetics factor, \( \sigma \) is the stoichiometric factor, \( D_{eff} \) is the effective diffusion coefficient, \( R \) is the mineral particle radius and \( K_C \) is the mass transfer coefficient in the liquid-solid film. \([Fe^{3+}]\) is the concentration of ferric ions in the leaching solution. The leaching rate of a given mineral species is related to the rate of decrease of the unreacted core radius through the mass balance.

### 2.2 Air transport through the bed

Air is mainly transported through the ore bed by convection (forced or natural). Oxygen may, however, be transported by diffusion in some zones of the heap. The density of the gas varies through the ore bed due to temperature changes caused by the exothermic reactions and due to changes in the air composition. Oxygen is consumed by the oxidation of the ferrous ions and the air humidity varies with the local temperature. The local velocity of air, \( q_g \), can be expressed as:

\[
q_g = -\frac{\rho_g k_{ns} k}{\mu} \nabla P
\]

(7)
where $k$ and $k_{rg}$ are the intrinsic and relative gas permeabilities of the bed, $\rho_g$ is the gas density, $\mu$ is the fluid viscosity and $\nabla P$ denotes the fluid pressure gradient. The pressure applied in the aeration channels and the air density variation cause the airflow in the bed.

The transport of oxygen in the gaseous phase is described by the Advection-Dispersion (AD) equation:

$$
\varepsilon_g \frac{\partial O_{2,g}}{\partial t} = \varepsilon_g D_g \nabla^2 O_{2,g} - q_g \nabla O_{2,g} - R_{O_2}
$$

(8)

where $O_{2,g}$ is the concentration of oxygen, $D_g$ is the dispersion coefficient in the gas phase and $\varepsilon_g$ the volume fraction of air.

### 2.3 Liquid flow

During leaching a solution of sulphuric acid is applied at the top of the bed at a given rate. The liquid flow is constant and is given by the irrigation rate and, in general, is independent on the permeability of the bed. The transport of solutes in the liquid phase is described by:

$$
\varepsilon_L \frac{\partial C_i}{\partial t} = D_L \varepsilon_L \nabla^2 C_i - q_L \nabla C_i + R_i
$$

(6)

where $C_i$ is the concentration of species $i$, $D_L$ is the dispersion coefficient, $\varepsilon_L$ the volume fraction of liquid, $q_L$ the liquid flow rate, and $R_i$ the reaction rate of species $i$. This equation may be applied to both ferric and ferrous ions and copper ions.

### 2.4 Energy balance

Due to the exothermic reactions, the temperature of the bed is increased. Energy is transported through the ore bed by conduction and convection. The energy transported by convection depends on both the liquid flow and the gas flow, which have opposite directions in the bed.

$$
C_{p,B} \rho_B \frac{\partial T}{\partial t} = k_B \nabla^2 T - \rho_L q_L \nabla H_L - \rho_g q_g \nabla H_g - \Delta H_R \frac{R_{ch}}{R_{ch}}
$$

(9)

where $C_{p,B}$ is the mean heat capacity of the ore bed, $\rho_B$ is the bed density, $k_B$ is the thermal conductivity of the ore bed, $\rho_L$ is the liquid density, $q_L$ and $q_g$ denote the liquid and the gas flow respectively, $H_L$ and $H_g$ denote the liquid and gas enthalpy respectively. $\Delta H_R$ denotes the heat of reaction per mineral dissolved.

### 3. CASES MODELLED

A mineral consisting of chalcocite and pyrite was considered in these simulations. The pile was assumed to have a flat top and to slope downwards at the edges at an angle of 45°. In the model, for the sake of simplicity, it is assumed that the ferrous ions are present in excess and that they are not a limiting factor for the oxidation reaction. Bacteria, the population of which is assumed to be constant, mediate the oxidation of ferrous ions. The ferric ions produced diffuse into the ore particle and react with the copper mineral and copper ions are leached to the solution. The ferric ion diffusion resistances, both in the film and the particle, are taken into account. The air in the bed is assumed to be water-saturated and local equilibrium is assumed between the temperature of the solid, the liquid and the gas. Two cases are modelled:
a. The central part of the heap, where the aeration takes place through a number of channels equidistantly separated. For reason of symmetry, only one channel needed be modelled, as shown in Figure 1.

b. The edge of the heap, where air is introduced by natural convection through the slope of the heap and by aeration channels at the bottom.

Five differential equations are solved in addition to the equation for the airflow. These equations consider the balances of ferric ions, copper ions, oxygen, and energy and the variation in the unreacted core diameter of the particle as a result of the leaching process. The conditions inside of the bed vary with time, since the diffusion-resistance is increased as copper is dissolved. For sake of simplicity, the model results will be shown only for a short time.

![Figure 1. Schematic picture of the heap showing the aeration channels and the modelled zone](image)

The parameters needed for these simulations are shown in Table 1. The simulation software FEMLAB® (http://www.femlab.com/) was used to solve the system of differential equations.

5. RESULTS AND DISCUSSION

This two-dimensional transient model was simulated only for short times, since our aim is to show the impact of the distance between the channels and the aeration rate on the leaching process. In the initial period the necessity of aeration is the largest. When the directly accessible copper has been depleted, the dissolution of copper will be slower and the needed aeration will be less. In the calculations, aeration is assumed to take place through a channel 0.2 m in width.

The simulations were done for a heap with a height of 10 metres. Figure 2a shows the relative oxygen concentration profiles for channels separated in 20 m and over-pressures of 1000 Pa. In spite of forced aeration, the results shown zones between the aeration channels where the oxygen concentration in the air is almost zero, with insignificant dissolution of copper in these zones, as consequence of the bad aeration. For a channel distance of 12 m (Figure 2b), the aeration is significantly improved, the relative oxygen concentration at the bottom is at all the locations higher that 0.30 (6-7 vol-%). Better aeration is, of course, obtained with shorter distances, but the ventilation costs are increased.
Figure 2. Relative concentration profiles for oxygen in the air for channel distances of 20m (on the left) and 12m (on the right)

Calculations were also done for other airflow rates by changing the air pressure at the aeration channel. Figure 3 shows the case where no over-pressure is applied on the channels and the air is introduced only by the effect of natural convection. As may be observed in the figure, the zones with deficient aeration are strongly increased.

Figure 3. Relative concentration profiles for oxygen in the air for channel distances of 20 m for natural convection

The results are summarised in Figure 4, where a relative measure of the extent of the leaching process is plotted for different channel distances for a heap height of 10m. The leaching process is estimated by integrating the copper concentration leaving through the bottom of the heap and dividing by the channel distance.

The results show that forced aeration has a beneficial effect on the leaching process; the amount of copper that is dissolved is increased. However, also the cost of inversion and operation are increased.

Table 1. Important parameters used in the simulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Value</th>
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<tbody>
<tr>
<td>Heap height</td>
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</tr>
<tr>
<td>Number of bacteria</td>
<td>Bacteria/m³ bed</td>
<td>1.010¹⁴</td>
</tr>
<tr>
<td>Michaelis constant</td>
<td>Kg/m³</td>
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</tr>
<tr>
<td>Liquid flow</td>
<td>L/m².s</td>
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</tr>
<tr>
<td>Gas permeability</td>
<td>m²</td>
<td>2.5 10⁻⁹</td>
</tr>
<tr>
<td>Ambient temperature</td>
<td>°C</td>
<td>20</td>
</tr>
<tr>
<td>Ore copper concentration</td>
<td>%</td>
<td>0.63</td>
</tr>
<tr>
<td>FPY parameter</td>
<td>Kg pyrite/Kg Cu₂S</td>
<td>2.0</td>
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</table>
6. CONCLUSIONS

A two-dimensional model bioleaching of copper sulphide mineral in heap was developed. The model includes aeration by natural and forced convection. The emphasis was set in the impact of the aeration (distance between the aeration channels and the aeration rate) on the leaching process.

The results show that the copper-leaching rate is increased when aeration is improved by using channels at the bottom of the heap. Even aeration channels without over-pressure, natural convection, helps to enhance the leaching process. It was found that a large separation between the channels cause a deficient aeration in zones located between the channels. When the distance between channels is decreased, the aeration is improved, however, this improvement is less significant if one continues to decrease the distance.
between channels. The optimal aeration rate is a function of several factors such as particle size, type of minerals, number of bacteria, and dimensions of the pile. However, the installation of aeration channels increases the equipment cost and the forced aeration increases the operation costs. The results may be used to determine the most favourable distance between channels and aeration rate for the leaching process.

REFERENCES

Optimal oxygen and carbon dioxide concentrations for thermophilic bioleaching archaea

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Abstract

Tank bioleaching reactors are currently sparged with air to satisfy both oxygen and carbon dioxide requirements in the reactors. Under high sulphide loading conditions, as is the case with high-grade concentrates, the microbial and chemical demand for oxygen is significantly increased during the bioleaching process in agitated tank reactors. Sparging with enriched oxygen gas may offer a potential solution in order to overcome the mass transfer difficulties at elevated temperatures. In the case of air sparging, the dissolved oxygen (DO) concentration in tank reactors could not be increased to a point where it would become inhibitory. The use of enriched oxygen in such reactors at large scale does, however, pose its own set of process risks. The first aim of this investigation was therefore, to determine the effects of various DO concentrations, in both the limiting and inhibitory ranges, on the microbial activity of \textit{Sulfolobus} sp. U40813. Secondly, the effect of carbon dioxide concentration on the rate of ferrous iron oxidation was investigated. Both the oxygen and CO\textsubscript{2} kinetics were examined in controlled batch cultures at 78°C, using ferrous sulphate and potassium tetrathionate as energy sources. The optimal DO concentration for iron oxidation was found to be between 1.5 to 4.1 mg L\textsuperscript{-1}. The use of elevated DO concentrations (above 4.1 mg L\textsuperscript{-1}) inhibited the ferrous oxidation rates. This inhibition effect increased progressively as the DO was increased above 4.1 mg L\textsuperscript{-1}. Due to the sensitivity of \textit{Sulfolobus} to elevated dissolved oxygen, the use of oxygen-enriched air to overcome low solubilities in tank bioleaching reactors at high temperatures will have to be strictly controlled. The optimal CO\textsubscript{2} concentration for ferrous iron oxidation is predicted to be between 7\% and 17\%. The iron oxidation rates were however, severely limited with CO\textsubscript{2} concentrations less than 7\%, indicating that the CO\textsubscript{2} supply was limiting in this range and inhibited the microbial growth rate.

\textit{Keywords:} \textit{Sulfolobus}, oxygen, carbon dioxide, thermophilic, bioleaching, archaea

1. \textbf{INTRODUCTION}

Mineral bioleaching, the process by which metals are dissolved from sulphide ore-bearing rocks by microorganisms, is an established technology for metal recovery (Rawlings, 1997; Marsh \textit{et al}., 1983). Although these technologies offer process and environmental advantages in certain instances, they are more prone to certain process upsets than non-biological hydrometallurgical extraction processes. One such important bioprocess condition is the requirement for a suitable dissolved oxygen concentration.
Tank bioleaching reactors are currently sparged with air to satisfy both oxygen and carbon dioxide requirements in the reactors. Under high sulphide loading conditions, as is the case with high-grade concentrates, the microbial and chemical demand for oxygen is significantly increased during the bioleaching process in agitated tank reactors. The increased demand for oxygen under these conditions is facilitated by increased aeration rates, higher impeller agitation rates and improved agitator designs. Agitation speeds cannot however be indefinitely increased to improve mass transfer limitations, as cell damage to bioleaching microbes becomes a limiting factor at high agitation speeds and power inputs in the presence of high pulp densities. Mass transfer limitations are not routinely encountered in commercial tank leaching operations at mesophilic (35-40°C) conditions when sparging with air. At thermophilic (65-80°C) bioleaching conditions, as are required to leach primary copper sulphide minerals such as chalcopyrite, mass transfer limitation due to the reduced solubility of oxygen is, however, a significant process challenge that cannot be overcome by simply increasing agitation speeds and aeration rates. Sparging with enriched oxygen gas offers a potential solution in order to overcome the mass transfer difficulties at elevated temperatures. The use of enriched oxygen in such reactors at large scale does, however, pose its own set of process risks. In the case of air sparging, the dissolved oxygen concentration in tank reactors could not be increased to a point where it would become inhibitory. The use of oxygen-enriched air, however, could potentially result in the increase of dissolved oxygen concentrations as high as 15 mg L⁻¹. Such elevated dissolved oxygen concentrations could have a detrimental effect on microbial cells as the capacity to dissipate oxygen derived free radicals enzymatically could become overloaded.

The concentration at which severe effects are encountered depends both on the species of microorganism as well as process and medium factors (Onken and Liefke, 1989). Su and Kelly (1987) investigated the effect of hyperbaric oxygen on the heterotrophic growth of *Sulfolobus acidocaldarius*. Elevated dissolved oxygen tensions were created through higher levels of air added to the gas phase while the total pressure was maintained at 50 bar. With agitation at 480 rpm, increasing the air partial pressure from one to two bar reduced the cell growth rates as well as the final cell density. The inhibition was even more severe at higher stirring rates. At 75°C, three bar air, or an oxygen partial pressure of 0.6 bar (estimated by Su and Kelly (1987) to be a dissolved oxygen tension of approximately 0.01 mg L⁻¹) resulted in the complete inhibition of growth.

Besides oxygen, carbon dioxide is a critical component for growth of the bacteria to meet their carbon assimilation requirements. In contrast to bacteria (mesophiles and moderate thermophiles) where CO₂ fixation occurs by means of the Benson Calvin cycle (Holuigue *et al*., 1987; Beudeker *et al*., 1980; Wood and Kelly, 1985), the high temperature mineral-oxidizing archaea such as *Sulfolobus* do not assimilate CO₂ via the Calvin cycle. Kandler and Stetter (1981) showed that CO₂ fixation in *Sulfolobus brierleyi* occurs via a reductive tricarboxylic acid pathway in which malic acid, aspartic acid, glutamic acid and citric acids play an important role. Enzyme assays by Wood *et al*. (1987) confirmed the absence of a Calvin cycle in *Sulfolobus brierleyi* and showed that a small proportion of CO₂ fixation could occur through the carboxylation of pyruvate and phosphoenolpyruvate, but provided no further evidence for the proposed reductive carboxylic acid pathway believed to operate in *Sulfolobus*. Subsequent work has found enzyme activities that support the operation of a modified 3-hydroxypropionate pathway in *Sulfolobus* (now *Acidianus*) *brierleyi* and in other thermoacidophilic archaea.
(Metallosphaera sedula and Sulfolobus metallicus) (Burton et al., 1999; Ishii et al., 1997; Menendez et al., 1999).

Nixon and Norris (1992) found that the yield in an air-sparged thiosulphate-limited continuous Sulfolobus culture (0.03% v/v CO₂) was only 60% of that of a culture grown with 5% CO₂ supplementation. In both cases however, the thiosulphate (20 mM in the feed) was completely oxidized by the cultures. Both Norris (1989) and Norris et al. (1989) reported that the rate of iron solubilization by Sulfolobus, in a pyritic medium, was reduced if the culture aeration was not enriched with CO₂. Various studies focused on the effect of CO₂ on the growth of mesophilic and moderate thermophilic bioleaching bacteria. Holuigue et al. (1987) found that an increase in the CO₂ concentration from 0.03% to 0.1% caused a reduction of more than half in the doubling time of A. ferrooxidans. A further increase to 5.4% CO₂ did not result in a further increase in the growth rate, although it seemed to enhance the total yield of bacteria as compared with 0.1% CO₂. Hazeu et al. (1986) supplemented the air supply to a thiosulphate chemostat culture with A. ferrooxidans with 2% CO₂ (v/v) and also observed a slight increase in the yield.

The optimal CO₂ concentration for growth of A. ferrooxidans during the bioleaching of a pyrite-arsenopyrite ore concentrate was predicted to be 3 to 7 mg L⁻¹ (estimated to be about 0.23 to 0.53% CO₂). The study also showed that CO₂ concentrations below the optimal levels lead to sharply reduced bacterial growth rates, whereas CO₂ concentrations in excess of 10 mg L⁻¹ (estimated to be about 0.73% CO₂) were inhibitory to the growth of A. ferrooxidans (Nagpal et al., 1993). Torma et al. (1972) observed that zinc extraction rates from a zinc sulphide mineral increased from 360 mg L⁻¹ h⁻¹ to 640 mg L⁻¹ h⁻¹ when the CO₂ concentration in air was increased to 0.23%. However, increasing the CO₂ further made no difference to extraction rates. In contrast, Norris (1989) and Norris et al. (1989) both found that the rate of pyrite dissolution of A. ferrooxidans was only slightly reduced by the use of air without additional CO₂. At moderate thermophilic temperatures, both the growth rate on ferrous iron (Wood and Kelly, 1985) and pyrite dissolution rate (Norris and Owen, 1993) were enhanced with CO₂ supplementation.

A number of significant problems exist when interpreting literature results in which CO₂ supplementation was tested as CO₂ supplementation cannot directly or even indirectly be related to dissolved CO₂ concentrations. Unlike for dissolved oxygen, no reliable dissolved CO₂ measuring instruments are available, particularly at high temperatures. Microbial cells in solution are only exposed to dissolved CO₂ concentrations. From existing literature no such measurements or comparisons can therefore be made. Furthermore, the interpretation of whether CO₂ supplementation was beneficial is often skewed by the fact that it did not result in improved bioleaching rates of the mineral under consideration. In cases where mineral dissolution effects are rate-limiting, CO₂ supplementation, and the resultant increase microbial growth rates and yields will not have an improved bioleaching effect. For this reason, the effect of CO₂ supplementation on microbial growth per se (and the potential use of CO₂ supplementation in bioleaching scenarios where mineral dissolution rates are not the limiting factor), has probably been overlooked in many instances. Another important factor that may influence the interpretation of the results is that some bacteria, for example, have been found to exhibit supplementary carbon fixing ability. According to this mechanism, some bacteria would switch to a different, more efficient, carbon fixing mechanism in environments where dissolved CO₂ is limiting (Norris, 1989).

The aim of this investigation was to determine the effects of various dissolved oxygen and CO₂ concentrations, in both the limiting and inhibitory ranges, on the microbial
activity of a representative thermophilic bioleaching archaea. Analyses of numerous continuous culture pilot scale tank reactors at laboratory facilities of BHP Billiton Johannesburg Technology Centre had revealed *Sulfolobus* sp. U40813 to be the most common mineral oxidizer across a wide range of mineral types at 78°C. This investigation, therefore, focuses on determining oxygen and CO₂ kinetics effects for this particular archaea at typical thermophilic tank bioleaching conditions.

2. MATERIALS AND METHODS

2.1 Microbial inoculum

The *Sulfolobus* sp. U40813 microbial inoculum used was maintained in an 8-L "fed-batch type" bioleaching mini-plant treating a chalcopyrite concentrate at 78°C under non-sterile conditions. Two litres of the reactor slurry was removed daily and replaced with fresh solids and nutrients. Mineral particles in the samples taken from the mini-plant were allowed to settle gravitationally for approximately 30 minutes. The resulting supernatant, with suspended cells, was used as inoculum. The microbial cell concentration in the inoculum were determined using a CellFacts biological particle analyzer (Microbial Systems Limited, UK), and were in the range of 4.4 x 10⁸ cells mL⁻¹ to 5.2 x 10⁸ cells mL⁻¹. A 100 mL microbial inoculum was added to 900 mL culture medium at the start of each run.

The microbial population in the bioreactor was identified using molecular microbiology techniques, including PCR amplification and denaturing gradient gel electrophoresis (DGGE). The sample was submitted to a series of low- and high-speed centrifugation steps using saline (0.856% m/v NaCl, pH 1.2) to remove debris and precipitates. DNA was extracted using the High Pure PCR template Preparation kit (ROCHE, Johannesburg, South Africa). A fragment of the 16S rRNA gene was amplified by PCR using primers annealing on either side of a variable region on the 16S rRNA gene of thermophilic archaea associated with the bioleaching of minerals. The PCR fragment was submitted to DGGE together with PCR fragments of known thermophilic archaea, which served as standards. The PCR product migrated in the same way as a *Sulfolobus* sp. (GenBank accession no. U40813).

2.2 Culture medium and growth conditions

Batch cultivations were carried out in a 1.5-L glass vessel, placed on a stirring hotplate with temperature feedback control and mixed with a magnetic stirrer bar at approximately 1000 rpm. The nutrient medium used in all the batch cultivations was the 9K medium supplemented with 3 g L⁻¹ potassium tetrathionate (Fluka, Steinheim, Switzerland) and 1 mL L⁻¹ of a trace element solution. The 9K medium comprised (per litre tap water): (NH₄)₂SO₄, 3 g; K₂HPO₄, 0.5 g; KCl, 0.1 g; Ca(NO₃)₂, 0.01 g; FeSO₄·7H₂O, 50 g (all ACE, Glenvista, South Africa) and MgSO₄·7H₂O, 0.5 g (Saarchem, Johannesburg, South Africa). The final concentration of the trace elements in the nutrient medium comprised (per litre); MnCl₂·4H₂O, 1.80 mg; Na₂Ba₄O₇·10H₂O, 4.50 mg; ZnSO₄·7H₂O, 0.22 mg; CuCl₂·2H₂O, 0.05 mg; Na₂MoO₄·2H₂O, 0.03 mg; VOSO₄·2H₂O, 0.03 mg and CoSO₄, 0.01 mg. The pH of the medium was initially adjusted between 1.5 and 1.55 with sulfuric acid and controlled at 1.5 during the cultivation with 4 N NaOH. Temperature was maintained throughout the experiments at 78°C. The redox potential was determined at 10 minute intervals using a combined electrode (Pt-Ag/AgCl in 3N KCl) connected to a 718 pH STAT Titrino (Swiss Lab Ltd, Rivonia, South Africa).
Dissolved oxygen and CO₂ was controlled by varying the proportions of nitrogen, carbon dioxide and oxygen in the influent gas through three 58505 S Brooks mass flow controllers (Alpret Control Specialists Ltd, Florida, South Africa). The gas mixture was sparged at a constant total flow rate of 1.5 L min⁻¹. The dissolved oxygen (DO, mg L⁻¹) concentration was measured with a polarographic oxygen probe (Knick, Berlin, Germany). An inlet CO₂ concentration of 5% was used in the oxygen cultivations, whereas the DO was maintained at 3 mg L⁻¹ in the CO₂ experiments.

3. RESULTS AND DISCUSSION

Although no specific precautions were taken to ensure aseptic conditions inside the bioreactor, the microbial population did not change during the duration of the experiments, due to the very selective culture conditions within the bioreactor. Prior shake-flask cultivations in the laboratory (data not shown) indicated that *Sulfolobus* sp. U40813 was not able to grow using sulphur in the form of potassium tetrathionate as sole energy source. Complete oxidation was, however, achieved using ferrous sulphate as sole energy source.

The effect of increasing dissolved oxygen concentrations on the rate of iron oxidation was investigated in a series of batch cultivations, each controlled at a different dissolved oxygen concentration. The microbial activity in each test was evaluated by monitoring the increase in redox potential and Fe³⁺ (g L⁻¹) over a 25 to 30 h period. Due to rapid precipitation on the redox probes at the high temperatures, the redox potential readings were not very reliable and, therefore, excluded from comparative interpretation of the effect of DO on microbial activity. Although the conditions inside the mini-plant (supplying the inoculum) were kept as constant as possible by daily additions of fresh solids and nutrients, the microbial activity of the inoculum was not identical for each batch cultivation.

In order to overcome variable inoculum activity, batch cultivations were conducted in sets of three, with a reference test (at a DO of 2.4 mg L⁻¹) included in every set of experiments. For every set of experiments, the maximum iron oxidation rate of the reference test was given a relative activity value of one, and the relative activity of the remaining two runs were expressed as a fraction of the maximum iron oxidation rate of the reference test. A relative activity of 1 is, therefore, equal to an iron oxidation rate of 0.524 (±0.081) g L⁻¹ h⁻¹. An example of a typical set of batch cultivations results is shown in Figure 1. The relative activities of the Fe³⁺ production curves at different dissolved oxygen concentrations are given in Figure 2. The relative activity data points were fitted using a four-parameter log normal fit function (Equation 1), where y and x are the relative activity and DO (mg L⁻¹), respectively.

\[
y = 0.1072 + 0.9029 \left[ -0.5 \left( \frac{\ln \left( \frac{x}{2.5077} \right)}{0.9908} \right)^{2} \right]
\]  

(1)
Figure 1. The Effect of a Dissolved Oxygen Concentration of 2.4 mg L\(^{-1}\) (●), 8.2 mg L\(^{-1}\) (○) and 9.0 mg L\(^{-1}\) (▼) on the Fe\(^{3+}\) Concentration During Autotrophic Growth of \textit{Sulfolobus} sp. U40813

Figure 2. The Effect of Dissolved Oxygen Concentrations on the Iron Oxidation Activity During Autotrophic Growth of \textit{Sulfolobus} sp.

Approximated by the fitted curve (Figure 2), a dissolved oxygen concentration between 1.5 and 4.1 mg O\(_2\) L\(^{-1}\) is required for optimal microbial activity. Within these ranges, the microbial activity was between 90% and 100% of the maximum activity obtained in the experiments. With an increase or decrease in the dissolved oxygen concentration outside this range, the microbial activity was affected negatively. The inhibitory effect was more severe at the very low dissolved oxygen concentrations (Figure 2) with a microbial activity of less than 50% of the maximum microbial activity resulting from a dissolved oxygen concentration of 0.7 mg L\(^{-1}\). However, although the inhibition at
the high oxygen concentrations (at and above a DO of 7.2 mg L\(^{-1}\)) (Figure 2) was not initially as severe as at the low oxygen concentrations, prolonged exposure to high oxygen concentrations resulted in a continual decrease in activity, which eventually resulted in a cessation of activity.

To investigate the effect of increased CO\(_2\) concentrations of the microbial activity, a series of batch cultivations in sets of three (similar as for the oxygen work) was conducted with increasing CO\(_2\) concentrations. An example of a typical set of batch cultivations results is shown in Figure 3. The relative activities for the individual experiments were determined as for the oxygen work, and are shown in Figure 4. A relative activity of 1 in this case is equal to 0.434 (±0.086) g Fe\(^{3+}\) L\(^{-1}\) h\(^{-1}\).

![Diagram](image)

**Figure 3.** The Effect of an inlet CO\(_2\) Concentration of 0.03% (▼), 5% (●) and 11% (○) on the Fe\(^{3+}\) Concentration During Autotrophic Growth of *Sulfolobus* sp. U40813

![Diagram](image)

**Figure 4.** The Effect of CO\(_2\) on the Iron Oxidation Activity During Autotrophic Growth of *Sulfolobus* sp. U40813

\[ R^2 = 0.96 \]
The relative activity data points were fitted using a four-parameter log normal fit function (Equation 2), where \( y \) and \( x \) are the relative activity and inlet CO\(_2\) concentration, respectively.

\[
y = 0.3450 + 0.8298 \left[ -0.5 \left( \frac{\ln \left( \frac{x}{11.02} \right)}{1.147} \right)^2 \right]
\]

Approximated by the fitted curve, 95\% or more of the maximum relative activity was achieved with inlet CO\(_2\) concentrations between 7\% and 17\%. With a further increase in the CO\(_2\) concentration (above a concentration of 17\%), the relative activities decreased slightly, but remained above 85\% of maximum relative activity. The iron oxidation rates were severely limited with a CO\(_2\) concentration less than 7\%, indicating that the CO\(_2\) supply was limiting in this range and inhibited the microbial growth rate.

**4. CONCLUSIONS**

Oxygen and carbon dioxide are essential nutrients for the growth of mineral-leaching bacteria. Maintaining optimal dissolved oxygen and CO\(_2\) concentrations will lead to improved mineral oxidation process and bacterial growth rates. This study indicated that the optimal dissolved oxygen concentration for growth was between 1.5 to 4.1 mg.L\(^{-1}\). At dissolved oxygen concentrations below 1.5 mg.L\(^{-1}\), oxygen was the growth-limiting nutrient and inhibited the ferrous utilization rate. The use of elevated dissolved oxygen concentration (above 4.1 mg.L\(^{-1}\)) also reduced the ferrous oxidation rates. This inhibition effect progressively increased as the DO was increased above 4.1 mg.L\(^{-1}\). Due to the sensitivity of *Sulfolobus* to elevated dissolved oxygen, the used of oxygen-enriched air to overcome low solubilities in tank bioleaching reactors at high temperatures will have to be strictly controlled in the narrow range.

The optimal inlet CO\(_2\) concentration for ferrous iron oxidation is predicted to be between 7\% and 17\%. Above 95\% of the maximum relative activity was obtained in these ranges. At elevated CO\(_2\) levels the ferrous oxidation rate was slightly lower, but remained above 85\% of maximum relative activity. The iron oxidation rates were however, severely limited with a CO\(_2\) concentration less than 7\%, indicating that the CO\(_2\) supply was limiting in this range and inhibited the microbial growth rate. Although this is much higher than is generally considered adequate for bioleaching, it is nevertheless the concentration that will allow for optimal microbial growth rates. The use of such a high concentration of CO\(_2\) would only be beneficial in cases where mineral dissolution is not a limiting factor, and where the additional cost could be justified against process improvement.

Based on the results from this investigation, it is suggested that tank bioleaching conditions should be maintained at a dissolved oxygen concentration of 2.8 mg.L\(^{-1}\) and that strict process control mechanisms should be in place to ensure that DO concentrations in tanks are maintained in the narrow range of 1.5 to 4.1 mg.L\(^{-1}\).

**REFERENCES**


Optimization study on bioleaching of municipal solid waste (MSW) incineration fly ash by *Aspergillus niger*

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Abstract

The bioleaching efficiency of heavy metals from MSW incineration fly ash depends on physical, chemical and biological factors as well as the leaching environment. Our research objective is to determine the factors that have a greater impact on the bioleaching process, as well as to determine the optimal bioleaching conditions and maximum leached metal concentrations. Four factors were investigated in this study: sucrose concentration, inoculum spore concentration, fly ash pulp density, and the time of addition of fly ash to the fungus *Aspergillus niger*. The Central Composite Design (CCD) was used in order to determine the co-optimum level of the factors, as well as to provide an insight into the interactions amongst these factors during bioleaching. Empirical models obtained through 2nd order (Taylor series approximation) regression provided the optimal bioleaching conditions. Results showed that sucrose concentration and pulp density were more important factors than spore concentration and the time of addition of the fly ash.

1. INTRODUCTION

Bioleaching processes are based on the ability of microorganisms to transform solid compounds, and result in soluble and extractable elements which can be recovered [1]. Bioleaching is affected by a number of parameters; its effectiveness is highly dependent on physical, chemical and biological factors such as (i) nutrient, (ii) oxygen and carbon dioxide supply, (iii) pH, (iv) temperature of leaching environment, (v) pre-culture period and inoculum used, (vi) resistance of microorganisms to metal ions, (vii) physical and chemical states of the solid residue, (viii) liquid-solid ratio, and (ix) bioleaching period [2]. Maximum yield of metal leaching can be achieved when these parameters have been optimized.

Most of the previous work on fungal bioleaching has been conducted using a "one-factor-at-a-time" technique. Unfortunately, this method fails to locate the region of optimum response, since the "one-factor-at-a-time" technique does not take into account any joint factor interactions on the bioleaching process. An alternative approach is the Response Surface Method (RSM), which simultaneously considers several factors at many different levels and determines the corresponding interactions among these factors using a smaller number of experimental observations. RSM has been employed to solve multivariate problems and optimize several responses in many types of investigations [3].

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In this study the RSM approach is adopted to locate the co-optimum levels of sucrose concentration, fly ash pulp density, spore concentration (i.e. inoculum concentration), and the time of fly ash addition, and to gain an insight into the interactions among these factors during the bioleaching process using *Aspergillus niger*. Using such an approach, the optimal of these four factors and the maximum concentration of the metal leached may be obtained.

2. MATERIAL AND METHOD

2.1 Characteristics of local fly ash

A locally-produced municipal solid wastes (MSW) incineration fly ash (at the Tuas South Incineration Plant, Singapore) was used in this optimization study. Some physical and chemical characteristics of the fly ash are shown in Tables 1 and 2 respectively.

Table 1. Physical characteristics of MSW fly ash

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Particle size Mean (µm)</td>
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</tr>
<tr>
<td>Particle size Median (µm)</td>
<td>15.62</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>0.4</td>
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<tr>
<td>Specific gravity (g/cm³)</td>
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</tr>
<tr>
<td>Surface area (m²/g)</td>
<td>5.75</td>
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<tr>
<td>Total pore volume (cm³/g)</td>
<td>0.01927</td>
</tr>
<tr>
<td>Porosity (cm³/cm³)</td>
<td>0.0062</td>
</tr>
</tbody>
</table>

Table 2. Chemical characteristics of MSW fly ash

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (mg/kg)</th>
<th>RSD</th>
<th>Element</th>
<th>Concentration (mg/kg)</th>
<th>RSD</th>
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<tr>
<td>Al</td>
<td>1,860</td>
<td>0.17%</td>
<td>Mg</td>
<td>9,110</td>
<td>0.19%</td>
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<tr>
<td>Ca</td>
<td>404,000</td>
<td>0.14%</td>
<td>Mn</td>
<td>71</td>
<td>3.4%</td>
</tr>
<tr>
<td>Cu</td>
<td>326</td>
<td>1.3%</td>
<td>Pb</td>
<td>2,070</td>
<td>2.89%</td>
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<tr>
<td>Fe</td>
<td>2,200</td>
<td>0.65%</td>
<td>Sr</td>
<td>185</td>
<td>0.39%</td>
</tr>
<tr>
<td>K</td>
<td>11,500</td>
<td>2.29%</td>
<td>Zn</td>
<td>7,890</td>
<td>1.38%</td>
</tr>
</tbody>
</table>

RSD: relative standard deviation

2.2 Fungi inoculum preparation

*Aspergillus niger* was obtained from Dr H. Brandl (University of Zürich, Switzerland) and was cultured according to the protocol in Bosshard *et al.* (1996) [4]. The number of spores was enumerated under a microscope (Olympus CX40) at 400x magnification using 0.1 mm depth haemocytometer (SUPERIOR MARIENFELD).

To culture in liquid medium, 1ml of spore suspension was added to 100 ml of standard sucrose medium [4] with composition (g/L): 100 sucrose (Biorad), 1.5 NaNO₃ (Merck), 0.5 KH₉PO₄ (Merck), 0.025 MgSO₄·7H₂O (Merck), 0.025 KCl (Merck), 1.6 yeast extract (Difco), and incubated in an incubator at 30°C with rotary shaking at 120 rpm. All reagents were of analytical grade. The liquid medium was autoclaved at 121°C for 15 minutes prior to inoculation. Bioleaching was performed in 250ml Erlenmeyer flasks with 100ml of sucrose medium.
2.3 Analytical method

Inductive coupled plasma-atomic emission spectrometry (ICP-AES) was used to analyze the metals in the bioleaching process. The concentration of glucose was measured using YSI 2700 biochemistry analyzer. Fructose, sucrose, citrate, oxalate and gluconate were analyzed using HP 1100 series high performance liquid chromatography (HPLC) with variable wavelengths detector (VWD) at 210 nm for organic acids detection and refractive index detector (RID) for fructose and sucrose detection. Operation conditions for HPLC consisted of a 30 cm x 7.8 mm i.d. Biorad Aminex HPX- 87H hydrogen resin ionic form analytical column (9 µm particle size). The analysis was carried out at temperature of 30°C. The mobile phase used was 5mM sulfuric acid (Merck, analytical grade), at flow rate of 0.5 ml/min.

2.4 Biomass determination

In the pure fungal culture, the mycelium was dried at 80°C for 24 hours. For biomass determination in the bioleaching tests, the mycelium together with fly ash, after filtration, was dried at 80°C for 24 hours, followed by ashing at 500°C for 4 hours in a Carbolite CWF 1100 furnace. Biomass was calculated gravimetrically after cooling in a dessicator [5].

2.5 Software for experimental design, statistical analysis, and optimization

The Central Composite Design, statistical analysis of the data, and the development of the regression equations were performed using the MINITAB package (Minitab Inc.). Generalized Algebraic Modeling System (GAMS, GAMS Development Corp.) was used to optimize the 2nd order statistical empirical models (see Equation (2)-(7) later).

3. RESULTS AND DISCUSSION

In bioleaching, the optimal solid-liquid ratio (pulp density) has to be determined. In fly ash fungal bioleaching, the fly ash is toxic to the fungus. Although an increase in pulp density may lead to a decrease in leaching efficiency, the metal concentration in the leachate may still increase. Too high a pulp density however will lead to toxicity and result in poor bioleaching. Hence, there must be a critical (optimal) value for the pulp density, which will result in the maximum metal concentration. For this reason, the pulp density and time of addition of fly ash are considered in this study. Sucrose was selected as another factor since it is the precursor of organic acid, which is the most important leaching agent in the bioleaching process. The choice of the inoculum has an important impact on bioleaching efficiency. Hence, the spore concentration was investigated in this study.

Table 3. Parameter levels of central composite design (coded value)

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Bioleaching Applications

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Table 4. Quantitative value of the coded parameter levels

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<td>X1</td>
<td>Sucrose concentration, g/l</td>
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<td>90</td>
<td>120</td>
<td>150</td>
<td>180</td>
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<td>X2</td>
<td>Spores concentration, *10^7</td>
<td>0.3</td>
<td>0.8</td>
<td>1.3</td>
<td>1.8</td>
<td>2.3</td>
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<tr>
<td>X3</td>
<td>Pulp density, w/v%</td>
<td>0.1</td>
<td>0.3</td>
<td>0.9</td>
<td>2.7</td>
<td>8.1</td>
</tr>
<tr>
<td>X4</td>
<td>Time of addition, days</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16</td>
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</tbody>
</table>

Bioleaching were conducted for duration of 26 days.

3.1 Central Composite Design (CCD)

The CCD design for this study consists of a 2^4 (i.e. 4 factors) full factorial design, with 2*4 axial points at (±α, 0, 0, ..., 0), (0, 0, ±α, 0), ..., (0, 0, 0, ..., ±α), and 7 central points at (0, 0, ..., 0), where α is the distance of the axial point from the center [3]. Random error (standard deviation) can be estimated from the 7 central points. For a four-factor design, α is usually set at 2.0 [3], since the distance of the axial points from the center point is given by α=2^4/4 [6]. Therefore a total of thirty-one (16 full factorial tests + 8 axial points + 7 central points) batch bioleaching tests were performed to satisfy a central composite design. Table 3 and Table 4 describe how each parameter was varied in the batch tests.

The data collected from the 31-batch runs were used to develop empirical models describing the experimental results. The models were generated using the method of least squares. The technique involved the estimation of model parameters for second order models of the form:

\[ E(Y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j \]

where \( E(Y) \) is the expected value of the response variable, \( \beta_0, \beta_i, \beta_{ij} \) are the model parameters, \( X_i \) and \( X_j \) are the coded factors being studied and \( k \) is the number of factors being studied [3]. In this study, \( E(Y) \) represents biomass concentration, metal concentration, and organic acid concentration for the different empirical model.
Numerous factors have varying impact on the bioleaching process. The relative importance of the four factors considered in this study, i.e. sucrose concentration, inoculum spore concentration, fly ash pulp density, and the time of addition of fly ash is manifested through the magnitude of the model coefficients, i.e. $\beta_i, \beta_j$.

3.2 Data analysis

It is necessary to perform an analysis of the residual from the model in order to determine the adequacy of the least squares fit. A normal probability of the residuals, and the residuals versus predicted values of the response variable are constructed to verify that the data follow a normal distribution [3]. The analysis of variance (ANOVA) table was used to analyze these data.

3.3 Models for Biomass, Al, Fe, Zn, citric acid and gluconic acid concentration

Equations (2)-(7) were obtained from the 31-batch runs using the MINITAB software. The data followed a straight line in the normal probability plot of the residual, thus representing a normal distribution and supporting the assumptions of the empirical model. The plot of the residuals versus predicted values of the response variable also supported the assumption of a normal distribution.

3.3.1 Biomass concentration model

Equation (2) shows that all the four factors, i.e. sucrose concentration ($X_1$), spore concentration ($X_2$), pulp density ($X_3$), and the time of addition of the fly ash ($X_4$) have an important impact on the biomass concentration in bioleaching process. Equation (2) also shows an interaction between sucrose concentration ($X_1$) and spores concentration ($X_2$), sucrose concentration ($X_1$) and pulp density ($X_3$), sucrose concentration ($X_1$) and the time of addition ($X_4$), spores concentration ($X_2$) and pulp density ($X_3$), and pulp density ($X_3$) and the time of addition ($X_4$).

\[
\text{Biomass} = 2.45 + 0.0131X_1 - 0.2312X_2 - 0.758X_3 + 0.1305X_4 - 0.3304X_1^2 - 0.1002X_2^2 \\
-0.1254X_3^2 - 0.1485X_4^2 - 0.1741X_1X_2 - 0.2637X_1X_3 + 0.2801X_1X_4 - 0.1049X_2X_3 - 0.1882X_3X_4
\]

(2)

It can be concluded that the sucrose concentration had a significant interaction with the spore concentration, pulp density and time of addition in the production of biomass, since sucrose is the only source of carbon in the process (and which is a limiting nutrient in the biomass production).

3.3.2 Al concentration model

Equation (3) shows that all the four factors, i.e. sucrose concentration ($X_1$), spore concentration ($X_2$), pulp density ($X_3$), and time of addition ($X_4$) exert an important influence on Al concentration in the bioleaching process. Equation (3) also shows an interaction between sucrose concentration ($X_1$) and pulp density ($X_3$), spores concentration ($X_2$) and pulp density ($X_3$), and spores concentration ($X_2$) and time of addition ($X_4$).

\[
\text{Al} = 5.58 + 1.56X_1 + 0.0564X_2 + 3.34X_3 + 0.115X_4 - 0.496X_1^2 - 0.181X_2^2 + 0.436X_3^2 - 0.182X_4^2 + 1.80X_1X_3 + 0.155X_2X_3 - 0.127X_2X_4
\]

(3)

The model showed that pulp density had a significant interaction with the sucrose concentration and spore concentration in the leaching of Al.
3.3.3 Fe concentration model

Equation (4) shows that the spore concentration ($X_2$) over the range investigated was not a factor in the leaching of Fe. The equation also shows that the pulp density ($X_3$) had a significant interaction with the sucrose concentration ($X_1$), and that there was only one interaction amongst the four factors, i.e. between sucrose concentration ($X_1$) and the pulp density ($X_3$).

$$Fe = 5.73 + 2.06X_1 + 3.24X_3 - 0.317X_4 - 0.89X_1^2 - 0.387X_4^2 + 2.05X_1X_3$$

(4)

3.3.4 Zn concentration model

Equation (5) shows that in the leaching of zinc, there was an interaction between sucrose concentration ($X_1$) and pulp density ($X_3$), between sucrose concentration ($X_1$) and the time of addition ($X_4$), and between spores concentration ($X_2$) and pulp density ($X_3$).

$$Zn = 35.69 + 1.89X_1 + 1.27X_2 + 18.64X_3 + 0.488X_4 - 1.89X_1^2 - 1.58X_2^2 - 2.7X_4^2 + 11.13X_1X_3 + 1.239X_1X_4 + 2.15X_2X_3$$

(5)

The pulp density had a significant interaction with the sucrose concentration in the Zn leaching, as the only source of Zn in the process is the fly ash. Sucrose is converted to citric acid and gluconic acid, which are the most important leaching agents in bioleaching process.

3.3.5 Citric acid concentration model

Equation (6) shows that there was no interaction between any of the four factors. Indeed, it is surprising that no interaction between sucrose concentration and pulp density was observed. Our results are similar to that of Crolla et al. [3], where there were no interactions between the various factors, and only linear and square terms were obtained [16].

$$Citric = 47.0 + 9.08X_1 - 0.928X_2 - 2.97X_3 + 3.34X_4 - 7.85X_1^2 - 8.39X_2^2 - 4.58X_3^2 - 6.73X_4^2$$

(6)

3.3.6 Gluconic acid concentration model

Equation (7) shows that there was an interaction between sucrose concentration ($X_1$) and pulp density ($X_3$), and sucrose concentration ($X_1$) and time of addition ($X_4$). Sucrose concentration had a significant interaction with the pulp density. Low pH (<3) inhibits the activity of the enzyme glucose oxidase. It is known that at pH > 3, the presence of fly ash presence activates glucose oxidase and results in the greater production of gluconic acid [7].

$$Gluconic = 27.3 + 69.4X_1 - 1.5X_2 + 37.8X_3 - 10.3X_4 + 28.7X_1^2 + 3.84X_2^2 + 20.6X_3^2 + 6.37X_4^2 + 20.8X_1X_3 - 5.82X_1X_4$$

(7)

3.4 Summary

Equations (2)-(7) are objective functions. In general, it can be seen from these equations that the coefficients of sucrose concentration ($X_1$) and pulp density ($X_3$) were larger than those of spore concentration ($X_2$) and time of addition ($X_4$). Since a higher coefficient represents greater importance, this optimization study showed that sucrose concentration and pulp density were more important than spore concentration and the time of addition of the fly ash.
The optimized values, obtained by the maximization of these objective functions are summarized in Table 5. The table also shows that gluconic acid (at 281mM) is the main bioleaching agent and is produced under similar optimal condition as the optimised values of the leached metals. The optimal time for the addition of fly ash for gluconic acid production is 0 days. This is consistent with the fact gluconic production is optimal at a high pH [7] (since the earlier the time of addition, the higher the pH). The optimal the time of addition of the fly ash for metal leaching varied from 6 to 9 days. The optimal sucrose concentration for metal leaching and gluconic acid production is about 150 g/l, a value 50% higher than Bosshard’s medium. The corresponding optimal pulp density is 2.7%.

### Table 5. Optimal value for bioleaching process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sucrose Conc (g/l)</th>
<th>Spores Conc. (*10^7/ml)</th>
<th>Pulp density (%)</th>
<th>Time of addition (days)</th>
<th>Maximum Value</th>
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<td>Biomass</td>
<td>153</td>
<td>0.8</td>
<td>0.1</td>
<td>16</td>
<td>50.3 g/l</td>
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<tr>
<td>Al</td>
<td>150</td>
<td>1.6</td>
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<td>8.5</td>
<td>12.3 mg/l</td>
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<tr>
<td>Fe</td>
<td>150</td>
<td>0.3-2.3</td>
<td>2.7</td>
<td>6.6</td>
<td>12.3 mg/l</td>
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<td>Zn</td>
<td>150</td>
<td>1.8</td>
<td>2.7</td>
<td>9.3</td>
<td>77.6 mg/l</td>
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<td>Citric</td>
<td>108</td>
<td>1.3</td>
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<td>9</td>
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<td>Gluconic</td>
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<td>2.7</td>
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### 4. CONCLUSIONS

Four factors (i.e. sucrose concentration, spore concentration, pulp density and the time of fly ash addition) were investigated in this bioleaching study. The Central Composite Design (CCD) was used in order to determine the co-optimum level of the factors, as well as to provide an insight into the interactions amongst these factors during bioleaching. Empirical models obtained through 2nd order (Taylor series approximation) regression provided the optimal bioleaching conditions. Results showed that sucrose concentration and pulp density were more important factors than spore concentration and the time of addition of the fly ash.

### ACKNOWLEDGEMENTS

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### REFERENCES


Production of an *Acidithiobacillus ferrooxidans* biomass using electrochemical regeneration of energetic substrate

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*Laboratoire d'Electrochimie et de Physico-chimie des Matériaux et des Interfaces, UMR 5631 CNRS-INPG-UFJ, ENSEEG BP75, 38402 St Martin d'Hères, France*

**Abstract**

The aim of this study was to develop and to optimise a process for the production of an *Acidithiobacillus ferrooxidans* biomass. This process includes a classical bioreactor associated with two working loops. The first loop involves a gas/liquid contactor, providing the dissolved dioxygen amount required for bacterial growth. A preliminary study of the biomass respiration has allowed to select static mixers because of their efficiency as gas-liquid transfer device [1]. The second loop is devoted to the regeneration of the bacteria substrate (Fe^{2+}) and includes an electrochemical reactor (E3P©: Pulsating Porous Percolated Electrode). Two bacterial culture modes have then been considered: a batch mode *i.e.* without substrate regeneration and a continuous culture mode involving the electrochemical regeneration of the substrate. With this latter, the bacterial growth is maintained in its exponential phase during more than 100 hours by applying a maximum intensity of 40 A. This operating configuration resulted in a protein concentration up to 110 mg L^{-1}, *i.e.* a production yield of *Acidithiobacillus ferrooxidans* eight times greater than that achieved under the batch mode. This process appears to be among the most efficient existing ones when comparing both its productivity and operating scale.

**Keywords:** *Acidithiobacillus ferrooxidans*, continuous electrochemical regeneration, static mixers, high-cell density

1. **INTRODUCTION**

*Acidithiobacillus ferrooxidans* is a well-known acidophilic, aerobic and chemolithotrophic bacteria which generates its own energy from the oxidation of iron and reduced sulphur compounds. Moreover, this organism is able to grow optimally at acidic pH. Therefore, it is of a great interest for industrial processes such as coal desulfurisation or core bioleaching. On the other hand, this bacteria can be used in wastewater treatment processes and specially for an efficient removal of metallic ions [2,3]. However such an application at a large scale requires important bacteria amounts. This is the reason why a part of our research works is focused on designing and optimising a process for the production of a high-concentrated *Acidithiobacillus ferrooxidans* biomass. This original process (Figure 1) is composed of a classical biological reactor associated with an electrochemical reactor and an aeration system.

* Nicolas.Gondrexon@lepmi.inpg.fr

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* Image: Logo of the 15th International Biohydrometallurgy Symposium (IBS 2003) held in Athens, Hellas. The logo features the event title and location.

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Figure 1. Scheme of the high-density biomass production process

2. MATERIALS AND METHODS

2.1 Strains and culture medium

The high-density biomass cultivation is performed with the DSM 583 strain in the so-called 9K Grenoble medium [2] derived from that proposed by Silverman [4]. Ferrous ions (Fe$^{2+}$) are the major compounds of this culture medium (see Table 1) and act as the single source of mineral energy for the *A. ferrooxidans* biomass growth.

<table>
<thead>
<tr>
<th>Table 1. 9K Grenoble Culture medium composition</th>
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<tr>
<td>Composition</td>
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<tr>
<td>FeSO$_4$.7H$_2$O</td>
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<td>MgSO$_4$.7H$_2$O</td>
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<td>K$_2$HPO$_4$</td>
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<tr>
<td>H$_2$SO$_4$</td>
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</table>

2.2 Electrochemical reactor

The electrochemical unit is a E3P® reactor (Pulsating Porous Percolated Electrode) [5], classically used to remove heavy metals from dilute aqueous solutions. Its role is to regenerate the substrate (Fe$^{2+}$) through Fe$^{3+}$ cathodic reduction, the ferric ions being issued from the Fe$^{2+}$ oxidation due to bacteria activity.

This electrochemical reactor involves a porous granular carbon cathode and a circular titanium anode separated by an anion exchange membrane (IONAC®). The culture medium containing *A. ferrooxidans* biomass flows throughout the cell under a pulsating mode. This flow mode prevents biomass attachment and biofilm formation onto the granular carbon matrix surface. Moreover, the three-dimension electrode offers a high active surface owing to the high values of both granular specific surface area and mass transfer coefficient [3].
2.3 Biological reactor and gas/liquid contactor

The bioreactor is a 50 L spherical pyrex tank. Its temperature is controlled at 30°C that is the optimal bacterial growth temperature. The reactor is aerated and continuously stirred by a closed recirculation loop involving static mixers (SMV Sulzer® DN20). This loop ensures the gas-liquid transfer for a suitable supply of dioxygen and carbon dioxide with respect to the growth and production of a high-density *A. ferrooxidans* culture.

3. MATERIALS AND METHODS

3.1 Culture conditions

Bacterial cultures in the batch mode were precultured at 30°C with the 9K Grenoble medium in a 4 L bioreactor. This laboratory-scale reactor was well stirred and aerated by a simple air flow. At the end of the preculture, the biomass was harvested to inoculate the 40 L reactor. The initial protein concentration in the culture medium was close to 1 mg L⁻¹.

3.2 Analytical method for the bacterial growth

The cultivated biomass was quantified by determining the protein concentration with the Lowry method [6].

The bacterial growth was estimated by measuring the bacterial metabolism characterised by the ferrous oxidation associated to the Fe²⁺/Fe³⁺ ratio. The determination of the substrate oxidation degree depends on the culture mode. For the batch mode, the Fe²⁺ concentration was determined directly by the phenanthroline method [7]. Total Fe concentration was estimated after prior reduction of Fe³⁺ ions by hydroxylamin.

For biomass cultures in continuous mode, the Fe³⁺/Fe²⁺ ratio was deduced, via the Nernst law, from the redox potential given by a redox probe placed directly within the culture medium.

4. RESULTS AND DISCUSSION

4.1 Experimental configuration for the determination of the growth model parameters

A bacterial culture was carried out within a 40 L reactor working in batch conditions. Static mixers provided the dissolved dioxygen necessary for *A. ferrooxidans* growing. Measuring the remaining substrate and biomass concentrations versus time allowed to determine the growth rate.

4.2 Growth model

The postulated model for the *A. ferrooxidans* growth in the 9K Grenoble medium is characterised by the following ordinary non-linear differential equations:

\[
\begin{align*}
\frac{dX}{dt} &= \mu X \\
\frac{dS}{dt} &= -\mu X \\
&= \frac{Y_{XS}}{Y_{XP}} \\
\frac{dP}{dt} &= \mu X \\
&= \frac{Y_{XP}}{Y_{XP}} \\
\end{align*}
\]
The substrate/product yield can be considered as equal to unity because of the stoechiometric coefficient related to ferrous iron oxidation by *A. ferrooxidans* according to:

\[
4 \text{Fe}^{2+} + \text{O}_2 + 4 \text{H}^+ \rightarrow 4 \text{Fe}^{3+} + 2\text{H}_2\text{O} \tag{4}
\]

and a simple relation between concentrations is thus obtained:

\[
Y_{X:S} = Y_{X:P} \tag{5}
\]

Assuming the bacterial growth kinetics obeys the Contois’ model [8], the growth rate can be expressed as:

\[
\mu = \mu_{\text{max}} \frac{S}{K_s + X} \tag{6}
\]

where appear the maximum specific growth rate $\mu_{\text{max}}$, the substrate concentration $S$, the bacterial concentration $X$, and the substrate accessibility constant $K_s$.

4.3 Implementation

The parametric identification for the Contois’ model was performed by fitting the growth experimental data owing to the Fletcher-Reeves optimisation [9] available with the Matlab® software. Parameters $\mu_{\text{max}}$ (h$^{-1}$) and $K_s$ (g mg$^{-1}$) have been computed despite the dispersion of the experimental data for biomass concentration. In this way, the yield parameters were fixed by calculating both ferrous iron and protein concentrations between the beginning and the end of bacterial cultures. The state variables $X_0$, $S_0$ and $P_0$ refer to the beginning of the bacterial culture. Values of both fixed and identified parameters are given in Table 2.

<table>
<thead>
<tr>
<th>Fixed values</th>
<th>$X_0$ (mg L$^{-1}$)</th>
<th>$S_0$ (g L$^{-1}$)</th>
<th>$P_0$ (g L$^{-1}$)</th>
<th>$Y_{X:S}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.3</td>
<td>5.8</td>
<td>0.8</td>
<td>2.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Identified values</th>
<th>$\mu_{\text{max}}$ (h$^{-1}$)</th>
<th>$K_s$ (g mg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.131</td>
<td>0.098</td>
</tr>
</tbody>
</table>

The evolution of protein and Fe$^{2+}$ concentrations in the culture medium versus time are shown in Figure 2. As it can be seen, the modelled growth curves are in good agreement with experimental results.
Figure 2. Biomass growth curve of A. ferrooxidans (DSM 583), 9K Grenoble medium: ● biomass cell, □ substrate, - from the model

It is thereby possible to determine the instantaneous biomass production rate (mg protein L⁻¹ h⁻¹) during the growth phase as well as the bacterial growth cycle during which A. ferrooxidans growth is maximal. Simulated curves resulting from the Contois’ growth model are shown in Figures 3 and 4. The maximal biomass production rate (0.87 mg protein L⁻¹ h⁻¹), deduced from Fig. 3, is obtained at the end of the exponential growth phase. This optimal value is reached when 65% of the initial substrate has been biologically oxidised (see Fig. 4.).

Figure 3. Simulated biomass production rate vs. time according to the Contois’ model
Bioleaching Applications

Figure 4. Simulated biomass production rate vs. Fe$^{3+}$/ total iron ratio within the culture medium

### 4.4 Continuous biomass production by electrochemically-assisted cultivation

Under these working conditions, the product of bacterial metabolism (Fe$^{3+}$) was reduced by using an E3P® electrochemical reactor. A schematic reaction diagram for this bio-electrochemical process is proposed in Figure 5.

![Chemistry Diagram](image)

**Figure 5. Diagram of the chemistry during electro-cultivation of A. ferroxidans in the process**

The substrate concentration (Fe$^{2+}$) was monitored owing to an oxidoreduction potential PID controller using Labview™ software. The redox potential set point value was selected to obtain the optimal bacterial growth conditions and particularly a 60%
Fe\textsuperscript{3+}/total iron ratio was fixed. Moreover, the pH was kept constant to 1.4 by addition of concentrated H\textsubscript{2}SO\textsubscript{4} solution preventing precipitation of jarosite.

Experimental results obtained in such conditions are illustrated in Figure 6. For 17 hours, the biomass oxidises the substrate (Fe\textsuperscript{2+}) and tends to develop in an exponential phase. Thereby, the Fe\textsuperscript{2+} concentration decreases down to approximately 40% of the total iron concentration present in the cell culture (2.64 g L\textsuperscript{-1}).

At the 18\textsuperscript{th} hours, the electrochemical reactor starts on to continuously regenerate the substrate in order to maintain such a physiological bacteria state for more than 100 hours. Fe\textsuperscript{2+} concentration is thus kept constant. The cell density increases from 1.3 mg protein.L\textsuperscript{-1} to more than 110 mg protein.L\textsuperscript{-1} at the end of the culture.

![Figure 6. Growth curves for A. ferrooxidans using electrochemically-assisted cultivation: ● biomass concentration, — Fe\textsuperscript{2+} concentration](image)

5. CONCLUSION

A biomass production process has been designed coupling a classical biological reactor, a E3P\textsuperscript{©} electrochemical reactor, and static mixers. After a 115-hour cultivation period, a protein concentration up to 110 mg L\textsuperscript{-1} was reached. Owing to its experimental performances and operating scale, the process proposed here may be regarded as one of the most efficient by comparison with previous results reported in literature [10-11]. Further experiments are now required to investigate the continuous production of A. ferrooxidans biomass for longer operating duration and to point out the possible limiting factors of the process.

NOMENCLATURE

\[
\begin{align*}
X_0 &= \text{Initial biomass concentration (protein) \quad g L}^{-1} \\
X &= \text{Biomass concentration (protein) \quad g L}^{-1}
\end{align*}
\]
$S_0$ = Initial substrate concentration (Fe$^{2+}$) g L$^{-1}$

$S$ = Substrate concentration (Fe$^{2+}$) g L$^{-1}$

$P_0$ = Product concentration (Fe$^{3+}$) g L$^{-1}$

$P$ = Initial product concentration (Fe$^{3+}$) g L$^{-1}$

$Y_{XS}$ = Biomass/substrate yield mg g$^{-1}$

$Y_{XP}$ = Biomass/product yield mg g$^{-1}$

$\mu$ = Biomass specific growth rate h$^{-1}$

$\mu_{max}$ = Maximum biomass specific growth rate h$^{-1}$

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Removal of dibenzothiophene from fossil fuels with the action of iron(III)-ion generated by *Thiobacillus ferrooxidans*: Analytical aspects

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Abstract

Among various classes and numerous kinds of organic compounds with sulphur, identified in fossil fuels, the most represented is dibenzothiophene (DBT) and its derivatives. Therefore, this compound can be considered to be the model substrate of organically bonded sulphur in fossil fuels. In focus of our interest is bacterial removal of sulphur (desulphurization) from oil (bituminous) shales as fossil fuel and potential/alternative source of “synthetic” hydrocarbons liquid fuels.

The application possibility of *Thiobacillus ferrooxidans* for removal of organic sulphur out of the fossil fuels is in the following idea: “To convert DBT into the (water soluble) sulphur-free form with the oxidation by bacterially generated-regenerated iron(III) sulphate from pyrite”!

The results presented in this paper are just an initial step in realization-checking of the mentioned idea and relate to the development and adaptation of well-known analytical procedures for the quantitative analysis and monitoring of changes on DBT molecule as the model substrate. The analytical methods are also checked in the interaction of DBT with potential-model solutions: sulphuric acid solution (pH 2.5), iron-free medium 9K, medium 9K and medium 9K in which iron(II) is chemically oxidated beforehand. The shake flasks testing technique has been applied in these experiments.

For the quantitative analysis, gas chromatography is the choice method, while the UV spectrophotometry is the most convenient for fast detection of changes in the DBT structure. $^1$H and $^{13}$C-NMR and mass spectrometry are the instrumental structural methods by means of which it is possible to monitor DBT transformation pathway.

*Keywords:* analytical aspects, dibenzothiophene, fossil fuels, removal, *Thiobacillus ferrooxidans*
1. INTRODUCTION

Reduction of emission of sulphur dioxide as the combustion product of various sulphur forms from fossil fuels is the imperative in protection of environment in forthcoming years which directly depends on the content of sulphur in them. Thus, solving the problem is brought down to reduction of sulphur quantity in fuels resp. their desulphurization in order to avoid post-combustion refining of gases from sulphur. This global ecological problem is followed also by the recommended an/or adopted strict legislature (in relation to the permitted emission of sulphur dioxide in the air and the total sulphur content in fuels), which varies in some countries and the regions of the world [1,2].

Sulphur in solid fossil fuels (coals and oil shales) is found in three basic forms: sulphate, pyrite and organic sulphur [3-5]. Elementary sulphur is also present in fossil fuels [6]. In crude oil are identified the most diversified classes of organosulphuric compounds: from simple alkylthiol-mercaptan to polycondensed benzophenanthrotiophene, their derivatives and polyaromatic compounds with more sulphur atoms and other heteroatoms [7, 8]. Over 200 organic compounds containing sulphur is identified in petroleum [9]. DBT and its derivatives are found in all fossil fuels and they are the main carriers of organic sulphur [10, 11].

![Structural formula of DBT and atoms numbering](image)

The amount of overall sulphur and individual forms, classes and compounds of sulphur in fossil fuels considerably varies subject to origin-source-deposit. Its distribution is not constant, not even within the same deposit [4-5].

Generally, desulphurization of fossil fuels, herein only bioprocessing is studied, understands separation of the basic sulphur forms. Sulphate sulphur is not a problem since sulphates are well soluble in water. Bacterial depyritization and segregation of elementary sulphur with high efficiency (over 90%) is possible by action of *Thiobacillus ferrooxidans* (*Th. ferrooxidans*) and mixed cultures *Th. ferrooxidans* i *Th. thiooxidans* [12-14] and by thermophilic *Sulfolobus acidocaldarius* [15,16]. The biggest problem is separation of organic sulphur. A great number of microorganisms in heterotrophic conditions of cultivation-growth, among which also *Sulfolobus acidocaldarius* [17] is tested as the “biological agent” for removal of organic sulphur, both from coals and from crude oil and hydrocarbons fuels at which different efficiencies are obtained but considerably less than depyritization [18-20]. DBT is a typical representative of organosulphuric compounds in fossil fuels and therefore it can be considered a model substance resp. a model substrate of organic sulphur. The pathways of microbiological separation of sulphur from DBT are different, but essentially reduced to: (1) removal of sulphur with no cleavage of carbon-carbon (C-C) bonds (“4S” pathway) with occurrence of diphenyl and its ortho-hydroxy derivatives [21], and (2) with cleavage of C-C bonds at which DBT is the sole source of carbon, sulphur and energy, while as a result are obtained in the final products of mineralization but also aromatic carbonylic compounds, subject to the applied bacterial species [22,23]. Sulphate is the product of microbiological desulphurization of DBT.
Oil (bituminous) shales (compact sedimentary rocks of homogeneous fine-grained composition) are potentially and alternatively an important source of "synthetic" hydrocarbons liquid fuels [24], due to which they are the subject of geochemical investigations and economic interests. Therefore, this fossil fuel is the subject of our many-year researches. Majority of oil shales (about 80%) are inorganic components viz. carbonates, alumino-silicates and pyrite. Kerogen, as insoluble and of heterogeneous macromolecular crosslinking structure, is a dominant organic substance (approx. 95% out of the total organic matter) while, in the organic solvents, soluble bitumen is present in the quantities of several percentages [25-26]. Fundamental organic-geochemical studies of kerogen require preparation of its concentrates with relatively pure and unaltered kerogen. Removal of carbonates and alumino-silicates is realized by the action of mineral acids (diluted hydrochloric and concentrated hydrofluoric acids). Partial removal of alumino-silicate is also possible by application of *Bacillus circulans* strains (bacterial desilification) [27-29]. Bacterial removal of pyrite which is closely associated with kerogen from crude oil shale and its concentrates by *Th. ferrooxidans* is exceptionally efficient (approx. 97%), at which rich concentrates of kerogen with unchanged organic substance are obtained [30-34].

The structure of bituminous coal with included pyrite crystals [35], shown in Fig. 1 is the most alike the characteristic crosslinked structure of the organic part of bituminous shales with pyrite.

![Figure 1. Representative structure of bituminous coal with included pyrite crystals. Arrows indicate the atoms of organically bonded sulphur](image-url)
1.1 Scientific hypothesis of DBT removal from fossil fuels by the action of iron(III)-ions generated by *Th. ferrooxidans*

Microbial desulphurization of DBT is a complex enzyme process. Our scientific hypothesis of DBT removal from fossil fuels by the action of iron(III) ion generated by *Th. ferrooxidans* is based on the following idea: "To convert DBT into the (water soluble) sulphur-free form with the oxidation by bacterially generated-regenerated iron(III) sulphate from pyrite"! The hypothesis can be schematically shown as in Fig. 2.

![Schematic presentation of hypothesis](image)

**Figure 2. Schematic presentation of hypothesis. DS-DBT-Desulphurized DBT. R and R' = -H and/or -OH**

Hypothetical molecular chemical equation resulting from the schematic hypothesis diagram would have the following form:

\[
R=S + 2 \text{Fe}_2\text{(SO}_4\text{)}_3 + 4 \text{H}_2\text{O} \rightarrow \text{RH}_2 + 4 \text{FeSO}_4 + 3 \text{H}_2\text{SO}_4
\]

Thermodynamic computations [36-39] of DBT desulphurization (R=S) to biphenyl (RH₂) by the action of iron(III) sulphate according to the equation (1) indicate that free energy of this process under standard conditions (unit activity and 298 K) and at pH 2.5 has the value \(\Delta G_{298}^{\circ} = -91 \text{ kJ}\). This means that the process can be spontaneous developed, being one of the proofs that theoretically the hypothesis is correctly set.

The presence of pyrite as the source of iron(III)-ion (oxydans) together with DBT should favor oxidation of thiophenic sulphur.

In case the hypothesis is proved, this form of desulphurization would be bioprocessing by an indirect mechanism.

1.2 Aim and scope of the study

This paper represents an initial step in realization of the stated idea-checking of its correctness. Therefore, its essential aim is characterization of DBT as a model substrate and checking and adaptation of analytical procedures for its determination. For that purpose it was necessary to solve the following issues:

a) to characterize the commercial DBT by the instrumental analytical methods;

b) to adapt the methods of UV spectrophotometry and gas chromatography for fast proving the changes of DBT structure and its determination; and

c) to check applicability of analytical procedures and DBT stability in the interaction with model solutions.

The literature data [40] indicate that the acceptable value for an average DBT concentration in fossil fuels is approx. 25 mg/kg(L). Therefore, the concentrations of
solutions for analytical purposes as well as in model solutions would be close to this value.

2. MATERIAL AND METHODS

2.1 Model substrate

DBT purity 98% (Aldrich, Catalog No. D3,220-2). Molecular formula C_{12}H_{8}S, relative molecular mass 184.26, CAS No. [132-65-0].

2.2 Model solutions and model systems

Solution of sulphuric acid (0.27 mL concentrated acid in 1 L solution) with pH about 2.5 (designation: pH 2.5), medium 9K free from iron(II) sulphate heptahydrate (designation: 0 K), medium 9 K [41] (designation: 9 K) and medium 9 K in which iron(II) sulphate is chemically oxidated into iron(III) sulphate (designation: 9 K 3). pH of all solutions is adjusted to about 2.5.

Model solution 9 K 3 is obtained by oxidation of medium 9 K with 30% (m/m) hydrogen peroxide with heating to complete decomposition of peroxide. Peroxide consumption is about 10 mL/L.

The concentrated solution DBT in ethyl acetate (EtOAc) is added in solutions so that the final concentration of DBT should be 25 mg/L. Then, the solvent is separated on the rotary vacuum evaporator. Thus obtained model systems are slightly opalescent and DBT is homogeneously suspended. They are further "cold sterilized" and poured into sterile Erlenmeyer flasks equipped with sterile microbiological stoppers.

2.3 Chemicals

All used chemicals are of pro analysi purity, resp. of the appropriate purity for application in analytical purposes. EtOAc was additionally refined by distillation. Demineralized water was used.

2.4 Shake flasks testing

Experiments were conducted in the room termostated at (28±1°C) on the reciprocating shaker (New Brunswick Scientific, model R-82) with 200 strokes/min. Erlenmeyer flasks of the same geometry and the total volume of 5 L with 1 L solution (volume ratio 1:5) were used. They were plugged with identical microbiological stoppers made of cotton and gauze to ensure constant and reproducible oxygen transfer in the same conditions. The tests lasted 15 days.

2.5 Analytical methods

The following methods and instruments were used.

**DBT extraction from model system.** pH solution was adjusted prior to DBT extraction by EtOAc. Then the solutions were saturated by the solvent (about 50 mL/L) and multiple-extracted in the ultrasonic bath for 30 min. Every time the organic phase was separated in the separatory funnel. The collected fractions were dried overnight by anhydrated sodium sulphate and after filtration the total volume was filled up to 100 mL.

**pH.** pH-meter (Radiometer, type PHM 26) with a combined electrode PHC 2401 of the same manufacturer.

**UV spectrophotometry.** UV-Vis spectrophotometer: Beckman, model DU-50. Quartz cuvettes 1 cm.
FT-IR spectra. KBr pellet (approx. 1:100). Instrument: Perkin Elmer, model PE 1725 X.

NMR spectra. $^1$H-NMR in acetone-$d_6$ (at 200 MHz), $^{13}$C-NMR in DMSO-$d_6$ (at 50 MHz), ambiental temperature. Internal standard TMS. Instrument: Varian, model Gemini 2000.

Mass spectrometry: Chemical ionization (CI) with iso-butane. Mass spectrometer: Finnigan-Mat, model 8239.

Gas Chromatography (GC). GC analysis was conducted on the gas chromatograph (Varian, model 3400) with flame-ionization detector (FID). Temperature program: 50-285°C, 15°C/min. Hydrogen flow rate 1 mL/min. Column (J&W Scientific): length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm and filling DB-5. DBT standards were prepared by weighing the required substance amount and by dissolving it in the appropriate EtOAc volume.

3. RESULTS AND DISCUSSION

3.1 Spectral DBT characterization

UV spectrum of solution of the model substrate, concentration 25.1 mg/L in EtOAc in relation to solvent, is shown in Fig. 3. Characteristics of the spectrum in ethanol on the basis of literature data [42] is shown in table on the figure. Compared with literature data it can be seen that the peak at 237 nm is missing which is the result of application of EtOAc as solvent and the instrument is double beam.

Sharp signals in the spectrum correspond to the positions stated in literature but are shifted by 3-6 nm toward the ultraviolet region, which is not unusual, the more so because different solvents are in question.

Since the characterization on the basis of UV spectrum is fast and simple, it stands as the optional method for quality analysis of changes in DBT structure in the future work.

![Figure 3. UV spectrum of DBT](image-url)
FT-IR spectrum of the used DBT is shown in Fig. 4. Signals in the range of 1600-400 cm\(^{-1}\) can be considered characteristical for DBT [43] which means that the changes in this spectrum range and appearance of other signals indicate the changes in DBT molecule structure.

![FT-IR spectrum of DBT](image)

**Figure 4. FT-IR spectrum of DBT**

The protonic NMR spectrum of the commercial DBT (Fig. 5) compared with the literature one (inserted table) [44] does not show any differences in spite of 98% purity. Signals at about 2.1 and 2.9 ppm are the solvent impurities.

![1H-NMR spectrum of DBT](image)

**Figure 5. \(^1\text{H}-\text{NMR spectrum of DBT}**

\(^{13}\text{C}-\text{NMR spectrum model substrate shown in Fig. 6. Compared with the literature data (inserted table) [42], as also in the case of} \(^1\text{H} spectrum, coincides well. Signals at about 40 ppm are the solvent impurities.**

![13C-NMR spectrum](image)
On the basis of NMR characteristics, it can be said that in the future work this method could be useful for determination of the structural changes of DBT molecules.

DBT mass spectrum is shown in Fig. 7. The signal at m/e 185, which is also the base peak (intensity 100%) corresponds to the molecular ion. All signals on larger masses than this one, and of small intensity, originate from impurities.

Peak at m/e 240, related to intensity, point to fragmentation and rearrangement of the basic molecule.

These results clearly show that the mass spectrometry in further researches can, together with NMR, be the key structural instrumental method for explanation of DBT transformation – desulphurization mechanisms.
3.2 Checking and adaptation of the gas chromatographic methods for determination of DBT

Gas chromatography is the analytical method used for determination of DBT. Characteristical gas chromatogram for the standard solution model substrate (25 mg/L) is shown in Fig. 7.

Figure 7. Gas chromatogram of standard DBT solution

The signal with retention time (RT) at somewhat more than 10 min originates from DBT. Another two outstanding peaks are impurities from the solvent which was confirmed by recording the chromatogram only for the solvent and because of which we used to check GC purity EtOAc prior to each analysis.

Linearity of the calibration diagram and detection limit were defined within the concentration range (c) 5 to 50 mg DBT/100 mL (attenuation 32, injected volume 1 µL), which in the described experimental conditions for testing of interaction with model solutions correspond to DBT concentrations in 1 L. By processing the data with linear regression for area counts \[ AC = f(c) \] by Microcal Origin 5.0 program, we obtained the following equation of the standard line in the explicit form:

\[
AC = 2726 \pm 124 c - 3725 \pm 3488 \tag{2}
\]

Statistical criteria \( r = 0.9939, \) SD = ±4976 and \( p < 0,0001 \) for \( n = 8 \) indicate that the method is accurate, precise and reliable. The method is also sensitive \( (\alpha \approx 90^\circ) \), and the detection limit is 1.4 mg DBT/100 mL(L) for \( AC = 100 \). All GC results indicate that DBT can be directly determined (without the internal standards) and that it is applicable for the quantitative DBT analysis in desulphurization experiments.

3.3 Conditions for DBT extraction from the model system

Since the model substrate resp. DBT in fossil fuels is in water suspension, it is indispensable to extract it beforehand in order to determine its concentration. Therefore, we have checked for the adopted DBT concentration in fossil fuels and at the initial pH of above 2.5 (model solution pH 2.5), the influence of the number of extractions and pH solution on DBT recovery, for which UV \( (\lambda_{\text{max}} = 255 \text{ nm}) \) are applied as faster and more simplified, resp. GC method.
For the triple extraction in the range of pH 1-14, the results shown in Fig. 8 are obtained.

The highest recovery (91.7%) is obtained at pH 7, which means that it is necessary to adjust pH model system to this value prior to extraction.

With the triple extraction at the stated pH, the extraction degree of 91.6 ± 2.8\% (n=5) is obtained, and the double extraction gave the recovery of 87.2 ± 4.9\% (n=5).

![Figure 8. Influence of pH on DBT recovery from the model system with solution pH 2.5](image)

### 3.4 DBT interaction with model solutions

For the purpose of checking the applicability of analytical and instrumental structural methods, as well as DBT stability/changes as the model substrate of the organic sulphur in fossil fuels in the interaction with model solutions which are by their composition very close to the expected ones in the real experiments for separation of the organically-bonded sulphur, with participation of *Th. ferrooxidans*, and generation-regeneration of iron(III)-ions from pyrite, we have conducted four series of testings with various, the aforegoing model solutions.

The qualitative changes were identified by recording UV spectra of the extracted DBT after the finished tests. Shifting of the maximum absorption or phenomena of new peaks is not noticeable in any model system. However, for model solutions 9 K and 9 K 3, the peak shape at 255 and 263 nm is somewhat changed, so that inflections are noticeable on the portion toward the lower of the first signal and the portion toward the higher wave lengths of the second peak. The changes are more expressive on the signals for the extracted DBT from the solution 9 K 3. This could indicate certain changes in DBT molecule by the action of iron(III) ion, which occurs by oxidation of oxygen from the air in the solution 9 K, resp. iron(III) found in the solution 9 K 3.

The results of the quantitative analysis are shown in Table 1.
Table 1. Results of the quantitative analysis of DBT in model systems

<table>
<thead>
<tr>
<th>Model solution</th>
<th>Recovery of DBT, [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 2.5</td>
<td>93.1</td>
</tr>
<tr>
<td>0 K</td>
<td>88</td>
</tr>
<tr>
<td>9 K</td>
<td>33.1</td>
</tr>
<tr>
<td>9 K 3</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Extraction degree for the first two solutions is expected on the basis of the results with standards, though there is certain possibility that the salts from the solution 0 K affect lower extraction efficiency. Small and extremely low recovery in case of other two solutions does not mean that significant transformations of DBT occurred (neither shown UV spectra), but that the extraction procedure is not efficient enough. Namely, during adjustment of the optimal pH for extraction, the voluminous precipitates of iron(II) and iron(III) hydroxide drop which, due to their sorptive, occlusive and inclusive properties (physical bonding) make aggregations with DBT which is not found in the real solution but only is well suspended. This enables an efficient contact of DBT particles with the solvent resp. reduces the extraction efficiency which is more expressive with iron(III) hydroxide. This is confirmed by our preliminary results [45], after which the usual extraction is carried out in the extraction funnel, with which the quantity of the extracted DBT was below the detection limit improved by the procedure with ultrasound.

On the gas chromatogram for DBT from 9 K 3, besides the signal for the solvent contamination there are also two peaks with RT close to DBT position. One is at the place which corresponds to a lower and the second to a higher polarity of DBT. Their surfaces are about 3, resp. about 5% in relation to DBT signal surface. These results too point at possible oxidation-desulphiruzation changes of DBT structure by the action of iron(III) sulphate. In the outstanding chromatograms, neither these nor other signals were identified except the expected ones.

4. CONCLUSIONS

All the results obtained and discussed point at the following conclusions:

1. Thermodynamic computations for hypothetic chemical equation of DBT desulphurization by the action of iron(III)-ion speak in favor of foundation of the idea related to the possibility of removal of the organic sulphur from fossil fuels in the described way;

2. At the same time the presence of pyrite and organically-bonded sulphur in fossil fuels, and in oil shales too, which are the subject of our special interests, may favor removal of DBT by oxidation with iron(III) sulphate which would be generated by *Th. ferrooxidans* from that pyrite, whereby the overall desulphurization would be realized in the same process;

3. The commercial DBT (purity 98%), as a model substrate, is characterized with the structural instrumental methods, viz. by: UV, FT-IR, 

4. UV and FT-IR spectra are suitable for the qualitative analysis of changes in DBT structure at which, due to simplicity and the speed of conducting the advantage is given to UV spectrophotometry;
5. Other spectral methods are applicable for determination of the product structure and mechanisms of DBT transformations;

6. Gas chromatography is the appropriate quantitative method for the analysis of DBT content;

7. The conditions for DBT extraction from reactive mixture are: pH 7 and minimum three times repeated procedure (recovery about 90 and more percentages);

8. Checking of applicability of analytical procedures and stability/changes of DBT at interaction with the model solutions by shake flasks test technique indicates that there are no changes in the structure of the model substrate when the solution of sulphuric acid with pH 2.5 and medium 9 K without iron(II) sulphate are applied, while in the case of the medium 9 K and the same medium with iron(III)-ion possible desulphurization is noticed, to which the changes in the signal structure in UV spectrum point out, resp. in UV spectrum and gas chromatogram; and

9. Aggregates of the iron hydroxides precipitates in model solutions with iron(II) and iron(III)-ion occurred at pH adjustment prior to extraction are physically bonding DBT, so that the recovery is not acceptable (this does not occur with iron-free solutions), expecially in the case of iron(III) hydroxide (only about 5%), which means that DBT extraction method from the model system resp. under real experimental conditions, represent a series analytical problem in preparation of DBT specimen for further testings, to which attention should be paid.

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Solids loading in the bioleach slurry reactor: mechanisms through which particulate parameters influence slurry bioreactor performance

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Abstract

Operation of the slurry bioreactor at maximal solids loading is a key factor in determining the economic performance of processes such as mineral bioleaching in which the solid phase represents the key nutrient or reactant in the system. Through studies of mesophilic and thermophilic minerals bioleaching systems, limits to the solids loading have been proposed. Similarly, limits on solids loading have been illustrated in the model yeast-quartzite slurry reactor system.

In the paper presented, the combined influence of solids loading and particulate parameters such as particulate size and mineral quality on process performance are presented across three microbial systems: the mesophilic bacterial leaching of mineral sulfides dominated by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*; the thermophilic microbial leaching of pyrite and chalcopyrite concentrates as well as concentrate – quartzite mixtures dominated by *Sulfolobus* species; and the *Saccharomyces cerevisiae* quartzite model system used for the study of slurry bioreactors. The influence is discussed in terms of process performance, specific activity of the microbial phase as well as structural variation in the biophase. Mechanisms explaining the altered performance under specified culture conditions are sought through an analysis of microbial cell damage resulting from the hydrodynamic environment.

Keywords: bioleaching, microbial cell damage, metabolic activity, solids loading

1. INTRODUCTION

It is well recognised that microorganisms express biological responses to stress incurred in their culture environment. These stresses include osmotic, oxidative, thermal, chemical and hydrodynamic stresses. While less well studied than other stress systems, responses to adverse hydrodynamic conditions are reported to include changes in specific growth rate, nutrient uptake rate, product formation rate and morphology of the micro-

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organisms (Logan and Dettmer 1990, Toma et al. 1991). The presence of particulates in bioprocesses may further aggravate the hydrodynamic response. Agitation of a microbial phase in the presence of a particulate phase is a well-recognised means of cell disruption for product liberation through the bead mill (Currie et al. 1972, Schutte et al. 1986). Yet it is frequently necessary to grow a microbial phase in the presence of a particulate phase, particularly where this particulate phase forms the source of nutrients or is critical to the microbial energy provision such as occurs in mineral bioleaching. The particulate phase may form through the precipitation of products formed such as the precipitation of metal sulphides formed during biological sulphate reduction or provide the support in immobilised cell systems.

In mesophilic mineral bio-oxidation systems, a critical solids loading has been reported above which the process is detrimentally affected (Gormley and Brannion 1989, Torma et al. 1992). Studies of solids loading in mesophilic processes have indicated that loadings of 18-20% (m/v) may be used routinely (Oguz et al. 1987). Beyond this, performance is impaired. While this may result from gas liquid mass transfer limitation restricting the oxygen or carbon dioxide available and is affected by the grade of concentrate or ore used (Torma et al. 1992, Bailey and Hansford 1993), the influence of mechanical stress has not been rigorously discounted. Limited study on the influence of the solids phase on thermophilic leaching performance is reported. Initial reports suggest that the thermophiles used for bio-oxidation appear to be sensitive to hydrodynamic conditions (Jordan et al. 1993, Clark and Norris, 1996) and the presence of solids (Norris and Bar 1988, Le Roux and Wakerley, 1988, Escobar et al. 1993, Torres et al. 1995, Nemati and Harrison, 2000). These disadvantages may be due to the fact that the Sulfolobus lack a rigid peptidoglycan cell wall (König and Stetter 1986, König 1988, Michel et al. 1980). In addition, an increase in temperature causes the fluidity of tetraether-based cellular membranes to increase (Kelly and Deming, 1988).

The effect of agitation intensity on cell damage has been investigated in the mineral bio-oxidation and animal cell-microcarrier systems. In the mineral bio-oxidation system, Hackl et al. (1989) found that an impeller tip speed of 5.3 m s⁻¹ was detrimental to the acidophilic iron and sulphur oxidising mesophiles. However normal leach rates were observed when reducing the tip speed of the Rushton turbine to 3.3 m s⁻¹. Investigating the growth of the acidophilic iron and sulphur oxidising mesophiles in the presence of 2% (v/v) pyrite, Pearce (1993) determined that the cell growth was inhibited at a tip speed of 2.6 m s⁻¹ for a 6-bladed Rushton turbine, while an impeller tip speed of 1.4 m s⁻¹ was not detrimental to the cells.

In this paper, the influence of the presence of the solids phase on microbial cell damage in the slurry bioreactor is presented across the following microbial phases: the model system Saccharomyces cerevisiae in both the exponential and stationary growth phases, the acidophilic iron and sulphur oxidising mesophiles used in mesophilic bioleaching and Sulfolobus metallicus used in thermophilic bioleaching. Parameters of the solids phase investigated include solids loading, particle size, nature of the solids phase used, agitation intensity and thereby energy dissipation rate as well as impeller tip speed. The studies consider both the performance of the bio-phase as well as the biological responses observed at a cellular level, By comparison across a range of microbial systems, generic findings are sought.
2. MATERIALS AND METHODS

2.1 Microorganisms and Reactor Systems Used

The studies were carried out over the following microbial systems: a mesophilic mineral bioleaching system using a mixed culture of acidophilic iron and sulphur oxidising mesophiles, two thermophilic mineral bioleaching systems using *Sulfolobus metallicus* BC, a model Saccharomyces system under growth conditions and a similar system under stationary phase conditions. Details of the microbial system, growth conditions, particulate phase and reactor configuration for each system is given in Table 1.

2.2 Analytical Procedures

The concentration of cells free in suspension was measured by direct counting using a Petroff-Hauser-type cell counter (haemocytometer) of 0.02 mm depth and 1/400 mm² area under the light microscope at magnification. Cell counting and cell size determination was also conducted by volume displacement using the Cell Facts cell sizer (Microbial Systems, U.K.). For the yeast cultures, cell viability was determined by methylene blue staining using a modified Ringer salt solution. Disruption of the yeast cells was determined by soluble protein release, using the method of Lowry *et al.* (1951). Maximum protein release was determined by a dual pass through the French Press. The transmission electron microscopy methodology is detailed in Lamaignere (2002).

The pH and redox potential were measured at room temperature. The redox electrode was a combined platinum/reference redox cell.

The concentration of ferrous iron in solution was determined by titration against 0.017 M potassium dichromate in the presence of N-phenyl anthranilic acid as an indicator (Vogel, 1989). To determine the concentration of total iron in solution ferric iron was reduced to ferrous iron using stannous chloride as reducing agent, followed by titration against potassium dichromate. The ferric iron concentration was estimated by difference between the ferrous iron and the total iron concentration. Since part of the iron was precipitated during leaching, the iron concentration was determined in the supernatant of both pre- and post acid washing of the suspension. Atomic absorption spectroscopy was used to determine copper ion concentrations. A Varian Spectra AA-200 Atomic absorption spectrophotometer incorporating Spectra AA 100/200 (version 1.1) software was used. The operational parameters were as follows: slit width of 0.2 nm; air/acetylene flame; lamp current of 4 mA; and wavelength of 217.9 nm. The concentration of iron and, where appropriate, copper in solution was determined using the supernatant of a centrifuged sample.

Table 1. Microbial systems and reactor conditions used across the five studies conducted

<table>
<thead>
<tr>
<th>System</th>
<th>System 1</th>
<th>System 2</th>
<th>System 3</th>
<th>System 4</th>
<th>System 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-organism</td>
<td><em>At.</em> Ferrooxidans</td>
<td><em>Sulfolobus</em> metallicus BC</td>
<td><em>Sulfolobus</em> metallicus BC</td>
<td><em>S.cerevisiae</em> (Baker’s yeast)</td>
<td><em>S.cerevisiae</em> (Baker’s yeast)</td>
</tr>
<tr>
<td>Growth phase</td>
<td>Growing system</td>
<td>Growing system</td>
<td>Growing system</td>
<td>Growing system</td>
<td>Stationary phase</td>
</tr>
<tr>
<td>Growth medium</td>
<td>9K medium</td>
<td>0.4 kg m⁻³ (NH₄)₂SO₄</td>
<td>0.5 kg m⁻³ MgSO₄·7H₂O</td>
<td>Minimal glucose medium</td>
<td>Phosphate buffered saline</td>
</tr>
</tbody>
</table>
Bioleaching Applications

<table>
<thead>
<tr>
<th>System</th>
<th>System 1</th>
<th>System 2</th>
<th>System 3</th>
<th>System 4</th>
<th>System 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>20% by volume, resulting in a cell concentration of 2-4 x 10^8 cells ml⁻¹</td>
<td>10% by volume</td>
<td>Dry biomass conc. of 53 kg m⁻³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particulate phase</td>
<td>Quartzite</td>
<td>Pyrite &amp; quartzite</td>
<td>Chalcopyrite &amp; quartzite</td>
<td>Quartzite</td>
<td></td>
</tr>
<tr>
<td>Std particle size</td>
<td>Mean: 53 µm</td>
<td>37-75 µm</td>
<td>600-850 µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactor configuration</td>
<td>2 hr stress period, 1 dm³ STR, followed by growth in a shakeflask in the absence of solid phase</td>
<td>1 dm³ baffled, aerated STR, with working volume of 0.7 dm³. Pitched blade impeller.</td>
<td>Baffled, flat bottom, aerated 2 dm³ STR. Working volume of 1.5 dm³.</td>
<td>Baffled, flat bottom, 3 dm³ STR. Working volume of 2.45 dm³.</td>
<td></td>
</tr>
<tr>
<td>Operating temperature</td>
<td>30°C</td>
<td>68 – 70°C</td>
<td>68 – 70°C</td>
<td>30°C</td>
<td>&lt;20°C</td>
</tr>
<tr>
<td>Agitation rate (rpm)</td>
<td>Std: 772 rpm R: 400-090 rpm</td>
<td>Standard: 560 rpm Range: 560-760 rpm</td>
<td>Std: 565 rpm R: 460-850 rpm</td>
<td>200-1000 rpm</td>
<td></td>
</tr>
<tr>
<td>Impeller tip speed</td>
<td>Std: 2.2 m s⁻¹ R: 1.1-3.1 m s⁻¹</td>
<td>Standard: 1.7 m s⁻¹ Range: 1.7-2.3 m s⁻¹</td>
<td>Standard of 2.3 m s⁻¹</td>
<td>0.77-3.87 m s⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

### 3. RESULTS

#### 3.1 The influence of solids loading in the slurry reactor on performance

The authors have studied the influence of solids loading on microbial cell damage in slurry bioreactors across the following microbial phases: the model system *Saccharomyces cerevisiae* in both the exponential and stationary growth phases, the thiobacilli used in mesophilic bioleaching and *Sulfolobus metallicus* used in thermophilic bioleaching.

Scholtz *et al.* (1997) have shown the increase in cell disruption of stationary phase *Saccharomyces cerevisiae* with increasing solids loading across the range of quartzite loading of 5 to 40% (v/v). Further they have illustrated that disruption in excess of 90% is found independent of solids loading across the range studied. The disruption is first order with respect to the concentration of intact micro-organisms present. The first order disruption rate constant, k, was a function of both the power input per unit volume (P/V) and the volume fraction of solids present (Φ):

\[
k = 7.11 \times 10^{-5} (P/V)^{0.56} \Phi^{1.64}
\]  

This study has been extended to study *Saccharomyces cerevisiae* in a growing system (Lamaignere 2002). The growth of *S.cerevisiae* in the presence of increasing solids loading of quartzite over a 28 hour time period is illustrated in Figure 1. The reduction in both the rate and extent of growth with increased solids loading is clearly illustrated by a decrease in the stationary phase population (Figure 1), a decrease in specific growth rate \( \mu_{\text{max}} \) and the decrease in biomass yield, \( Y_{X/S} \) (Table 2). A critical solids loading exists
below which the solids loading exhibits an insignificant effect on cell growth. At a 1.5 and 2% solids loading, a decrease in the performance was observed with time. At 5% (v/v) solids loading, growth ceased and the cell number decreased. These observations are consistent with the hypothesis of increasing cell death with increasing volume fraction of inert particles.

![Graph showing the effect of solid loading across the range 0 to 5% on total Saccharomyces cerevisiae cell concentration in the agitated, aerated slurry bioreactor under growth conditions (system 4).](image)

**Figure 1.** Effect of solid loading (quartzite) across the range 0 to 5% on a volume basis on total *Saccharomyces cerevisiae* cell concentration in the agitated, aerated slurry bioreactor under growth conditions (system 4)

**Table 2.** Effect of solid loading across the range 0 to 5% on a volume basis on growth parameters of *Saccharomyces cerevisiae* (system 4)

<table>
<thead>
<tr>
<th>Solid loading</th>
<th>t lag (h)</th>
<th>μ max exp (h⁻¹)</th>
<th>Yx/s (10⁹ cells/g)</th>
<th>% of viable cells at the end of the growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0%</td>
<td>3.0 (±1.0)</td>
<td>0.254 (±0.026)</td>
<td>7.53 (±1.08)</td>
<td>98.2 (± 1.3)</td>
</tr>
<tr>
<td>0.5%</td>
<td>4.0</td>
<td>0.158</td>
<td>5.87</td>
<td>94.6</td>
</tr>
<tr>
<td>1.0%</td>
<td>3.5 (±0.5)</td>
<td>0.135 (±0.018)</td>
<td>7.15 (±0.96)</td>
<td>88.5 (± 5.6)</td>
</tr>
<tr>
<td>1.5%</td>
<td>5.0</td>
<td>0.134</td>
<td>5.90</td>
<td>93.0</td>
</tr>
<tr>
<td>2.0%</td>
<td>6.5</td>
<td>0.112</td>
<td>5.50</td>
<td>85.5</td>
</tr>
<tr>
<td>5.0%</td>
<td>0.0</td>
<td>0.000</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

The influence of the solids loading on the acidophilic iron and sulphur oxidising mesophiles was studied by exposure of the microbes to agitation in the presence of varying volume fractions of quartzite (nominal diameter 53 µm) over a 2-hour period. The growth and rate of ferrous iron oxidation in the absence of a solid phase was monitored following this exposure to identify any effect on microbial performance. The results, shown in terms of change in Eh due to a change in the ratio of ferrous to ferric iron, are given in Figure 2. Here it is clearly seen that microbial damage or reduction in physiological condition of the bacteria induced by exposure to agitation in the presence of the solids phase caused an increased lag phase or period of adaptation prior to active metabolism of the bacteria. This lag phase was extended with increasing volume fraction of solids.

The acidophilic thermophilic microorganisms exhibiting iron and sulphur oxidation potential are archae, possessing a different cell envelope structure to both the bacteria and yeasts. Owing to their perceived reduced structural resilience, comprehensive studies have been undertaken to quantify their performance under varied solids loading conditions. Nemati and Harrison (2000) reported the growth and performance of *Sulfolobus metallicus* BC under conditions of increasing pyrite loading across the range 3 to 18% (w/v) using a
pyrite size fraction of 53 to 75 µm in a one litre stirred tank reactor at an agitation rate of 500 to 550 rpm. The results reported, summarised in Figure 3 in terms of iron release, showed little effect of solids loading in the range 3 to 9% loading, a two phase leaching rate in the presence of 12 and 15% solids in which the leach rate was further impaired in stationary phase over exponential growth. At 18% loading, the system failed when an agitation rate of 550 rpm was applied and rapid cell death was observed. While microbial growth and physicochemical conditions were monitored, the changing physicochemical conditions did not allow the contribution of the solids loading to the reduced leaching performance to be established clearly.

Figure 2. The rate of ferrous iron oxidation, given in terms of Eh, of the acidophilic iron and sulphur oxidising mesophiles subsequent to their exposure to and agitation in the presence of increasing volumetric loadings of quartzite of nominal diameter of 53 µm and an agitation rate of 772 rpm (system 1)

Figure 3. The effect of mineral pulp density on the bioleaching of pyrite through Sulfolobus metallicus in a 1-litre laboratory stirred tank reactor

In order to establish the effect of solids loading while restricting the changing of physicochemical conditions, two further studies of the performance of Sulfolobus metallicus BC were conducted. In these, a constant mineral concentration was maintained of 3% (w/v) pyrite and 3% (w/v) chalcopyrite respectively. In each study, the solids loading was varied by the addition of inert quartzite of the same particle size distribution (38 to 53 µm nominal particle diameter) in 3% (w/v) increments across the range 0 to 24% and 0 to 18% for the pyrite and chalcopyrite studies respectively. The resultant bioleach
rates for these studies are presented in Figures 4 and 5 respectively. The leach rate of pyrite decreased from 0.113 kg m^-3 h^-1 ($r^2 = 1.00$) in the absence of quartzite through 0.095 kg m^-3 h^-1 ($r^2 = 0.98$) in the presence of 6 to 15% quartzite to 0.057 and 0.035 kg m^-3 h^-1 ($r^2 = 0.98$ and 0.99) in the presence of 21 and 24% quartzite respectively. Extent of leaching achieved varied from 91% in the absence of quartzite through 86% in the presence of 6 to 15% quartzite to 62% and 35% in the presence of 21 and 24% quartzite respectively. Similarly, on the leaching of 3% chalcopyrite in the presence of increasing loadings of inert quartzite across the range 0 to 18%, a similar decrease in the rate of solubilisation of both iron and copper was displayed (Figure 5). The rate of copper solubilisation decreased from 0.032 kg m^-3 h^-1 in the absence of quartzite to 0.029 kg m^-3 h^-1 in the presence of 9% quartzite. Thereafter the rate decreased linearly to 0.022 kg m^-3 h^-1 in the presence of 18% quartzite. The rate of iron solubilisation decreased from 0.054 kg m^-3 h^-1 in the absence of quartzite to 0.039 kg m^-3 h^-1 in the presence of 9% quartzite. Thereafter the rate decreased linearly to 0.022 kg m^-3 h^-1 in the presence of 18% quartzite.

Microbial cell growth was determined in terms of the planktonic cell concentration. Both Nemati and Harrison (2000) and Sissing and Harrison (2003) have shown that planktonic cells account for the dominant active microbial population under the tank leaching conditions employed. In Figures 6 and 7, the specific growth rate determined during the exponential growth phase (typically in the range 20-70 hours), is shown to decrease as a function of increasing solids loading in the presence of 3% (w/v) pyrite and chalcopyrite respectively. In the presence of pyrite, the decreasing growth rate is reported...
at solids loadings in excess of 9% (> 6% quartzite) while in the presence of chalcopyrite this decrease is seen at a loading of 9% and greater. At both a 27% solids loading in the presence of 3% pyrite and a solids loading of 15% or greater in the presence of 3% chalcopyrite, negative specific growth rates reported illustrate cell death under extreme hydrodynamic stress. Similarly this was reported at a solids loading of 18% by Nemati and Harrison (2000).

![Figure 6. Microbial growth rate and biomass yield (Y_{X/Fe}) in terms of microbial cells produced per kg iron oxidized as a function of total solids loading in system 2 (comprised of 3% pyrite, the remainder quartzite)](image)

![Figure 7. Microbial growth rate as a function of total solids loading in system 3 (comprised of 3% chalcopyrite, the remainder quartzite)](image)

### 3.2 The influence of particulate size on performance

The particle size of the particulates present influences the nature of the interaction between the microbial particle and the non-biological particle in terms of frequency of collision, momentum of collision and path of the particle with respect to fluid flow in the reactor. Further the size distribution of the particulate phase may influence the physicochemical properties of the suspension.

In our initial studies of the yeast system, the influence of particle size on the disruption of stationary phase yeast was studied. This data is presented in terms of the first order rate constant for disruption (k) and the extent of disruption achieved during a 2-hour exposure in Figure 8. Below a particle size of 300 µm, the disruption rate constant is less than 20% of that achieved at greater particle diameters while complete cell disruption is not achieved in a 2-hour period. Increase of the particle size beyond 700 µm showed no
further effect on the disruption rate constant. These findings were consistent with the bead sizes recommended for optimum cell disruption in the bead mill (Schutte et al. 1986). Harrison et al. (2003) have illustrated that this dependence of disruption rate constant on particle size can be correlated in terms of particle momentum.

![Graph showing the influence of particulate size on disruption rate constant and extent of disruption](image)

**Figure 8.** The influence of particulate size (given as geometric mean diameter) on the disruption of stationary phase *Saccharomyces cerevisiae* (system 5) on agitation at an impeller tip speed of 2.2 m s\(^{-1}\) (772 rpm) and a solids volume fraction of 0.20 is given in terms of the first order disruption rate constant (k) and the extent of disruption expressed as the fraction of protein released over that available for release (Ri/Rm).

The effect of particle size across the range 53 to 255 µm on the hydrodynamic stress response of acidophilic iron and sulphur oxidising mesophiles on agitation in the presence of a 0.05 volume fraction of particulates is presented in Figure 9. Samples were taken before and after a 2-hour agitation period in the stirred tank reactor and their performance monitored by ferrous iron oxidation in shake flask culture in the absence of a solid phase over 5 days. In all cases, a lag phase was induced by the exposure of the culture to agitation in the presence of particulates. These data are consistent with Figure 2. A similar lag period was induced across mean particle sizes of 53 to 161 µm.

![Graph showing the rate of ferrous iron oxidation](image)

**Figure 9.** The rate of ferrous iron oxidation of the acidophilic iron and sulphur oxidising mesophiles subsequent to their exposure to and agitation in the presence of quartzite of varying size, given as nominal diameter, at a volume fraction of 0.05 and an agitation rate of 772 rpm (system 1).
Studies on the effect of particle size on the leaching of pyrite in the presence of *Sulfolobus metallicus* on both the microbial growth and leaching performance have been conducted. Nemati *et al.* (2000) report the increasing rate of leaching with decreasing particle size across a particle size range of 37 to 150 µm nominal diameter. This is in accordance with predictions based on enhanced particle surface area and thereby area for reaction to occur. Their studies were extended to consider smaller particle size distributions. At a nominal mean particle diameter less than 25 µm, it has been found that both pyrite leaching performance as well as specific growth rate of *Sulfolobus metallicus* is reduced with decreasing particle size (Figure 10). These studies suggest that the particle size distribution may also affect the physicochemical properties of the slurry to provide a limiting environment for bioleaching. For example, it is well known that slurry viscosity increases inversely to particle size (Thomas 1965). Further study is required to provide a mechanistic understanding of these findings.

**Figure 10.** The influence of pyrite particulate size, given as nominal particle diameter, on the iron leaching rate and specific growth rate of *Sulfolobus metallicus* (system 2)

### 3.3 The influence of energy dissipated through agitation on performance

The influence of agitation rate in the slurry bioreactor was investigated in system 1 (acidothiobacilli) over the range 400 to 1100 rpm, corresponding to impeller tip speeds in the range 1.1 to 3.1 m s⁻¹. As shown on investigation of other solids parameters, agitation in the presence of the particulate phase resulted in an extended lag phase in subsequent culture of some 40 hours (Figure 11). While little difference is seen between the performance at agitation rates of 400 and 770 rpm (impeller tip speeds of 1.1 and 2.2 m s⁻¹), the lag phase was further extended on agitation at 1090 rpm, corresponding to an impeller tip speed of 3.3 m s⁻¹. Further, the extent of reaction was also reduced. Previously Hackl *et al.* (1989) reported impairment of bioleaching performance at impeller tip speeds of 5.3 m s⁻¹ relative to that at 3.3 m s⁻¹. The impairment at lower impeller tip speeds in our experiment is expected owing to the use of a Rushton impeller.

The effect of agitation rate on the bioleach performance in the presence of *Sulfolobus metallicus* was investigated by increasing the agitation rate in system 2 in the range above the critical impeller speed used to ensure fully suspended solids. The agitation rates investigated were 560, 660 and 760 rpm, corresponding to tip speeds of 1.67, 1.97 and 2.27 m s⁻¹ respectively, with a solids loading of 3% pyrite and 15% quartzite. These results are presented in Figure 12. The system failed at an agitation rate of 760 rpm (tip speed 2.27 m s⁻¹), where the rate of damage to the cells exceeded their growth rate. The rate of iron release and the extent of pyrite solubilisation was higher at 660 rpm than at 560 rpm due to the higher initial microbial cell concentration at 660 rpm. However, the specific growth rate of the microorganisms was slightly lower at the higher agitation rate in
accordance with the increased hydrodynamic stress. The specific activity of the microorganisms at 560 rpm was higher than that at 660 rpm, suggesting an adverse effect of higher agitation rate on the microorganisms. This may be due to increased damage on increased energy dissipation at the higher agitation rate. A specific cell death rate of 0.026 h\(^{-1}\) was observed at an agitation rate of 760 rpm.

Figure 11. The rate of ferrous iron oxidation of the acidophilic iron and sulphur oxidising mesophiles subsequent to their exposure to and agitation in the presence of quartzite at varying agitation rate and a volume fraction of 0.05 (system 1)

Energy dissipation rate was investigated with respect to the model *Saccharomyces cerevisiae* slurry system through varying the agitation rate across the range 460 to 850 rpm, equivalent to impeller tip speeds in the range 1.9 to 3.5 m s\(^{-1}\), using system 4. As illustrated in Table 3, an optimum agitation rate was found to be in the impeller tip speed range of 2.3 m s\(^{-1}\). While cell viability reduced with increasing impeller tip speed, it is apparent that a minimum energy dissipation rate was required to provide the mass transfer and mixing necessary for maximum specific growth rate.

Figure 12. Performance of *Sulfolobus metallicus* BC as a function of agitation rate under conditions of complete suspension and a solids loading of 3% pyrite and 15% quartzite (w/v): A. Rate of iron release and extent of pyrite dissolution B. Specific growth rate and microbial activity
Table 3. Effect of impeller speed on growth parameters of *Saccharomyces cerevisiae* (system 4)

<table>
<thead>
<tr>
<th>Agitation rate (rpm)</th>
<th>Impeller tip speed (m s⁻¹)</th>
<th>Lag time (h)</th>
<th>Max. specific growth rate (h⁻¹)</th>
<th>Stationary phase cell concentration (10⁶ cells ml⁻¹)</th>
<th>Yₓₛ (10⁹ cells g⁻¹)</th>
<th>% of viable cells at the end of the growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>460</td>
<td>1.9</td>
<td>4.0</td>
<td>0.077</td>
<td>38</td>
<td>5.14</td>
<td>94.7</td>
</tr>
<tr>
<td>565</td>
<td>2.3</td>
<td>3.5</td>
<td>0.135 (± 0.018)</td>
<td>55</td>
<td>7.15</td>
<td>88.5</td>
</tr>
<tr>
<td>600</td>
<td>2.5</td>
<td>3.5</td>
<td>0.125 (± 0.018)</td>
<td>50</td>
<td>6.17</td>
<td>86.2</td>
</tr>
<tr>
<td>850</td>
<td>3.5</td>
<td>4.0</td>
<td>0.114</td>
<td>43</td>
<td>4.92</td>
<td>77.4</td>
</tr>
</tbody>
</table>

3.4 Response of the biophase to solids-induced stress in the slurry bioreactor

In order to ensure that the response to hydrodynamic stress may be predicted, and where possible negative responses may be overcome, it is necessary to generate an understanding of these at the cellular level. Studies have been initiated to investigate the influence of hydrodynamic stress generated in the slurry reactor on metabolic activity, cell morphology and metabolic efficiency.

During the exponential growth phase of *Sulfolobus metallicus* on pyrite in the presence of quartzite, Figure 13 illustrates that a higher specific activity (rate of iron released per microbial cell) was observed at lower solids loading, i.e. 0-15% quartzite exhibited higher activity than 21% quartzite (24% total loading), and 24% quartzite (27% total loading) exhibited the lowest activity. The similarity in activity across the solids loading range during the stationary phase suggested that the efficiency of the microorganisms in converting ferrous iron present to ferric iron at the various solids loading was similar in the absence of microbial growth. However, the microbial cell concentration decreased with solids loading. Hence the decrease in pyrite oxidation with solids loading in the stationary phase corresponded to a lower microbial cell concentration as opposed to lower microorganism activity. In the exponential growth phase, both reduced specific activity and a decreased biomass concentration contributed to the decreased leaching performance.

![Figure 13. Microbial cell activity in terms of specific pyrite oxidation rate as a function of solids loading and duration of experiment](image)

Further, changes in morphology of Sulfolobus in response to hydrodynamic stress were reported qualitatively by Nemati and Harrison (2000). The change in morphology of
*Sulfolobus metallicus* has been observed quantitatively in terms of cell size in response to growth on chalcopyrite in the presence of varying quartzite loadings. As illustrated in Figure 14, *Sulfolobus metallicus* is typically found to have a cell diameter of approximately 1.0 to 1.1 µm under conditions of good physiological status. Exposure to increased hydrodynamic stress, associated with reduced leaching performance (Figures 4 and 5) and reduced specific growth rates (Figures 6 and 7), were accompanied with a decrease in the cell size shown in Figure 14. The decrease of some 25% on a diameter basis corresponds to a decrease of some 40% on a volume basis. This is consistent with the reduced cell size and change in phase brightness reported on growth of *Sulfolobus metallicus* in the presence of pyrite only while investigating both solids loading and size effects (Nemati and Harrison 2000, Nemati *et al.* 2000).

Figure 14. Effect of solids loading on diameter of *Sulfolobus metallicus* as determined on exposure to 3% chalcopyrite and 0 to 18% inert quartzite, using the Cell Facts cell counter

Further the yield of *Sulfolobus metallicus* biomass based on iron solubilised (Y_{X/Fe}) decreased with increased solids loading in the presence of 3% pyrite (Figure 6). From the results of similar studies conducted with *Saccharomyces cerevisiae* in the slurry bioreactor under growth conditions (Table 1), a decreasing biomass yield (Y_{X/glucose}) was also reported with increasing solids loading across the range 0 to 5%. Concomitantly, a decrease in cell viability was found. As the solubilisation of iron and glucose, respectively, are required for energy generation in the two systems investigated, the decreased yield indicated a decrease in the fraction of energy generated that was used for cell synthesis, suggesting a greater energy requirement for cell maintenance.

Using transmission electron microscopy, analysis was conducted to detect change in cell shape and size following growth of Saccharomyces cerevisiae under hydrodynamic stress in the slurry bioreactor. Yeast cell shape was modelled as an ellipsoid. No significant difference could be detected in mean length and mean width on comparison of growth at 0% and 1% solid loading (Table 4). Dimensions recorded were in good agreement with the literature (Srinorakutara 1998, Smith *et al.* 2000). However the appearance of the yeast differed with the level of stress applied. Figure 15 compares S.cerevisiae grown in the absence of a solid phase (at t=28h) and at 1% solids loading (at t=28h). Cells grown with 1% solid are visibly damaged compared to those grown in the absence of solids. Cell walls are not clearly defined and cell shape is less regular.
Table 4. Size and shape of *S. cerevisiae* as a function of solids loading during growth (system 4)

<table>
<thead>
<tr>
<th>Solids Loading</th>
<th>Mean Length (nm)</th>
<th>Mean Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% solid</td>
<td>3678 ± 266</td>
<td>2738 ± 400</td>
</tr>
<tr>
<td>1% solid</td>
<td>3614 ± 710</td>
<td>2797 ± 415</td>
</tr>
<tr>
<td>Srinorakutara (1998)</td>
<td>3330 ± 70</td>
<td></td>
</tr>
<tr>
<td>Smith <em>et al.</em> (2000)</td>
<td>3420 ± 620</td>
<td></td>
</tr>
</tbody>
</table>

Figure 15. Comparison between yeast grown in (a) the absence of a solid phase and (b) at 1% solid loading at t=28h (system 4)

Cell wall thickness was measured across a range of samples taken under three levels of hydrodynamic stress: agitation at 560 rpm in the absence of a solids phase, agitation at 560 rpm in the presence of 1% quartzite loading, and agitation at 850 rpm in the presence of 1% quartzite. The cell wall thickness of 71 ± 11 nm determined in the absence of hydrodynamic stress compared well with literature values (Moor and Muhlehaler 1963, Srinorakutara *et al.* 1998). On increasing the hydrodynamic stress, an increase in cell wall thickness was observed (Figure 16). In quantifying the cell wall thickness, it was observed that a certain percentage of cells exhibit a thin wall even for 1% solid and these increased with time. As dead cells could not be differentiated from living cells with TEM analysis, it was postulated that the thin walled cells were dead cells, while cells adapting to the hydrodynamic stress required thickened cell walls.

Figure 16. Influence of time and hydrodynamic stress on cell wall thickness (■ 0% solid loading, ■ 1% solid loading)
4. CONCLUSIONS

Biohydrometallurgy is an important example of the use of microorganisms in slurry bioreactor systems. In such systems, efficient microbial performance is essential while exposed to the increased hydrodynamic stress of the slurry environment. In this study, we have investigated the influence of this particulate phase on three microbial systems under differing operating environments in order to determine both the influence of the slurry system on active minerals bioleaching, as well as to seek a generic understanding of responses found. In all cases, a critical solids loading was found in the laboratory scale reactor above which the process performance is impaired. The critical solids loading determined varied as a function of microorganism used, its growth phase as well as the mineral phase used. In all cases, decreased performance was associated with a decreased microbial phase. Further, reduced microbial activity was reported in the growing systems.

Reduced particulate size distribution resulted in improved process performance in the mineral system across the range 37 to 150 µm through the provision of an increased surface area. Further the yeast study suggested that a minimum particle size to cell ratio of approximately 45-75 is required for disruption of the microorganisms. This is in accordance with bead mills studies. Poor performance of the bioleaching system at very low particle size distribution (<15 µm in diameter) was unexpected. It is postulated to result from altered physicochemical properties of the suspension resulting in process limitation.

Yeast cell disruption can be modelled as a power law function of energy input per unit volume (Scholtz-Brown et al. 1997). Similarly Lamaignere has proposed that the cell death constant in the growing S.cerevisiae system is a function of power input per unit volume. In the mineral bio-oxidation studies, an optimum agitation rate is proposed for maximum performance. It is postulated that lower agitation rates lead to process limitation while increased rates result in cell damage or death. Correlation of damage in terms of energy dissipation rate and impeller tip speed remains to be investigated.

At the microbial level, biological response to hydrodynamic stress has been shown to influence cellular structure and morphology through reduced cell size (S. metallicus), irregular appearance (S. metallicus and S. cerevisiae) and a thickened cell wall (S. cerevisiae). Further, an increasing first order death rate constant is proposed, thereby decreasing the apparent specific growth rate. In all cases, reduced performance is associated with a reduced microbial phase. Under specific conditions, the activity of this phase is also reduced. Further, it is apparent that the biomass yield coefficient is reduced on exposure to hydrodynamic stress. From this observation, it is postulated that an increased maintenance energy results to overcome the stress to which the cells are exposed.

ACKNOWLEDGEMENTS

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REFERENCES

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The development of a hybrid biological leaching-pressure oxidation process for auriferous arsenopyrite/pyrite feedstocks

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Abstract

A process has been evaluated, on a continuous pilot plant scale, to treat refractory gold arsenopyrite/pyrite concentrates. The process consisted of biological leaching to partially oxidize sulphides, followed by pressure leaching for complete sulphide oxidation and arsenic precipitation, followed by liquor neutralization and cyanidation of the autoclave residue for gold recovery.

This paper provides a brief description of the treatment flowsheet and discusses some of the main parameters and results of the process.

Keywords: refractory gold, arsenopyrite/pyrite, piloting, biological leaching, pressure oxidation, gold recovery, arsenic precipitation

1. INTRODUCTION

For several years, TVX Hellas Company has been developing the Olympias property in Greece. The deposit consists of a pyrite concentrate stockpile, a zinc flotation tailings stockpile and in-situ ore reserves. Run-of-mine ore was proposed to be milled and floated in a two-stage flotation circuit to produce lead and zinc concentrates, followed by flotation of the remaining sulphides in the zinc tailings to produce a gold-containing arsenopyrite/pyrite concentrate.

Previous studies indicated that pressure oxidation of the Olympias pyrite concentrate, as compared to bacterial oxidation, resulted in similar or slightly higher gold recovery at significantly lower cyanide consumption. Capex estimate of the pressure oxidation process was higher than bacterial oxidation, but a significant portion of the arsenic was precipitated in the autoclave as an environmentally stable compound.

A feasibility study was conducted at SGS Lakefield Research facilities to investigate the possibility of treating the refractory pyrite concentrates using a combination of bacterial oxidation (BIOX®) and pressure oxidation (POX) technologies. By combining the two technologies, the majority of the sulphide sulphur present in the concentrate (approximately 70%) could be oxidized with air, by applying the BIOX® process, leaving a relatively small portion of the sulphides to be oxidized with oxygen in an autoclave, to complete the oxidation and precipitate the arsenic.
The BIOX® section of the dual process could be designed to produce a partially oxidized product containing sufficient residual sulphur to ensure auto-thermal operation of the autoclave. Based on extensive bench-scale testwork and previous pilot plant campaigns, the recommended process flowsheet is illustrated in Figure 1.

Figure 1. Suggested flowsheet for the treatment process of the Olympias concentrate

As a part of the bankable feasibility study, TVX Hellas conducted a series of fully integrated pilot plant campaigns at SGS Lakefield Research in 1999 to 2000 to simulate the flowsheet illustrated in Figure 1. Each unit process of the pilot plant will be discussed in sequence. The base metal flotation section, and concentrate wash circuit are not discussed in this paper.

TVX Hellas constructed a BIOX® pilot plant, according to the design and equipment specifications provided by GoldFields Limited to treat 100 kilograms concentrate per day. The partially oxidized BIOX® product was used as feedstock for the continuous autoclave testing.

The oxidation pilot plant circuit consisted of the following unit operations:
1. BIOX® circuit
2. Thickening (by decantation) of the BIOX® discharge to remove ~10% BIOX® liquor volume (this portion of the liquor by-passed the autoclave and was forwarded directly to the neutralization circuit).
3. Pressure oxidation of the thickened, partially oxidized BIOX® pulp (plus a small portion of untreated pyrite concentrate) in a continuous pilot plant autoclave operation.

4. Thickening/washing of the POX discharge to produce PLS for neutralization.

5. Continuous neutralization of the POX thickener overflow and a portion of the BIOX® liquor.

6. Thickening of the neutralized product in a ‘high density sludge’ mode.

7. Cyanidation/CIL of the washed autoclave discharge samples and compartment samples. These tests were conducted in a batch mode.

The overall objectives of the pilot plant campaign were to: evaluate and optimize the BIOX® circuit; evaluate the behaviour of the partially oxidized BIOX® slurry in the autoclave; evaluate arsenic dissolution/precipitation in the autoclave; evaluate gold recovery; neutralize POX and BIOX® liquor to produce environmentally acceptable effluent and stable waste product.

2. DESCRIPTION OF FEED SAMPLES

It was proposed that the oxidation plant would treat pyrite concentrates from three sources: Stockpile and reclaimed tailings pyrite concentrate, run-of-mine (ROM) pyrite concentrate, and so-called post-East concentrate (PEC). The average chemical analyses of the three concentrate samples treated are shown in Table 1.

Table 1. Chemical analyses of the concentrate samples

<table>
<thead>
<tr>
<th>Concentrate</th>
<th>Au, g/t</th>
<th>Ag, g/t</th>
<th>S%,</th>
<th>Fe, %</th>
<th>As, %</th>
<th>Fe:As molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stockpile</td>
<td>23</td>
<td>22</td>
<td>39</td>
<td>38</td>
<td>8.6</td>
<td>6.0</td>
</tr>
<tr>
<td>ROM Blend</td>
<td>23</td>
<td>32</td>
<td>29</td>
<td>30</td>
<td>11.2</td>
<td>3.6</td>
</tr>
<tr>
<td>PEC Blend</td>
<td>25</td>
<td>41</td>
<td>32</td>
<td>32</td>
<td>10.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Continuous flotation pilot plant campaigns were conducted at SGS Lakefield Research on the ROM 2001-2003 and PEC ores. The flowsheet involved the use of cyanide as pyrite depressant during base metal flotation. It was observed that the cyanide reacted with sulphides to form thiocyanate, which "adsorbed" onto the pyrite concentrate surfaces. Thiocyanate is highly toxic to the BIOX® bacteria, even at a fairly low concentration in solution. Therefore, washing of the concentrate prior to the BIOX® circuit was found to be extremely important.

3. PILOT PLANT DEMONSTRATIONS

3.1 BIOX® circuit

The BIOX® program and circuit operation were directed by TVX Hellas and GoldFields Mining Services Ltd. representatives. The circuit was operated on a 24-hour basis for 160 days by TVX and SGS Lakefield personnel.

The main objective of the continuous BIOX® circuit was to produce semi-oxidized BIOX product as feed to the continuous POX circuit. These pilot plant campaigns presented the first opportunity to treat the fresh concentrate blends in a continuous mode. All previous piloting had been conducted on the Stockpile concentrate only. The BIOX process uses a mixed culture of thiobacillus ferrooxidans, thiobacillus thiooxidans and leptospirillum ferrooxidans to break down the sulphide mineral matrix. The active
The inoculum produced during small pilot plant operations in Greece was shipped to Lakefield for reactivation and inoculation in the pilot plant reactors.

The BIOX® plant consisted of two identical trains of one primary reactor and two secondary reactors fabricated from stainless steel. Figure 2 is a photograph showing the BIOX® pilot plant, and Figure 3 - the continuous autoclave.

The primary reactors had an unaerated volume of 622 liters each and the secondary reactors, 208 liters each. The plant was designed to treat 100 kilograms of concentrate per day, at a retention time of 5 days, 3 days in the primary stage and 1 day per each secondary stage. This arrangement allowed a reduction in retention time by taking the secondary reactors off line, should this prove to be necessary.

The pilot pant was controlled at the following operating conditions:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid/Solid Ratio</td>
<td>4/1 and 5.7/1</td>
</tr>
<tr>
<td>Slurry Temperature</td>
<td>40 to 45°C</td>
</tr>
<tr>
<td>Slurry pH</td>
<td>1.2 to 1.8</td>
</tr>
<tr>
<td>Dissolved Oxygen Concentration</td>
<td>2 to 6 mg/L</td>
</tr>
</tbody>
</table>

The pilot plant was fed on a continuous basis at a predetermined rate to give a specific retention time. The required nutrient salts, consisting of ammonium sulphate, ammonium phosphate and potassium sulphate, were added to the feed make-up tank.

The slurry pH was controlled by manual addition of limestone slurry. Limestone was also added to the feed make-up tank to give a total carbonate content of 3% in the feed to the plant.

The oxygen uptake rate, which is an instant measurement of the rate of oxygen depletion in an active inoculum and probably the most important criterion in assessing bacterial activity, was measured routinely. Ferric and ferrous iron concentrations were also measured routinely, as well as redox potential.

The following samples were regularly taken from the circuit: feed samples, profile samples from each reactor, and final products from each train. Typical results from the continuous operation are illustrated in Figure 4 and summarized in Table 2.

The bacterial activity was very high during the pilot plant operation, with an average sulphide sulphur removal over 90% after 5 days; however, the sulphide oxidation to sulphate was considerably lower due to the formation of elemental sulphur or poly sulphide species.
The elemental sulphur content in the BIOX® discharge was initially in the order of 10-11%, but later stabilized between 7-9%. The reasons for the presence of too much elemental sulphur were not clearly understood. The analytical data suggested that there was no clear correlation between the operating pH, iron and arsenic dissolution and the formation of elemental sulphur. It is likely that the formation of elemental sulphur in the BIOX® discharge is due to the slower kinetics of the last step of conversion of elemental sulphur to sulphate. More elemental sulphur is produced as the overall sulphide removal increases, but only a fraction of the elemental sulphur further reacts to form sulphate. The iron and arsenic dissolution averaged 60-75% and 80-90% respectively, with the BIOX® liquor containing up to 85 g/L Fe (mostly as ferric) and 40 g/L As.

Table 2. BIOX® pilot plant results

<table>
<thead>
<tr>
<th>Blend</th>
<th>Tank</th>
<th>Retention time days</th>
<th>Solids Analyses</th>
<th>Solution Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S^-</td>
<td>S^o</td>
</tr>
<tr>
<td></td>
<td>Feed</td>
<td>-</td>
<td>39</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Primary</td>
<td>3</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Secondary R1</td>
<td>1</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Secondary R2</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Feed</td>
<td>-</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Primary</td>
<td>3</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Secondary R1</td>
<td>1</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Feed</td>
<td>-</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Primary</td>
<td>3</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Secondary R1</td>
<td>1</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 4. Sulphide oxidation as a function of retention time

The BIOX® circuit discharge was forwarded to a pressure oxidation stage to complete the oxidation of residual sulphides and other sulphur species. A percentage of the BIOX® solution was decanted from the slurry and bled directly to the neutralization circuit. The design target of the bleed was 10% volume, with the actual volume varying between 10 and 25%, depending on the BIOX® discharge slurry density. The BIOX® product must
contain enough residual sulphides and elemental sulphur to maintain auto-thermal operation of the autoclave. Therefore, it was established that the BIOX® plant only required a primary and one secondary stage to achieve the required level of sulphide oxidation. The residual sulphide grade in the BIOX® discharge was controlled by varying the number of reactors on-line and the reactor configuration.

Based on the pilot plant results, the following plant operating parameters were recommended by GoldFields Mining Services:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Slurry Density</td>
<td>20% solids</td>
</tr>
<tr>
<td>Operating pH in primary and secondary reactors</td>
<td>1.2 to 1.7</td>
</tr>
<tr>
<td>Operating Temperature</td>
<td>40 to 45°C</td>
</tr>
<tr>
<td>Retention Time</td>
<td>Primary reactors 3 days</td>
</tr>
<tr>
<td></td>
<td>Secondary reactors 1 day</td>
</tr>
</tbody>
</table>

### 3.2 POX circuit

The main objective of the POX circuit was to oxidize all sulphides and sulphur present and to expose gold for extraction by cyanide leaching. Additional objectives were to precipitate out arsenic as a stable precipitate and to produce final effluents meeting industrial standards. The test program was designed in consultation with SNC-Lavalin and TVX Hellas representatives.

SGS Lakefield’s continuous horizontal autoclave, constructed of Grade 12 titanium, is 172.7 cm in length with an inside diameter of 25.0 cm, and is divided into six compartments by means of weir plates. Oxygen gas is sparged at controllable flow rates into all compartments. The oxygen is normally distributed with greater than 80% of the total flow directed into the first two compartments. Total oxygen flow is typically in the order of 28–40 liters per minute.

Three continuous integrated pilot plant campaigns were conducted on each pyrite concentrate blend, partially oxidized by the BIOX® process. Autoclave feed comprised principally of BIOX® product, with small additions of untreated pyrite concentrate in order to achieve the target sulphide plus sulphur grade of ~15%. This value was determined by the MetSim model as the minimum amount of sulphide sulphur for auto-thermal autoclave operation. The target solid content varied between 15 and 20% solids. The autoclave feed contained approximately 10% elemental sulphur. Quebracho or Lignosol were added at a rate of 5kg/t and 2.5 kg/t respectively, as an elemental sulphur dispersant, to prevent occlusions by sulphur of unreacted sulphide particles. Autoclave target operating conditions were 225°C, 100 psig oxygen overpressure and 30 to 70 minutes nominal residence time.

Filtered and washed autoclave discharge and compartment samples were submitted for neutralization-cyanidation/CIL for the recovery of gold and silver. The tests were conducted in a batch mode. The samples were neutralized at 30% solids with hydrated lime to pH 11, for at least 12 hours in order to reach a stable pH prior to cyanidation. Activated carbon was added at 10 g/L solution and CIL was carried out at 0.5 g/L NaCN for 24 hours. A summary of the autoclave campaign results is presented in Table 3. The results indicated that sulphide sulphur oxidation was typically greater than 95% after 30 minutes in the autoclave. Elemental sulphur conversion to sulphate was greater than 98% after 10 minutes in the autoclave.
Table 3. Pressure oxidation pilot plant results

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Campaign 1</th>
<th>Campaign 2</th>
<th>Campaign 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POX Feed</td>
<td>POX Discharge</td>
<td>POX Feed</td>
</tr>
<tr>
<td><strong>A/C Conditions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>- 225</td>
<td>- 225 225 225</td>
<td>- 225</td>
</tr>
<tr>
<td>Time, min</td>
<td>- 70</td>
<td>- 70 40 30</td>
<td>- 65</td>
</tr>
<tr>
<td><strong>Solution Analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As, g/L</td>
<td>20 13 15 5 8 9 14 7 7 10</td>
<td>65 60 48 22 24 34 48 34 35 45</td>
<td>140 300 80 190 190 200 93 190 185 230</td>
</tr>
<tr>
<td>Fe, g/L</td>
<td>65 60 48 22 24 34 48 34 35 45</td>
<td>65 60 48 34 35 45 45 36 35 45</td>
<td>140 300 80 190 190 200 93 190 185 230</td>
</tr>
<tr>
<td>SO₄₂⁻, g/L</td>
<td>140 300 80 190 190 200 93 190 185 230</td>
<td>140 300 80 190 190 200 93 190 185 230</td>
<td>140 300 80 190 190 200 93 190 185 230</td>
</tr>
<tr>
<td>FA, g/L</td>
<td>&lt;10 47 &lt;10 62 56 44 &lt;10 41 46 37</td>
<td>&lt;10 47 &lt;10 62 56 44 &lt;10 41 46 37</td>
<td>&lt;10 47 &lt;10 62 56 44 &lt;10 41 46 37</td>
</tr>
<tr>
<td>Fe/As Molar Ratio</td>
<td>4.4 6.2 4.3 5.9 3.9 5.2 4.6 6.5 6.7 6.0</td>
<td>4.4 6.2 4.3 5.9 3.9 5.2 4.6 6.5 6.7 6.0</td>
<td>4.4 6.2 4.3 5.9 3.9 5.2 4.6 6.5 6.7 6.0</td>
</tr>
<tr>
<td><strong>Solids Analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As, %</td>
<td>3 6 2 5 5 5 2</td>
<td>3 6 2 5 5 5 2</td>
<td>3 6 2 5 5 5 2</td>
</tr>
<tr>
<td>Fe, %</td>
<td>20 22 16 22 21 22 18</td>
<td>20 22 16 22 21 22 18</td>
<td>20 22 16 22 21 22 18</td>
</tr>
<tr>
<td>S⁺, %</td>
<td>15 0.2 6-11 0.1 0.1 0.7 11 0.23 0.19 0.19</td>
<td>15 0.2 6-11 0.1 0.1 0.7 11 0.23 0.19 0.19</td>
<td></td>
</tr>
<tr>
<td>S⁻, %</td>
<td>10 &lt;0.5 7-10 &lt;0.5 &lt;0.5 &lt;0.5 9 &lt;0.5 &lt;0.5 &lt;0.5</td>
<td>10 &lt;0.5 7-10 &lt;0.5 &lt;0.5 &lt;0.5 9 &lt;0.5 &lt;0.5 &lt;0.5</td>
<td>10 &lt;0.5 7-10 &lt;0.5 &lt;0.5 &lt;0.5 9 &lt;0.5 &lt;0.5 &lt;0.5</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Au Extraction, %</td>
<td>98 98 98 97 96 97 98</td>
<td>98 98 98 97 96 97 98</td>
<td>98 98 98 97 96 97 98</td>
</tr>
<tr>
<td>As precipitation, %</td>
<td>- 50 - 85 79 72 - 86 85 75</td>
<td>- 50 - 85 79 72 - 86 85 75</td>
<td>- 50 - 85 79 72 - 86 85 75</td>
</tr>
</tbody>
</table>

The fast kinetics of oxidation of sulphide and elemental sulphur indicated that the risk of sulphide occlusion by elemental sulphur was minimized by the addition of Lignosol. In association with the sulphide and sulphur oxidation, iron conversion from ferrous to ferric was greater than 98%. Similarly, arsenic oxidation to As(V) was greater than 98%; the residual As(III) solution concentration was about 120 mg/L. Kinetic profiles of sulphide oxidation versus gold recovery and arsenic dissolution/precipitation are also illustrated in Figures 5 and 6.

The results showed that gold recoveries closely followed the sulphide sulphur oxidation profile. Gold extraction from the autoclave discharge was excellent and averaged 96-98%, leaving a residue assaying 0.3-0.9 g Au/t cyanidation residue. Sodium cyanide and lime consumptions for the autoclave discharge composites were in the range of 1 kg/t and 80-90 kg/t of cyanidation feed, respectively.

Arsenic precipitation efficiency was evaluated based on arsenic distribution between the solid and solution phases. The distribution of arsenic to the solids increased from 20 to 40% in the feed to 60-80% in the discharge.

The profile of arsenic precipitation across the autoclave suggested that arsenic precipitated as an unstable compound in the first compartment, followed by redissolution in the second compartment and re-precipitation as a stable compound in the last two compartments. The re-dissolution of the precipitate in the second compartment appeared to coincide with an increase in free acid formation through sulphur oxidation.
In simple arsenic-acid systems, it is well known that the solubility of arsenic increases with increasing acidity, with a minimum solubility in the pH range 3 to 5. The high acid level in the autoclave discharge, 40-60 g/L, must be one of the reasons for the high residual arsenic in solution.

The results obtained during the autoclave pilot plant campaigns indicated that the residual arsenic and iron in the autoclave discharge depended to a certain extent on the initial arsenic and iron concentration in the POX feed/BIOX® discharge, with relatively high soluble iron and arsenic in the feed to POX (campaign 1) leading to relatively high arsenic in solution in the POX discharge. Arsenic precipitation in the autoclave was only ~50% in campaign 1, versus ~85% in campaigns 2 and 3.

The iron to arsenic ratio is probably also important, since work by Monhemius and Swash¹ has shown that the precipitation of scorodite or other ferric-arsenate components is inhibited at higher Fe/As ratios, in the presence of sulphuric acid. Mineralogical analyses of selected autoclave discharge samples suggested that the major phase present was a basic iron sulphate and anhydrite. Arsenic was present as a low-level constituent of the iron sulphate.
3.3 Solid/liquid separation and liquor treatment

The autoclave discharge slurry and the small bleed of the BIOX® liquor that bypassed the autoclave were combined and treated in a multi-stage continuous CCD/neutralization circuit. The main objectives of this stage of the pilot program were to: demonstrate, at a pilot plant scale, that As(III) present in solution can be effectively oxidized to As(V) using SO₂ and air as an oxidant; precipitate arsenic as a stable product suitable for disposal; and produce final effluent that meets industrial regulatory limits. As(III) oxidation was carried out in a series of three cascading 45L tanks, with additions of sodium metabisulphite (Na₂S₂O₅) and air into each oxidation tank. Retention time within the oxidation circuit was 4.2 hours.

The results indicated that the autoclave discharge could be thickened to 55-60% solids. The SO₂/air oxidation was an effective process for As(III) oxidation. On average, the oxidation feed As(III) concentration was decreased from 125 mg/L to below 20 mg/L. Average consumption of SO₂ during the oxidation stage was 2.5 g/L of CCD overflow solution. The SO₂ consumption was not optimized during the pilot plant campaign.

Overall alkali consumption, during the neutralization stage, including limestone and lime as CaO equivalent, was in the range 500-600 kg/t of autoclave discharge solids. The final neutralized solution met industrial effluent standards for the required elements.

In order to determine the stability of the final waste products, samples of the neutralized sludge, the CCD underflow and the CIL residue and were submitted for TCLP 1311 leachate testing. The results showed that the concentrations of all the elements in the leachates for samples tested were below regulatory limits. However, elevated concentrations of manganese (Mn) and zinc (Zn) were observed in the leachates of the neutralization sludge samples.

4. CONCLUSIONS

From intensive and integrated pilot plant campaigns, it was confirmed that:

- Acceptable sulphide oxidation could be achieved by two-stage oxidation with BIOX® (4 days) followed by POX (40 minutes at 220°C).
- Gold extractions from the POX discharge were excellent, ranging from 96-98%, with only 1 kg/t NaCN consumed.
- Arsenic precipitation in the autoclave varied between 50-80%.
- Neutralization of the plant liquors (BIOX® and POX) was successful, resulting in effluents meeting effluent standards.
- The results of TCLP 1311 testing of the final waste products yielded leachates that were below regulatory limits, confirming that arsenic had been stabilized.

The Olympias process could be simplified, and capital and operating costs significantly lowered, if all of the BIOX® liquor was allowed to bypass the autoclave directly to the neutralization circuit. In this regard, the following factors are pertinent:

- The precipitate produced during neutralization under atmospheric conditions is as stable as the ferric arsenate compound produced in the autoclave, as indicated by the most recent research findings (Monhemius and Swash).
- The total residence time in the autoclave was determined by the rate of ferric arsenate precipitation (50-70 minutes) rather than the rate of sulphide and sulphur oxidation (30-40 minutes). The autoclave size could therefore be significantly reduced, if the autoclave design was based solely on the requirement for efficient gold recovery.
• Bypassing the BIOX® liquor around the autoclave will reduce the iron, arsenic and sulphate concentration in the feed to POX, which will in turn increase the efficiency of precipitation of any remaining arsenic in the autoclave.
• It will also allow the feed to the autoclave to be adjusted to the required density and sulphide plus sulphur concentrations for auto-thermal autoclave operation. Therefore, it will not be necessary to blend untreated pyrite concentrate with BIOX® discharge in the POX feed to achieve the required heat balance. This will further reduce the size of the autoclave and lower capital costs.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the work of all investigators and pilot plant operators who have been involved in the technical evaluation of the Olympias project. The permission of TVX Hellas in publishing this paper is gratefully acknowledged.

REFERENCES

The development of the first commercial GEOCOAT® heap leach for refractory gold at the Agnes mine, Barberton South Africa

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Abstract

Facing lower margins, gold mine operators throughout the world are seeking ways to reduce costs for treating refractory sulphide ores. Fortunately, oxidation technologies for refractory ores need not be complex or costly. GeoBiotics, LLC. has developed the GEOCOAT® heap biooxidation technology for the treatment of refractory gold ores and concentrates. GEOCOAT® uses simple low cost unit operations common to heap leaching of gold and copper ores. The cost effectiveness and flexibility of GEOCOAT® heap biooxidation allows mining companies to reduce cut-off grades and increase mine life. In addition, GEOCOAT® can be utilized to supplement or replace existing expensive or environmentally unfriendly oxidation processes such as roasting, autoclaving, or agitated tank biooxidation.

The GEOCOAT® technology involves coating sulphide concentrates onto sized support rock, stacking in a heap environment, irrigating with acidic nutrient solutions and supplying low-pressure air to the heap base. After biooxidation the concentrate is stripped from the support rock, neutralized and leached in conventional cyanidation facilities.

African Pioneer Mining (APM) has adopted the GEOCOAT® technology for use at the redeveloped Agnes Mine in Barberton, South Africa. This paper details the development of the GEOCOAT® process including laboratory testing and construction and commissioning of the first commercial GEOCOAT® plant. The Agnes Mine is expected to initially produce approximately 25,000 ounces of gold per year with the potential for expansion.

Keywords: biooxidation, refractory ore, sulphide, pyrite, GEOCOAT®

1. INTRODUCTION

The GEOCOAT® process incorporates elements of two successful and commercially proven technologies: heap leaching and biooxidation. Gold-bearing sulphide minerals are concentrated by flotation and thickened. The resulting slurry is thinly coated onto crushed, screened support rock, stacked on a lined pad, and allowed to biooxidize. Coating is accomplished by spraying the concentrate slurry onto the support rock as it discharges from the end of a stacking conveyor onto the biooxidation heap as shown in Figure 1. The
coating solids density is highly dependent on the slurry viscosity and densities of 50-65% have been successfully coated at scale.

The hydrophobic nature of the concentrate assists in the formation of a coating on the support rock. No binding agents are required. The concentrate naturally adheres to the support rock and does not wash out of the heap during solution application or during heavy rainstorms.

**Figure 1. Coating Operation**

The support rock is uniformly sized, in the range of 6 to 25 millimeters in diameter and the concentrate coating is relatively thin, less than one millimeter in thickness. The weight ratio of support rock to concentrate is in the range of 5:1 to 10:1. Figure 2 illustrates the concentrate coated support rock from a pilot test.

**Figure 2. Concentrate Coated Support Rock**

Depending on the desired temperature of operation, the heap is inoculated with naturally occurring sulphide-oxidizing bacteria. Nutrients are added to the heap via recirculating solutions. As biooxidation progresses, the sulphides in the concentrate are oxidized and the solubilized iron, arsenic and sulphate are carried from the heap by the recirculating solution. A portion of the solution stream is bled from the circuit for neutralization to maintain a maximum iron or arsenic level.

The relatively uniform size of the support rock leads to large interstitial spaces within the heap and subsequently a low resistance to air and liquid flows. Sufficient air for biooxidation and heat removal is supplied to the heap by low-pressure blowers through a system of perforated pipes laid in the drain rock below the base of the heap.
After biooxidation the coated rock is unloaded from the pad and the oxidized concentrate removed by trommeling or wet screening. The concentrate residue is then neutralized and subjected to conventional gold recovery methods. The support can be recycled or, in the case of low-grade sulphide ore, a portion can be bled out for gold recovery and replaced with fresh gold bearing ore. Figure 3 presents a simplified schematic representation of the process\(^{(1,2)}\).

**Figure 3. GEOCOAT® flowsheet**

The Agnes mine, located in Barberton, Mpumalanga, South Africa, decided to employ the GEOCOAT® technology as it presented a lower cost alternative refractory gold treatment scenario. Additionally, the process is simple and easy to control. The mine, owned by African Pioneer Mining, is scheduled to produce approximately 25,000 ounces of gold per year from an ore throughput of 500 tonnes per day from the Galaxy deposit. The GEOCOAT® plant is designed to treat approximately 50 tones per day of sulphide concentrates containing 50 g/t Au, 15% Fe, 1% As and 15% sulphide sulphur.

The GEOCOAT® plant is operating at full capacity with respect to concentrate delivery and the first biooxidized concentrates are expected to be treated in the CIL circuit by the end of May 2003. The plant was commissioned using temporary materials handling equipment but the balance of the plant including stacking and reclaim conveyors, trommel, CIL, neutralization and cyanide destruction are scheduled to be commissioned at the same time as the first concentrates are completing their biooxidation cycle. This paper details the testwork employed to develop the plant and the ongoing construction and commissioning.

### 2. LABORATORY TEST PROGRAM

A series of biooxidation tests have been performed at Lakefield Research Africa to define the performance of the GEOCOAT® process on the Galaxy and Princeton concentrates. These tests include amenability testing and column tests. The amenability tests are conducted as batch stirred tank biooxidation and the column tests are conducted in 150mm diameter by 6m high columns. Several process variables have been investigated.
including coating ratio, heap height, temperature regime, and grind. Additionally, mineralogical investigations were undertaken before any testwork was initiated.

The amenability tests are conducted at approximately 5% solids w/v using a heated and aerated stirred tank reactor. Periodic solution and solids samples are removed to determine the extent of biooxidation and the gold extraction. Both amenability and column tests employ adapted bacteria that is maintained using the concentrate under investigation. Approximately 2 months of adaptation is required prior to the commencement of any biooxidation testing.

The columns tests are conducted by batch coating the concentrate onto representative substrate at a known ratio and loading into the column. The columns are equipped with zone heating to ensure uniform temperatures. Low pressure humidified air is applied to the base of the column at rates in excess of stoichiometric.

Acid solutions are applied to the top of the column via a peristaltic pump, the effluent solutions are collected separately at the column base. Effluent solutions are generally recycled. However, solution is removed on a periodic basis to maintain the desired PLS profile. The column is fitted with sampling ports that allow for both solid and liquid sample removal. At the termination of the biooxidation cycle the column is acid/water rinsed, allowed to drain and then emptied. The concentrate residue is removed from the support rock by simple wet screening. The biooxidized concentrate is then subjected to CIL testing to determine gold extraction and reagent consumptions.

2.1 Mineralogy

The Galaxy and Princeton deposits are composed of a very similar mineral makeup. The primary gold carrier is pyrite (FeS$_2$) with minor amounts of arsenopyrite (FeAsS). The primary gangue material is quartz (SiO$_2$) with appreciable quantities of siderite (FeCO$_3$) and ankerite (CaCO$_3$*(Mg, Fe, Mn)CO$_3$). Both ores are refractory due to the small gold grains encapsulated within the sulphide matrix. The majority of the gold is less than 5um. The Princeton ore is a higher sulphur grade and is more refractory, providing a lower baseline gold extraction with direct cyanidation. Table 1 shows the average composition of the Princeton and Galaxy flotation concentrates.

As shown from Table 1, the Princeton concentrate has a much higher sulphide content but at a lower gold grade. The Agnes orebody is also comprised of a variety of other reefs but most are similar to either the Galaxy or Princeton veins. Table 2 shows the complete testwork program for the Agnes orebody.

**Table 1. Princeton and Galaxy Concentrate Mineralogy**

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<th>Galaxy</th>
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Key: 1 - Svengali 1 4 - Giles 2 7 - Galaxy 1 10 - Princeton 2
      2 - Svengali 2 5 - Woodbine 1 8 - Galaxy 2 11 - Princeton 3
      3 - Giles 1 6 - Woodbine 2 9 - Princeton 1 12 - Princeton 4

2.2 Batch amenability biooxidation testing

Batch biooxidation amenability tests were carried out at Lakefield on nine samples of concentrates from five different ore zones. Initial samples (Svengali 1, Woodbine 1, Giles 1, and Princeton 1) were relatively low-grade, a result of very high mass yields in the flotation tests. Subsequently, the batch amenability tests were repeated on higher grade concentrates (Svengali 2, Woodbine 2 and Giles 2). Only one concentrate was produced from the Galaxy ore for amenability. The results of this work are summarized in Table 3.

As shown by Table 3, all concentrates were extremely amenable to biooxidation producing sulphide oxidations ranging from 74.3% to 100% with gold extractions ranging from 92.5% to 96.1%. Figure 4 shows these results graphically.

Figure 4 shows that the concentrates exhibit a wide-ranging level of refractoriness as shown by the baseline CIL extractions. Gold extractions range from 47.9% to 76.0% for the unoxidized concentrates. It is also apparent that in order to achieve high overall extractions, near complete oxidation is required.

The reagent consumption of these BAT tests was extremely dependant on the concentrate employed and the subsequent elemental sulphur formed during the biooxidation process. Generally, the biooxidation process becomes more efficient at elemental sulphur removal as the bacterial adaptation period is lengthened(3).
Table 3. Batch Amenability Tests

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<th>Biooxidation time days*</th>
<th>Conc. Head Grade Au g/t</th>
<th>Sulphur Oxidation %</th>
<th>Gold Extraction %</th>
<th>Reagent Consumption**</th>
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<th>CaO kg/t</th>
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<td>50.6</td>
<td>0.0</td>
<td>76.0</td>
<td>9.7</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>82.1</td>
<td>93.5</td>
<td>21.9</td>
<td>8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>98.6</td>
<td>95.5</td>
<td>22.3</td>
<td>8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>99.3</td>
<td>95.7</td>
<td>18.1</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100.0</td>
<td>96.1</td>
<td>17.0</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 0 bio-oxidation time represents the concentrate sample as-received

** reagent consumptions - kg/t unoxidized concentrate
2.3 Column Biooxidation Testing

The batch amenability tests were followed by a series of GEOCOAT® column tests. Column tests are designed to simulate conditions in a GEOCOAT® heap. Support rock type, coating ratio, solution application rate, and solution management scheme are all selected to duplicate the operation of the full-scale heap. Column diameter is 150mm and the height is typically 2m. However, a final test was carried out in a 6m tall column, designed to simulate fully a GEOCOAT® heap stacked to the design height of 6m. This test will provided additional information on solution chemistry and confirmed the solution management strategy proposed for the Agnes project.

The concentrates for each of the first three column tests were produced from Princeton ore, while a fourth column test was carried out on Galaxy concentrate produced from the Agnes plant. Separate concentrates were produced for each of the column tests. The first test used a barren support rock, while the other tests were run using the Alpine waste rock that it is proposed to use in the full-scale heap.

Intermediate solids samples were extracted from each of the columns twice during the course of the tests. At the termination of the test, the columns were emptied and the contents wet screened to separate the support rock from the oxidised concentrate. As with the intermediate samples, the oxidised concentrate slurry was filtered, washed and dried, and the solids analysed and subjected to cyanidation testing. Table 4 shows the results of the biooxidation column testing.

As shown, the column tests produced similar gold extraction results to those of the BAT tests with lower cyanide consumptions. The use of a 6m column had no impact on the biooxidation process and excellent results were achieved.
### Table 4. GEOCOAT® Column Test Results

<table>
<thead>
<tr>
<th>Column No.</th>
<th>Cyanidation Conditions</th>
<th>Reagent Consumption*</th>
<th>Reagent Consumption*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bioox. Time days</td>
<td>Sulphur Ox %</td>
<td>NaCN ppm</td>
</tr>
<tr>
<td>Prin 1</td>
<td>0</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>(Conc. 2)</td>
<td>20</td>
<td>23.1</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>53.0</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>77.8</td>
<td>2000</td>
</tr>
<tr>
<td>Prin 2</td>
<td>0</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>(Conc. 3)</td>
<td>21</td>
<td>68.4</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>81.0</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>53 (final)</td>
<td>91.5</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>53 (final)</td>
<td>91.5</td>
<td>2000</td>
</tr>
<tr>
<td>Prin 3</td>
<td>0</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>(Conc 4)</td>
<td>24</td>
<td>39.1</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>68.8</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>86.7</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>95.1</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>95.1</td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>95.1</td>
<td>2000</td>
</tr>
<tr>
<td>Galaxy 2</td>
<td>0</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>(6m)</td>
<td>24</td>
<td>63.9</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>89.6</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>93.0</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>93.0</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>93.0</td>
<td>2000</td>
</tr>
</tbody>
</table>

* based on bioleach concentrate feed, ** 24hr CIL tests if not stated

### 3. GEOCOAT® PLANT CONSTRUCTION

The Agnes mine began construction of the GEOCOAT® plant in October of 2002 and the first concentrate was stacked on the pad at the beginning of February 2003. This despite construction being delayed for a month by the Christmas season. It is expected that the recovery of the first biooxidized concentrate will commence by the middle of May 2003 coinciding with plant completion.

The biooxidation cycle is expected to require approximately 60 days to complete. The first heap will require a slightly longer period as the support and base rock need to be pH stabilized due to their carbonate content. Additionally, the speed of the process will improve as a large population of adapted mesophilic bacteria is formed. Currently bacteria is grown on site for heap inoculation in 3-10m³ fermentors. The heap has been biooxidizing at an average rate of 1.7% sulphide oxidation per day, above the design rate of 1.5% and the heap is maintaining its desired operating temperature between 35-45°C.

The Agnes GEOCOAT® plant was designed and built to provide African Pioneer Mining with flexibility for future expansion. The pad base was enlarged as was the air supply to facilitate the treatment of other concentrates such as those to be derived from the Princeton deposit. The Table 5. provides the design statistics of the Agnes plant.
Table 5. GEOCOAT® Plant Statistics

<table>
<thead>
<tr>
<th></th>
<th>Design (for Galaxy only)</th>
<th>Actual/Proven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stacking Rate</td>
<td>34.5 tph</td>
<td>100 tph</td>
</tr>
<tr>
<td>Concentrate Rate</td>
<td>4.6 tph</td>
<td>8.0 tph</td>
</tr>
<tr>
<td>Biooxidation Time</td>
<td>60 days</td>
<td>N/A</td>
</tr>
<tr>
<td>Irrigation Rate</td>
<td>10-30 L/m²/hr (max 80m³/hr) Using Wobblers®</td>
<td>10-30 L/m²/hr (max 120m³/hr) Using Wobblers®</td>
</tr>
<tr>
<td>Aeration</td>
<td>Centrifugal Fan 360 m³/min at 2.5 kPa</td>
<td>Centrifugal Fan 3 x 360 m³/min at 2.5 kPa</td>
</tr>
<tr>
<td>Aeration Method</td>
<td>Perforated pipes in drain rock base</td>
<td></td>
</tr>
<tr>
<td>Pad Size</td>
<td>50 x 75 m</td>
<td>50 x 150m</td>
</tr>
<tr>
<td>Heap Size</td>
<td>6 x 45 x 60m</td>
<td>6 x 45 x 130m</td>
</tr>
<tr>
<td>Pond Size</td>
<td>1 x 2500 m³</td>
<td>1 x 7000 m³</td>
</tr>
<tr>
<td>Stacking Method</td>
<td>Slewing radial stacker with automated material handling</td>
<td></td>
</tr>
<tr>
<td>Concentrate Recovery</td>
<td>FEL, Trommel, Thickener</td>
<td></td>
</tr>
<tr>
<td>Gold Recovery</td>
<td>24 hr CIL - 6 x 11 m³ Tanks</td>
<td>24 hr CIL - 6 x 20 m³ Tanks</td>
</tr>
<tr>
<td>Effluent Disposal</td>
<td>Heap bleed solutions are neutralized by mixing with carbonate float tails. CIL cyanide is destroyed using excess acid bleed.</td>
<td></td>
</tr>
<tr>
<td>Performance Monitoring</td>
<td>Solution analysis, solid sampling, and temperature monitoring.</td>
<td></td>
</tr>
</tbody>
</table>

The following pictures document the construction of the plant.

Figure 5. Site – Sept 2002

Figure 6. Site showing pad and pond – Oct 2002
4. CONCLUSIONS

The first commercial GEOCOAT® plant for the treatment of refractory gold sulphides is currently being commissioned in Barberton, Mpumalanga, South Africa at the Agnes Gold Mine. African Pioneer Mining has selected this technology based on its cost
advantages, its inherent simplicity and safety. Laboratory testwork has shown that the process should yield sulphide biooxidations well above 90% in under 60 days with gold extractions also above 90%. The commercial heap has yet to complete its first biooxidation cycle but indications are that it will yield the desired results. The heap is now operating within the desired temperature range and solution and solids assays indicate an average sulphide biooxidation rate of 1.7% per day, which is in excess of design.

ACKNOWLEDGEMENTS

The authors would like to thank African Pioneer Mining for the permission to write this paper and also all of those people who assisted in making this project a success.

REFERENCES

The electrochemistry of chalcopyrite bioleaching using bacteria modified powder micro-electrode*

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Abstract

The anode behavior of chalcopyrite in the presence and absence of bacteria in 9K mediums was studied using a combination of standard electrochemical techniques by bacteria modified powder microelectrode at 30°C. It was found that the anode oxidation process of chalcopyrite includes many intermediate transient reactions. During the dissolution, it is exist the intermediate production of chalcocite and covellite. At lower scanning potential, the iron of chalcopyrite is extracted by ferrous form, but at the relative high potential, it is the ferric. When in the presence of *Thiobacillus ferrooxidans*, the peak current and reversibility of oxidation reaction increased, and the oxidation potential negatively moved. It demonstrated that apart from enhancing the metallic ion extracting oxidation reaction, the *Thiobacillus ferrooxidans* has also contribution to the oxidation of element sulfur formed on the surface of chalcopyrite during the intermediate process. The effect of ferric and pH on the oxidation of chalcopyrite was also investigated.

Keywords: chalcopyrite, *Thiobacillus ferrooxidans*, electrochemistry, bioleaching, oxidation, bacteria modified, powder microelectrode, mechanism

1. INTRODUCTION

The bioleaching research has been a great success for metallurgy industry. As a result of this work, a significant number of commercial applications have emerged and are able compete with conventional processing, especially the application for the copper recovery. Furthermore, bioleaching treatments have the great advantage of being environment friendly [1, 2]. However Bioleaching applications for copper extraction are mainly concentrated on the treatment of secondary copper minerals. chalcopyrite is the most abound ore of the sulfide minerals of copper [3], but it gives very slow kinetics and limited recovery, so it need to elucidate the oxidation mechanism. We know that the microorganism can catalyze the copper dissolved.

It has demonstrated that chalcopyrite and most other metallic sulfide are dissolved by the electrochemistry mechanism, and the most of sulfides are semiconductors, using the electrochemistry methods to study the oxidation mechanism of sulfides is affective [4-10].

* supported by national science foundation of China [50204001]
Furthermore, there are large number of studies on the anode dissolved mechanism of chalcopyrite in different media including culture media or other experimental condition [11, 12], however there are fewer information on the experiments when microorganism in presence. One of the most important reasons is it is difficult to guarantee the affective attachment of microorganism on the chalcopyrite surface when the electrochemical quick scan carrying on, and the convention polished nature massive specimen used as work electrode and the microorganism added in solution media [13]. For this reason, we used a new method of the bacteria modified powder microelectrode as the work electrode to over the above said difficulties. Based on the characters of the microelectrodes, apart from the affective attachment of leaching bacteria on the surface of chalcopyrite powder, the more information about transient intermediate reaction and other useful information during electrochemistry scanning can be obtained [14]. Although it is reported that some microorganisms like Sulfulobus are more effective on the chalcopyrite resolution, but in ordinary temperature, thiobacillus ferrooxidans is still the main microorganism for bioleaching [15], so in this work, we use thiobacillus ferrooxidans as bioleaching microorganism to do some preliminary investigations.

2. MATERIALS AND METHODS

2.1 Ore

The nature chalcopyrite was get from Hunan museum, with high quality and showing no foreign inclusions under the microscope. The massive specimen was crushed and milled under N₂ ambience in order to prevent the surface oxidation. The size of particle was under 50um.

2.2 Bacterial culture

The mixed cultures of acidophilic bacteria were obtained from Guangdong Dabaoshan copper mining. The _thiobacillus ferrooxidans_ was isolated from mixed cultured in laboratory, The standard composition of the nutritive media was 9K media, g/L: (NH)₂SO₄, 3.0; KCl, 0.1; K₂HPO₄, 0.5; MgSO₄·7H₂O, 0.5; Ca(NO₃)₂, 0.01. The water used in the experiment was ferrous ion free.

2.3 Electrode

Prepared an micro plate electrode using platinum wire, and the diameter is 100 um, corroded the micro electrode surface to a small 100um deep pitch; washed the chalcopyrite powder with acetone prior to use, added the bacteria to chalcopyrite powder, blended evenly and keep for few hours in order to guarantee the bacteria attached and adhered to the powder surface absolutely, so the bacteria modified sulfide powder was prepared, and compressed it into the electrode pitch using a glass plate to make the powder electrode surface flawless. The detail method has described in other papers [14, 16]. The powder microelectrode is composed of a micro plate electrode and a thin layer electrode. The structure of the electrode is shown in Fig. 1.
2.4 Electrochemical experiment

The electrochemical measurements were performed in a typical cell (500mL) with three electrodes: the working electrode (bacteria modified chalcopyrite powder microelectrode), the counter electrode (Pt plate), and the reference electrode (KCl-saturated calomel electrode). The cell was kept at constant temperature by connecting it to a circulating thermostatically controlled water loop. The electrolyte used in the experiment was standard 9K media without ferrous ion. The used water was ion removed. The electrochemical experiments were carried out using Solartron 1287. In the paper, all the potentials value is Vs SCE.

3. RESULTS AND DISCUSSION

3.1 Influence of bacteria

Fig. 1 and Fig. 2 are the cyclic voltammograms of the chalcopyrite powder microelectrode when in absence and presence of bacteria. The initial sweep potential is the rest potential. Comparing different sweep cyclic we can see that the oxidation of chalcopyrite include multi intermediate steps. From Fig.1 and Fig.2 we know that from initial potential the shape of the anode prewave of first cyclic is different from the second and third cyclic, comparing the anode direct oxidation reaction and cathode direct reduction reaction, it demonstrates that during the oxidation there is a thin layer product covered on the chalcopyrite surface, and the anode process is controlled by diffuse steps. This is similar to the results described by Biegler et al using massive chalcopyrite electrode in acid medium [17-20]. It is predicted that in the anode oxidation process, there are element sulfur and other intermediate phase will be created on the chalcopyrite surface.

In Fig. 1, A represents the "prewave", in which chalcopyrite is transformed to CuS, through an intermediated non-stochiometric phase (Cu_{1-x}Fe_{1-y}S_{2-z}), producing S^0 and Fe^{2+}, the oxidation of chalcopyrite through the following reaction:

\[ \text{CuFeS}_2 \rightarrow \text{CuS} + \text{Fe}^{2+} + S^0 + 2e^- \quad E^0=0.055 \]

In the potential zone from −0.2V to 0.5V (the initial potential of peak A has a small movement to negative during second and third cyclic sweep in anode direction. It is possible to conclude that because of the deform of the crystal lattice of chalcopyrite by
polarized and the intermediate production formed by cathode reduction reaction, the oxidation reaction of Cu$_2$S may occur by:

\[
\begin{align*}
\text{Cu}_2\text{S} & \rightarrow 2\text{CuS} \quad \text{E}^\circ=-0.021 \\
\text{CuFeS}_2 & \rightarrow \text{Cu}^{2+} + \text{Fe}^{2+} + 2\text{S}^\circ + 4\text{e} \quad \text{E}^\circ=0.231
\end{align*}
\]  

Figure 1. The cyclic voltammograms of the chalcopyrite in absence of bacteria (r$_a$=5×10$^{-5}$ m, 5 mVs$^{-1}$, T=25°C, pH=2, initial sweep direction: anode)
1-first cyclic, 2-second cyclic, 3-third cyclic

Figure 2. The cyclic voltammograms of the chalcopyrite in presence of bacteria (r$_a$=5×10$^{-5}$ m, 5 mVs$^{-1}$, T=25°C, pH=2, initial sweep direction: anode)
1-first cyclic, 2-second cyclic, 3-third cyclic
At the potentials more positive than 0.7V, the Fe\(^{2+}\) and S\(^{0}\) would be oxidized to Fe\(^{3+}\) and SO\(_4^{2-}\) for further steps in the C range. The overall dissolution of chalcopyrite takes place through the following reactions:

\[
\text{CuFeS}_2 \rightarrow \text{Cu}^{2+} + \text{Fe}^{3+} + 2\text{S}^{0} + 5e^-\quad E^o=0.274
\]

\[
\text{CuFeS}_2 + 8\text{H}_2\text{O} \rightarrow \text{Cu}^{2+} + \text{Fe}^{3+} + 2\text{SO}_4^{2-} + 16\text{H}^+ + 17e^-
\]

It is consistent to the Macillan et al’s results using massive electrode, but the peak of the oxidation of intermediate process is more clearly. Potential range from about 0.3V to 0.7V (peak B) represents the chalcopyrite oxidation reaction:

In the reverse scan, the range of D and E may represent the reduction of Fe\(^{3+}\) and Cu\(^{2+}\) produced in anode reaction respectively, the peak F may represents the reverse reaction of (3), by the express of Biegler et al [20-23]. We know that during the cathode scanning there would be forming a reduction layer on the chalcopyrite particles surface. At the peak G and H, there would occur a series of reaction as following:

\[
2\text{CuS} + 2\text{H}^+ + 2e^- \rightarrow \text{Cu}_2\text{S} + \text{H}_2\text{S}
\]

\[
\text{S}^{0} + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{S}
\]

The peak may represent the oxidation of element sulfur formed on the surface of chalcopyrite during the anode and cathode process. In this range the reverse reaction of (7) would occur.

Comparing Fig. 1 and Fig. 2, we know that thiobacillus ferrooxidans can enhance the oxidation of chalcopyrite, because in Fig. 2, the shapes of the oxidation reactions represented by prewaves of A, B and C are more apparently, especially the peak B and C. The anode sweep results of currents and potentials of the reaction can be shown in Table 1. From the results we know that when in the presence of thiobacillus ferrooxidans, the peak current density and reversibility of the oxidation reaction increased, and the oxidation potential negatively moved. As described above, the reaction of (4), and the conversion of ferrous to ferric and the element sulfur to sulfate on the ore surface have elevated. In cathode sweep the peak D in Fig.2 is more clearly than in Fig. 1, it demonstrate that there are more mount of ferric have been produced on the surface layer during anode process when the thiobacillus ferrooxidans in presence. The peak I in Fig. 2 is more flat than that is in Fig1, it is show that there are less amount of element sulfur formed on the surface when in the presence of thiobacillus Ferrooxidans during the anode oxidation and cathode reduction process. It demonstrated that the attachment of Thiobacillus ferrooxidans on the surface can accelerate the oxidation reaction of chalcopyrite, especially the oxidation of ferrous, apart from enhancing the metallic ion extracting, it also has contribution to the oxidation of element sulfur formed on the surface.

### Table 1. The anode sweep results of currents and potentials by cyclic voltammogram (first cyclic)

<table>
<thead>
<tr>
<th>Peak ranges</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactions</td>
<td>(1)</td>
<td>(3)</td>
<td>(4)</td>
<td>(7)</td>
</tr>
<tr>
<td>Inoculated</td>
<td>6.59</td>
<td>22.33</td>
<td>18.70</td>
<td>1.51</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>3.17</td>
<td>7.66</td>
<td>6.27</td>
<td>2.76</td>
</tr>
<tr>
<td>Initial potentials of prewave (V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>-0.23</td>
<td>0.26</td>
<td>0.68</td>
<td>-0.68</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>-0.20</td>
<td>0.32</td>
<td>0.75</td>
<td>-0.53</td>
</tr>
</tbody>
</table>
3.2 Influence of pH

Fig. 2 is the cyclic voltammograms of the chalcopyrite in the presence of bacteria under different acid condition.

![Figure 2. The cyclic voltammograms of the chalcopyrite in the presence of bacteria](image)

From the results we know that when the pH decrease from 2 to 1.5, the anode oxidation potentials of chalcopyrite (especially prewave B and C) become more electropositive, the current density decrease, and the anode oxidation reaction is inhibited slightly. This result is similar to the description by C. Comze et al [11, 20, 21]. At the cathode sweep, the shifts of at least 0.15V in the initial potential of F and G reactions towards the more electropositive zone because of the decrease of pH from 2.0 to 1.5 have been observed. It is means that there is a clear influence of pH to the cathode scan by the following reactions:

\[
\text{2CuS} + 2\text{H}^+ + 2e \rightarrow \text{Cu}_2\text{S} + \text{H}_2\text{S}
\]

\[
\text{S}^\circ + 2\text{H}^+ + 2e \rightarrow \text{H}_2\text{S}
\]

In which we know the increase of pH would enhance the reactions towards right hand direction, and the E-pH dependence can be expressed as: \(E=E-0.059\cdot\text{pH}\).

3.2 Influence of Fe\(^{3+}\)

Fig. 3 is the cyclic voltammograms of the chalcopyrite when the Fe\(^{3+}\) free and added in the electrolyte. At the anode scan, the current density of prewave of A, B, C and I increased and the initial potentials of prewave negatively moved. The addition of Fe\(^{3+}\) makes the reaction rates of the anode oxidation of chalcopyrite increased greatly, and we know that the Fe\(^{3+}\) can oxidize the intermediate CuS or Cu\(_2\)S film formed on the chalcopyrite. At the same time, from the information given by prewave of I, we know that by the increase of oxidation rates there are more element sulfur formed on the chalcopyrite surface, although the diminish effect by \textit{thiobacillus ferrooxidation}.
Figure 2. The cyclic voltammograms of the chalcopyrite in presence of bacteria (\(r_a=5\times10^{-5}\ m, 5\ mVs^{-1}, T=25^\circ C,\ pH=2,\ initial\ sweep\ direction:\ anode\) 

1—\([Fe^{3+}]=0.000\ mol.dm^{-3}\)  
2—\([Fe^{3+}]=0.179\ mol.dm^{-3}\)

In the reverse scan, the peak current density of the relative reduction reactions corresponding to the oxidation reactions increased and the initial potentials electro positively moved. Comparing with the cyclic without added ferric and attachment of bacteria on chalcopyrite, the prwave of the Fe\(^{3+}\) reduction reaction:

\[
Fe^{3+} + e \rightarrow Fe^{2+}
\]

is more clearly when added ferric shown by peak D. On the freshly prepared surface of chalcopyrite there is almost no reduction reaction occurred in Fig.1, due to the slow kinetics and the irreversibility of the Fe\(^{2+}/Fe^{3+}\) couple on this sulfide. It demonstrate that the thiobacillus ferrooxidans and ferric have positively affected on the dissolution of chalcopyrite. Because of the more production produced during the anode oxidation process, the current density of the other reduction reactions in cathode scan increase relatively.

4. CONCLUSIONS

The anode oxidation process of chalcopyrite includes many intermediate transient reactions. During the dissolution, it is exist the intermediate production of chalcocite and covellite. At lower scanning potentials the iron of chalcopyrite is extracted by ferrous form, but at the relative high potential it is by the ferric. When in the presence of *Thiobacillus ferrooxidans*, the peak current density and reversibility of the oxidation reaction increased, and the oxidation potential negatively moved. It demonstrated that apart from enhancing the metallic ion extracting reaction, the *Thiobacillus ferrooxidans* has also contribution to the oxidation of element sulfur formed on the chalcopyrite surface during the intermediate process. The decrease of pH from 2.0 to 1.5 gives slightly inhibition to the dissolution of chalcopyrite. The Fe\(^{3+}\) added in the medium can enhance the anode oxidation of chalcopyrite.

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REFERENCES

The influence of crystal orientation on the bacterial dissolution of pyrite

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Abstract

In order to understand the influence of crystallographic orientation on the mechanism of pyrite bioleaching, single crystals cut to expose plane orientations of 100, 111 and 110 were used for the study. Experiments were carried out both in the presence and absence of *Thiobacillus ferrooxidans*. Experiments to compare the extent of dissolution of the pyrite surfaces in bacterial and sterile solutions under similar solution conditions were also undertaken by matching the conditions in sterile solutions to those in bacterial leaching using an electrolysis cell. Differences in the reaction rates of the pyrite surface planes in both sterile and bacterial solutions have been observed. Furthermore, the results for the comparison between the bacterial and sterile leaching of pyrite samples under similar conditions indicate higher dissolution rates in the presence of bacteria. The microbial corrosion patterns generated on the surfaces were further used to study the leaching process. Microbial leaching of pyrite was observed to create surface corrosion patterns distinct from those of sterile leached samples. In addition the morphology of corrosion patterns arising from microbial leaching were found to slightly differ from one crystal plane to another while those in sterile leaching generally reflected the symmetrical arrangement of the crystallographic planes in the lattice on which they formed. The variation of corrosion patterns observed on the surfaces of bioleached samples seems to indicate a variation in cell-surface interaction from one crystal plane to the other. The results show that the surface properties of mineral sulphides may control the evolution of corrosion patterns and the initial oxidation kinetics in acid bacterial leaching. The overall analysis seems to indicate an influence of the primary cell-mineral interaction during the early leaching stage.

Keywords: bacterial attachment, pyrite, crystal orientation, corrosion patterns

1. INTRODUCTION

Pyrite is the most common metal sulphide in the mineral processing industry and is normally found in three main crystal forms, the most common being the cubic form where (100) surfaces predominate. This surface is close to ideal, and has a bulk termination of Fe and S species with a five-fold coordination of Fe sites and three-fold coordination of S sites, these being the respective bonding environments for the uppermost surface of Fe and S sites on a flat terrace. It is also found as octahedral and pyritohedral crystals, terminated by the (111) and (210) surfaces respectively. According to Guevremont *et al* (1998), the
111 surfaces can either be sulphur or iron terminated with the surface Fe atoms and S₂ groups being three coordinated and with each bonded to three species (S₂ or Fe atoms respectively) in the layer below. Rarely, pyrite is found in the form of dodecahedral crystals terminated by the (110) surfaces. This surface structure has a four-fold coordination and lies perpendicular to the 110 direction, which according to Edwards et al (1998) is the direction characterized by a high density of disulphides in the pyrite lattice.

Due to its ubiquity an understanding of the reactivity of pyrite is important especially for such applications as froth flotation and leaching, as well as for geochemical processes like the production of acid mine waters. All of these processes involve reactions at pyrite surfaces and, as a result, it is essential to understand the nature of the reactions occurring at these surfaces. Although some research has been done on the effect of crystallographic orientation on the dissolution process of pyrite, there are relatively few studies in the field of bioleaching that explain the aspects involved, especially the association if any, between the surface structure and the leaching process in the presence of surface attached bacteria. The main objectives of the work described in this paper are to investigate the influence of crystallographic orientation on the bacterial leaching of pyrite crystals by undertaking an examination of the dissolution rates and the surface corrosion patterns associated with the dissolution process and establishing the correlation, if any, between the surface attached bacteria, the observed dissolution pits, and the surface structure of the planes.

2. MATERIAL AND METHODS

2.1 Bacteria culturing

The strain of *Thiobacillus ferrooxidans* used in this experimental study was originally obtained from University College, Cardiff and was propagated in 9K medium at pH 1.8 (Silverman and Lundgren, 1959). The same medium was modified accordingly and used for experimental processes. The number of bacterial cells was estimated by direct counting using the improved Helber counting chamber. The final cell concentration used for experimental inoculation was approximately 1x10⁸ cells/ml.

2.2 Pyrite sample preparation

Single crystal cubes (approximately 1 cm³ and weighing 4.5-8g) of natural pyrite were characterised by the Laue X-ray diffraction method (Phillips Analytical X’Pert Data Collector). The 100 planes were found to be the principally occurring planes on the faces of the samples. The samples were cut with a diamond saw and polished from initial symmetric cubes parallel to the plane orientations of 100, 110 and 111. The samples were prepared with each surface of the required face to limit the leaching to about 0.5-1 cm² exposed area and covering the remaining surface with araldite epoxy resin. These were then washed with ethanol and dilute HCl before each experiment to remove any soluble material on the surface. The samples were made in pairs so that matching faces could be used for duplicate experiments.

2.3 Matched leaching experiments

The aim of the experiments was to compare the extent of dissolution of the different crystallographic pyrite surfaces in bacterial and sterile solutions under similar solution conditions, using a method previously adopted by Driessens *et al* (1999) in the study of sphalerite. In the present study, experiments were done by simulating conditions experienced in bacterial leaching by controlling the redox potential in sterile leaching through ferrous to ferric iron electrolytic oxidation. The apparatus used was a two-
compartment electrolysis cell in which the two compartments were separated by an ion exchange membrane (Figure 1). The sterile leaching reactions were done in the working compartment of the electrochemical cell, whilst the bacterial leaching experiments were done in a separate reactor. Both the cathode and anode were made of platinum foil. The redox potential in the working compartment was matched to those measured in parallel bacterial leaching experiments, by manipulating the current to the platinum cathode. Thus, in sterile leaching a current was applied to oxidise the ferrous to generate the same solution redox potentials as measured in the bacterial experiments. The redox potentials were measured using a platinum electrode with a silver/silver chloride reference electrode.

![Figure 1. Schematic diagrams of the apparatus for re-oxidation of ferrous iron in chemical leaching experiments](image)

The reaction for pyrite mineral dissolution is:

$$FeS_2 + 8H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 16H^+ + 14e^-$$

While reoxidation at the platinum electrode is defined mostly by,

$$Fe^{2+} \rightarrow Fe^{3+} + e^-$$

### 2.3.1 Leaching media

The leaching experiments were carried out in 9K basal medium (3.00 g (NH$_4$)$_2$SO$_4$, 0.50g K$_2$HPO$_4$, 0.50g MgSO$_4$7H$_2$O, 0.01g Ca(NO$_3$)$_2$ and 0.10 g KCL dissolved in 1000ml distilled water) at a pH of 1.8 adjusted with sulphuric acid. A cell suspension (10% v/v.) with an initial population of 1x10$^8$ cells/ml was added to the bioleaching solution. Initially, a ferrous iron solution giving a total Fe (II) concentration of 0.05M was added to each solution to provide a source of energy for the bacteria in the bioleaching experiments and to gradually generate ferric ions by electrolytic oxidation in the sterile leaching experiments. The leaching volume used for each experiment was 100ml.

### 2.4 Analytical methods

A scanning electron microscope, JEOL JSM T220, was used to monitor bacterial adhesion and surface changes occurring on the samples during leaching. The samples were removed from solution, washed with acidified water, then acetone, dried, coated with a very thin layer of gold and observed under SEM. The samples were observed at weekly
intervals. The total iron concentration in the bio-oxidation and chemical leaching solutions was measured at 2-day intervals using a Perkin-Elmer 1100B atomic absorption spectrophotometer with an air/acetylene flame.

3. RESULTS AND DISCUSSION

3.1 Leaching rates

The results for the leaching experiments indicated that the surfaces of pyrite react at different rates (Figure 2a and 2b). This is most likely due to the differences in the surface atomic geometry and chemical surface states that predominate on pyrite surfaces. However, since these states are only present on the pyrite surfaces rather than in the bulk crystal structure, it is possible they will play a major role only during the early stage of the reaction process.

![Figure 2. Leaching trends of the planes in (a)-bioleaching and (b)- sterile solutions under matched conditions. Leaching conditions: bacterial leaching: 9K medium, 10% (vol/vol.) bacteria inoculumn, 0.05M Fe²⁺ solution concentration, pH 1.8. Sterile leaching: 9K medium, 0.05M Fe²⁺ solution concentration, pH 1.8](image)

The experiments were carried out in duplicate, corresponding to the matching faces of the cut crystal surfaces, A and B. While the behaviour of the matching faces of the 100 and 110 planes was not different, those of the 111 surfaces varied (Figure 2a and 2b). From surface analysis by SEM, a lower pit density was observed on one 111 surface layer, whilst the other adjacent surface had a higher pit density. In addition, the surface with a high pit density had a lower dissolution rate compared to the other over the first seven days of leaching. Since for pits to develop, it is necessary that dissolution proceed faster in the direction of the pit than on the surface surrounding it, it can be suggested that the plane with a lower pit density had the most reactive atoms on its surface. Conversely, the one with a higher pit density had the least reactive surface atoms. Furthermore, since the dissolution rate was measured in terms of released iron, it can then be assumed that the plane with a lower pit density is the iron-terminated surface (thereafter referred to as A), and the other one with the higher pit density is taken as the sulphide terminated surface (B plane). The overall reaction rate trend observed at the end of the 14-day leaching period for both sterile chemical and bacterial leaching solutions was:

111A > 111B > 110 > 100
3.1.1 Bacterial leaching

The 111A surface showed the highest overall iron dissolution after 8-10 days of leaching. However, this surface generally showed a lower dissolution rate compared to the 100 and 110 planes during the early stages of leaching when the redox potential was low. The iron released from the 111A plane only increased above that of the other planes after a sharp increase in solution potential, Figure 2(a). In addition, although the 111A plane showed an overall higher release of iron in the presence of bacteria compared to that achieved in sterile solutions, the degree of leaching enhancement (ratio of iron released under bacterial leaching to that released in sterile leaching) did not vary much, remaining generally between 1.15 and 1.20. The 111B surface, which is assumed to be the sulphur-terminated plane, showed the lowest dissolution during the early leaching stages, but this gradually increased to give an overall iron release greater than that of the 110 and 100 planes, but slightly less than that of the 111A plane. The surface geometry and lower surface atom coordination leads to the 110 plane being more reactive and producing a higher amount of dissolved iron compared to the 100 plane. In addition, the results indicated a higher amount of dissolved iron for the 100 and 110 surfaces in the presence of bacteria under similar conditions during the early stages of leaching. This seems to indicate that during this initial stage of leaching, the presence of bacteria at low concentrations of ferric iron in solution enhances the initial oxidation process. This is further discussed in Section 3.2.

3.1.2 Sterile matched leaching

In general, simulation of conditions experienced in bacterial leaching through control of the solution potential greatly increased the dissolution of pyrite under sterile leaching conditions. Sasaki et al. (1998), have reported that chemical dissolution of Fe is more rapid from pyrite than oxidation of S species in the lattice, leading to the formation of elemental sulphur on pyrite. As a result, passivation due to the inaccessibility of Fe sites to the leach media occurs. In acid solutions, the overall oxidation reactions involve the formation of sulphur and/or a metal-deficient sulphide at low overpotentials and sulphate at high overpotentials. It is possible that by maintaining a high redox potential, bacteria catalyse the sulphate-forming reactions, preventing the accumulation of a sulphur layer. Thus, if conditions in sterile leaching are matched to those of bacterial leaching, high redox potentials are maintained and sulphate, instead of sulphur, formation is promoted. Significantly, pyrite dissolution is enhanced and it becomes possible to obtain characteristic bioleaching rates in an abiotic system provided that the redox potential is kept high. A further comparison of the results for matched leaching experiments and for experiments undertaken in sterile solutions whose redox potentials were not matched to the bacterial conditions indicated that the amount of iron released for non-matched sterile conditions is on average about 20-25% of that released under matched conditions. This shows the significant influence of the solution potential and hence ferric iron in the overall dissolution process.

3.2 Pitting morphology

3.2.1 Bacterial leaching

One of the objectives of this study was to establish the role if any, of cell-mineral interactions on the evolution of corrosion pits in bacterial leaching during the early stages of leaching in the absence of significant amounts of ferric ion. This was to be done by determining whether pits were established at locations on the mineral surface where there were attached bacteria. However, although bacteria cells were observed on the 111B, 110
and 100 surfaces, their distribution was random and there was no close proximity to the corrosion pits as observed by Bennet and Tributsch (1978), Rodriguez-Levia and Tributsch, (1988). No significant bacterial colonisation was observed on the 111A surfaces. On the other hand, the observed corrosion patterns, although irregularly distributed over the surface, showed a significant orientation. It was observed that the surface planes with high ratios of sulphur/iron atoms e.g. the 111B, 100 and 110 generally generated elongated pits similar in shape to bacteria, but much larger than the bacteria cell dimensions (Figure 3a and 3b).

![Figure 3](image)

**Figure 3.** (a)-Corrosion patterns on (a)-110 plane; note the circular pits in close proximity to well-developed elliptical pits and (b)-corrosion pits on 111B planes

One other interesting aspect was the observation of surface films on these 110, 111B and 100 surfaces (Figure 4a). These were observed both in the early stages and after about a week of leaching and have previously been observed by other researchers on pyrite particles (Rodriguez-Levia and Tributsch, 1988, Edwards *et al*, 2001). Unlike in the work of these authors however, no bacteria were generally observed in close proximity to these films.

![Figure 4](image)

**Figure 4.** (a)-Surface films observed on 110, 100 and 111B surfaces. Inset shows enlarged view of the film possibly generated by bacteria. (b)-pitting on the 111A plane (bar size 10µm).
The film layers exceeded the amount of surface covered by the cells indicating that the film spreads beyond the area of bacterial contact (inset Figure 4a). The fact that surface films were observed mostly on those planes developing elongated dissolution pits seems to indicate that the extension of the film beyond the area of direct bacteria contact extends the zone/compartment of cell interaction with the surface, subsequently generating larger pits. The fact that not all the planes developed this film (111A did not) and on those where it did, only certain areas of the surface were covered, suggests that film formation depends on bacteria recognizing certain sites on the surface for colonisation, with the subsequent formation of a film. Furthermore, since the surface films were observed even after two weeks of leaching without any bacteria being observed in close proximity to the films or the pits, this may suggest that the film contains some constituent that enhances leaching after the bacterial cells have moved from these specific sites. Calculations of pit depths and pyrite lattice layers consumed for all the planes further support this hypothesis. Thus, it seems that it is the initial recognition of active sites on crystal surfaces and subsequent attachment of bacteria on these specific sites that controls the initial attack on the surface and subsurface layers (Ndlovu and Monhemius, 2003).

It has been mentioned in the literature that bacteria seem to have a preferential attachment to specific sites such as sulphur enriched zones (Mustin et al 1993, Edwards et al 1998) and they preferentially oxidise sulphur during the lag phase, while the released iron, despite being preferentially leached into solution, remains in the ferrous state (Sasaki et al 1995, 1998, Jae-Young et al 2001). The crystal structure of pyrite indicates that the directions of high sulphide density are the <110> and the <100>. If sulphur removal occurs in a crystallographically controlled manner, then the attachment of bacteria should be concentrated along these sulphur-enriched zones. This is supported by the observed high density of the corrosion pits on the 110 and the sulphide-terminated 111 surfaces (Figure 3a and 3b). The leaching patterns observed on the 110 planes were found to lie parallel to the 110 directions. On the other hand, the 111 plane is bounded by 110 directions and, significantly, corrosion patterns on the 111B plane were observed to occur parallel to the edges of the surface. Both surfaces were covered with elliptical/rod-like pores and quasi-circular pits. In general the elliptical pits developed in pairs and circular pits were found mostly in close proximity with the developed elliptical ones (Figure 3a). This suggests that bacteria possibly grow and divide in-situ with the daughter cell developing a pit near to the mother cell creating pairs of pits as illustrated in Figure 3a and 3b.

The importance of the surface structure on the leaching process of the pyrite crystal can also be understood by considering the leaching trend of the 111B plane (Section 3.1.1). This crystal plane is dominated by an immediate sulphide sulphur layer, which initially hinders the direct exposure of iron to the solution. Therefore, initially iron release is slow and sulphur release is favoured until, due to the action of attached bacteria, the surface becomes relatively enriched in iron and iron release becomes favoured. Thus in the early stage of leaching, the rate of erosion of the sulphide surface is limited by the rate that sulphur can be removed from the surface by either the bacteria or the chemical reaction (in sterile leaching). Consequently, as the leaching period progresses, the rate of iron dissolution increases for this plane. By assuming that pit formation is the result of enhanced bacterial attack at the attachment sites during the initial leaching stage, correlations between substrate-based interactions as defined by pit density measurements and solution analysis further indicated that the initial leaching on pyrite surfaces by bacteria is dominated by the reactions occurring at the cell-mineral interfaces (Ndlovu and Monhemius, 2003).
On the 100 plane the leaching patterns appeared as both circular pores that tended to penetrate into the crystal face and elliptical pores that penetrated into and grew along the plane, although not to the same extent in axial length as those observed on the 110 plane (<20µm on 100, compared to up to 50µm for 110 planes). If the elliptical pits arise as a result of the persistence of disulphide atoms along a crystallographic direction, by considering the checkerboard structure of alternate disulphide and Fe atoms existing on the 100 planes, their development will be bounded/restricted by the Fe atoms. As a result they will not develop to a large extent along the 100 directions, resulting in a tendency to circular pit formation. The 111A surface did not reveal any corrosion pits during the first days of leaching. With the gradual production of ferric iron however, circular pits appeared (Figure 4b). This behaviour can be explained from both surface and solution analysis (Section 3.1). The absence of a significant bacterial colonisation and elliptical pits on this surface compared to other surfaces in the early stages of leaching, followed by the presence of mostly quasi-circular etch pits at later stages, suggests that cell-mineral interaction is minimal. Therefore, the dissolution on these surfaces occurs mainly due to the conditions created by the bacteria in bulk solution, that is, ferrous to ferric oxidation. Furthermore, the leaching trend of the 111A plane as observed in Section 3.1 indicated an increase in the dissolution rate with a sharp increase in solution potential and hence ferric iron. Since the dissolution of pyrite by ferric iron is taken as an indirect mechanism, this further suggests that this mechanism contributes significantly in the dissolution process of this pyrite surface. In addition, a comparison of the active surface area as calculated from the pit morphology analysis and solution chemistry confirmed a low cell-surface interaction with a non-localised surface-wide phenomenon governing the dissolution process for this surface (Ndlovu and Monhemius, 2003).

3.2.2 Sterile Leaching

Sterile chemical leaching was characterised by leaching patterns distinct from those observed on bioleached samples. There were slight differences between the pitting morphologies observed from one crystal plane to the other, with the corrosion patterns having symmetries related to the crystallographic surface orientations. Figure 5(a) shows typical corrosion pits observed on the 110 and 100 surfaces, while Figure 5(b) shows the pits observed on the 111 surfaces.

![Figure 5](image.png)

(a) (b)

Figure 5. Corrosion pits observed under matched leaching conditions. 5(a) -110 and 100 surfaces, rhombic pits; label 1-pit faceting and 2-merging of individual rhombic pits. Figure 5(b) shows triangular pits on the 111 surfaces (see enlargement)
Bioleaching Applications

The 100 and 110 surfaces were characterised by rhombic pits, whilst the 111 surfaces were characterised by triangular pits. The pit density and pit wall dimensions changed gradually as leaching proceeded, with some individual pits merging to form dissolution channels (Figure 5a). The pits further showed defined faceting indicating that the influence of crystalline orientation is clearly dominant in the relatively slower reactions that occur in the matched leaching experiments, where the concentration of the ferric iron gradually increases. The formation of faceted pits on the crystal surfaces suggests a contribution of an orientation-controlled type of dissolution mechanism in the leaching process. This suggests a relationship between the etch figures, crystal orientation and the overall dissolution processes of the surface.

4. CONCLUSION

The work carried out has shown differences in the initial reaction rates of the pyrite surface planes in both sterile and bacterial solutions. This has been explained as most likely being due to the influence of the differences in the surface atomic geometry and chemical surface states that dominate on pyrite surfaces. In addition, the correlation between the attached bacteria, the appearance of the surface organic films and the generation of elongated pits supports the influence of cell-mineral interaction in the initial oxidation process and the initiation of the leaching patterns (as observed by SEM analysis) on the crystal surfaces at low concentrations of ferric iron in solution. Most importantly, the absence of bacterial colonisation and elongated pits on the iron terminated 111 surface in the early stages of leaching seems to indicate that the most significant step in the early leaching process is probably the recognition and subsequent attachment of the bacteria to the active (sulphur) sites on the initial surfaces. This initial process subsequently controls the leaching progression and defines the type and evolution of pits.

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REFERENCES


The influence of temperature and pH on the bioleaching of copper from a flotation concentrate of chalcopyrite

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Abstract

The process of bioleaching of copper has been improved through the use of several chemical compounds and environmental and operational conditions in shake flask and tank leaching. The main objective of this project was to increase the knowledge on the effects of temperature and pH values on the bioleaching of copper from a chalcopyrite flotation concentrate, by using a native *Thiobacillus thiooxidans* strain. The effect of temperature on the copper extraction showed that the asymmetry of the curve is typical. Usually the curve is steeper at supraoptimal temperatures than in the suboptimal region. The effect of pH is similar to that of temperature in that an optimal pH value exists. Nevertheless, the shape of the curve in not quite the same and a broader plateau is observed.

**Keywords:** chalcopyrite, copper bioleaching, temperature, pH

1. INTRODUCTION

In the biological leaching of copper from chalcopyrite concentrates, the process has been improved through the use of several chemical compounds and environmental and operational conditions in shake flask and tank leaching where technical and economical parameters should be determined (1). Biohydrometallurgical extraction of copper from chalcopyrite can be described by the following electrochemical reactions (3):

Anodic:

\[
2 \text{CuFeS}_2 + 16 \text{H}_2\text{O} + \text{H}_2\text{SO}_4 \quad \rightarrow \quad 2 \text{Cu}^{2+} + 2 \text{Fe}^{3+} + 5 \text{SO}_4^{2-} + 34 \text{H}^+ + 34 \text{e}^-
\]

Cathodic:

\[
34 \text{H}^+ + 34 \text{e}^- + 8\frac{1}{2} \text{O}_2 \quad \rightarrow \quad 17 \text{H}_2\text{O}
\]

Sum:

\[
2 \text{CuFeS}_2 + 8\frac{1}{2} \text{O}_2 + \text{H}_2\text{SO}_4 \quad \underset{\text{bacteria}}{\longrightarrow} \quad 2 \text{CuSO}_4 + \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O}
\]
With this information in mind, Minera Mexicana de Avino S.A. de C.V. a mine company in Durango State, Mexico, has been interested in applying microbial leaching techniques by use of baffled Erlenmeyer flasks in tests in order to improve oxygen transfer conditions at this level and several temperature and pH values. Experiments were carried out in order to remove copper from a chalcopyrite flotation concentrate.

2. MATERIALS AND METHODS

2.1 Microorganism

An adapted and native culture identified and characterized as *Thiobacillus thiooxidans* used in this study was originally isolated from samples of acid mine waters obtained from Minera Mexicana de Avino S.A. de C.V. Company. The culture was maintained on a modified Silverman and Lundgren 9K medium (4), in which chalcopyrite concentrate was used as the source of energy.

2.2 Substrate

The chemical assay of the chalcopyrite flotation concentrate was as follows: Pb, 5%; Cu, 29.6%; Fe, 15.6%; S, 25.88%; insolubles, 16.4%.

2.3 Shake flask experiments

These tests were carried out in 500 ml baffled Erlenmeyer flasks on a gyratory incubator shaker NBS Model G-25 at 120 rpm, 100 ml of iron-free 9K medium, at pH (1.5-3.0), temperature (30-70°C), 20% (w/v) of pulp density, and 20% (v/v) of inoculum.

2.4 Analytical techniques

The oxidation of ferrous sulphate was monitored by determining its residual concentration in the medium following the 1,10-phenantroline method (5) and from the redox potential measurements. In order to measure the concentration of total iron in solution the ferric iron was reduced to ferrous, after filtration of the medium, using hydroxylamine as reducing agent and determining this concentration by the previously mentioned method. Subsequently, the concentration of ferric iron in solution was determined by difference between the ferrous and total iron concentrations. Redoxpotential was measured by using a redox electrode with a combination platinum / reference (Ag / AgCl). Soluble copper was determined by the iodometric method (5).

3. RESULTS AND DISCUSSION

3.1 Effects of temperature and pH

The effect of temperature on the copper extraction given in Figure 1 shows that the curve is asymmetry. Usually the curve is steeper at supraoptimal temperatures than in the suboptimal region.

Although an Arrhenius equation of the type $\mu = \exp(-E/RT)$ is suitable for describing the temperature effect below the optimal temperature, this model fails to predict the behavior at optimal and supraoptimal temperatures. Few mathematical models have been published that are adequate for describing the complete curve. Moreover, they do not refer, specifically, to bioleaching system (2).
Figure 1. Relationship between copper release and temperature values

This work has demonstrated that the culture cited above can be adapted to the leaching of copper from a chalcopyrite concentrate with high efficiency. The high extraction (92%) obtained shows that the problem of incomplete chalcopyrite leaching typically associated with the use of *T. ferrooxidans* as a leaching organism does not occur when *Thiobacillus thiooxidans* is used. Our native culture suggests that *Thiobacillus thiooxidans* could offer a more economically attractive route for the bioleaching of chalcopyrite than the process using *T. ferrooxidans*. This aspect was observed in our laboratory.

One point that could be of interest to explore is related to the operation at supraoptimal temperatures, where an increase in the leaching activity is to be expected due to a higher ratio of energy metabolism to biomass formation as a function of the copper extraction in the chalcopyrite concentrate.

Figure 2. Relationship between copper release and pH values

The effect of pH shown in Figure 2 is similar to that of temperature in that an optimal pH value exists. Nevertheless, the shape of the curve is not quite the same as that of temperature and a broader plateau is observed in this case.
It has been shown that the growth of *T. ferrooxidans* in a defined medium with Fe$^{2+}$ as energy source produces a rise in pH due to proton consumption. The situation is similar in the leaching of actual ores. Acid must be added to keep the pH at the desired value, especially in the earlier stages of leaching. The acid consumption is increased when using tailings, which have been obtained in our laboratory (2).

REFERENCES

The role of chemolithotrophic bacteria in the oxide copper ore heap leaching operation at Sarcheshmeh Copper Mine

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Abstract

The role of chemolithotrophic (inc. mesophilic) bacteria in the oxidation of copper sulfide minerals in oxide copper ore heap leaching operation of Sarcheshmeh Copper Complex has been investigated. In the present study, it was determined that about 34% of the heap copper content, existed in the form of sulfide minerals such as chalcopyrite and chalcocite, the remaining were oxide copper ores. Hence, the heap consisted of a mixed sulfide and oxide copper ore. Sulfide minerals are insoluble or partially soluble in the chemical heap leaching conditions. However, studies showed that about 30% of the heap sulfide copper content has been leached naturally during the pad’s irrigation. Previous samplings from the heap showed there were a lot of native chemolithotrophic bacteria belonging to the genera Acidithiobacillus, Leptospirillum and Sulfobacillus. To investigate a probable relation between the existence of bacteria and the leached sulfide ores, several samples were taken from different depths of a newly leached pad and analyzed for their bacterial number, pH, Eh, total soluble iron (TSI) and pyritic iron. It was found that bacteria had an important effect on the value of pH, Eh, and TSI of the heap. As these are very active oxidizing bacteria, they created a suitable condition for copper sulfide ore oxidation. Copper extraction from sulfide ores in the heap can then be attributed to the bacterial activity. It was claimed that the remained sulfide copper ore in the heap can be leached biologically after finishing chemical leaching.

Keywords: copper heap, acidithiobacillus, sulfide ores

1. INTRODUCTION

Natural bioleaching of sulfide minerals has been taking place as long as the history of the world, but it is only in the last few decades that we have realized that bioleaching is responsible for liberating some metals. The application of the bioleaching reactions for copper has been exploited and used to develop suitable methods to recover copper from copper bearing solutions [1].

The heap leaching of copper has been practiced for several decades, mostly with oxide ores. Probably bacteria aided some of these oxide operations, but this was without

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serious study. This is the subject of the current paper for determining the role of chemolithotrophic bacteria in the chemical (acid) heap leach operation at Sarcheshmeh Copper Mine. It should be noted here that the copper ore used for the heap leaching operation at this mine can be considered as a mixed oxide/sulfide copper ore which is composed of 66% copper in the form of oxide minerals and 34% as sulfide minerals mainly in the form of chalcocite and chalcopyrite. There are some industrial experiences that for such mixed copper ores a process of chemical and then biological operation has been practiced [2, 3]. No considerations were taken for the biological operation part while Sarcheshmeh copper heap was constructing. Copper sulfide minerals are insoluble or partially soluble at the relatively short time (about 90 days) considered for dissolution of copper oxide ores, but studies in this project showed that about 30% of total copper in the form of sulfide minerals has been leached naturally. Several samplings from different parts of the heap operation showed there were a lot of bacteria (more than $10^5$/ml) from the genera, Acidithiobacillus, Leptospirillum and Sulfobacillus [4]. Hence, sulfide copper dissolution can be attributed to the bacterial activity and therefore it may be possible to recover the copper (which amounts to about 28000 tones) from the buried sulfide minerals. So the first aim of this project was to determine the role of the above bacteria on some of the heap factors and then, propose some processes that may be useful for recovering the left copper in the heap.

2. MATERIALS AND METHODS

In order to find out the role of chemolithotrophic bacteria in the oxidation of sulfide ore fraction of heap leaching operation of Sarcheshmeh Copper Mine, a newly finished irrigation pad was selected in September. The temperature in this month at Sarcheshmeh Copper Mine is around 26.3°C. Three sampling area, each with a distance of 20 meters were marked and then from the surface of the pad down to the depth 2.75 m, several samples of the leached ore were taken each at intervals of 0.25 m and then a total of 36 samples were transferred to the laboratory. In order to measure the pH and the Eh values, bacterial count and total soluble iron concentration in each solid sample, a one kg sample was added to one liter of acidic (pH=1.9) distilled water in a 5 liter volume beaker and mixed thoroughly for 20 minutes. After settling, a sample of the clear supernatant was used for determining the above parameters. The bacteria were counted microscopically using a slide counting chamber. pH and Eh values were measured by a WTW pH/Eh meter model 323. The total iron was analyzed by the AAS method. The solid residue of each sample was washed, dried, pulverized and analyzed for iron by the AAS method.

For each specific depth, an average of the above three sampling sites was recorded.

3. RESULTS AND DISCUSSION

Regarding the environment temperature (around 26.3°C) at the time of sampling, the dominant bacteria belonged to the genera Acidithiobacillus and leptospirillum. In warmer months, moderately thermophiles were also isolated from this oxide heap [4]. Table 1 shows the temperature at Sarcheshmeh Copper Mine in different months.

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<td>7.1</td>
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<td>16.5</td>
<td>22.1</td>
<td>27.4</td>
<td>28.4</td>
<td>27.2</td>
<td>26.3</td>
<td>18.1</td>
<td>13.6</td>
<td>7.8</td>
</tr>
</tbody>
</table>
A theory of bacterial growth in a heap holds that the major area of bacterial growth is in the top 1.5 meters of a leach pile [1]. In this paper it was decided to take some samples from the top to a deeper depth of 1.5 meters and then compare the results.

Figure 1 shows the number of bacteria and the Eh changes at different depth of the pad. There is a close direct relation between these two factors. At the top of the heap, there are few bacteria and a low Eh value as well. This may be because of the very rapid environment changes at the top of the heap such as different day and night temperatures, sunshine and the high evaporation of introducing solutions to the heap. Beneath the surface where the harsh environment conditions disappeared, the number of bacteria and the Eh increased considerably. After that, down to the depth 0.75 m, the above factors decreased; there was no special reason here. There might not be sufficient sulfide minerals to support the bacterial growth. From the depth 0.75 down to 1.25 m, again the bacterial number and the Eh value increased. The lower depths showed a relatively constant number of bacteria. These are nearly non-growing cells washed from the higher levels. According to Schnell, the oxygen level at lower depths of 1.5m in a nonaerated heap (like the present case) drops to below 5% [1]. It can be concluded here that bacteria may have a determining role in the value of oxidation-reduction potential of the mixed copper ore heaps.

Figure 1. Bacterial count and Eh value changes at different depths of the pad

A better understanding of the bacteria and their role in the heaps has been shown in figure 2, which demonstrates the bacterial number and the pH value changes at different depths of the pad. At the top of the pad, the pH of the leached ore was high together with few bacteria. Beneath the surface, down to the depth 1.25 m, along with the more active bacteria, the pH was lowered. From this point down to the lower depths, the pH value started to increase gradually.

The pH value of the leached solution determines the solubility of ferric ion, which is a key factor for sulfide mineral oxidation. Figure 3 shows the pH and the TSI variations at different depth of the pad. There is an antithetic relation between these two factors. The low concentration of TSI was due to the long time irrigation of the pad. During this time, the total iron in the acid soluble ore form has been leached and what had remained, was the iron in the form of pyrite. So the TSI changes could be attributed to the bacterial activity.

The results of bacterial growth and activity on the oxidation of pyrite have been shown in figure 4. Supposing the homogeneous distribution of pyrite and the complete oxidation of acid soluble iron ores, one can observe following bacterial activity at the upper depths, ferric ions were produced and while going down the heap, oxidized the existence pyrite down to the 1.25 m. Around this point bacterial activity was going to
cease and hence, down to the depth 2.75 m, pyretic iron started to increase gradually as there was not enough ferric ions and bacterial growth for continuing pyrite oxidation.

Figure 2. Bacterial count and pH value changes at different depths of the pad

Figure 3. TSI concentration and pH value changes at different depths of the pad

Figure 4. Bacterial count and iron (in the form of pyrite) changes at different depths of the pad
4. CONCLUSIONS AND SUGGESTIONS

- According to the results, native bacteria have a determining role in the values of pH, Eh, TSI and sulfide ores at the upper surfaces of the copper heap operation. They create a suitable condition for sulfide mineral leaching.

- It should be noted that the results in this research came from a chemical heap leach operation in which no preparations were made for bacterial activity.

- Regarding the mixed nature of the ore used for chemical (acid) heap leaching at Sarcheshmeh Copper Mine, it would be useful if some preparations were made for copper sulfide ore oxidation, while heap construction. Now, the leaching of the heap operation has been completely terminated. So, attentions should be paid to the huge amounts of copper in the form of sulfide minerals especially chalcopyrite that has been buried in the heap.

- In order to leach the copper sulfide ores left in the heap, it may be possible to bioleach the upper two meters of the heap first, then discard it and run a same process for the next two meters and going down to the bottom of the heap.

- Another possible process may be applying a ferric sulfate leaching system in which the produced ferrous ion will biologically be oxidized to ferric ion.

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Three-stage revolving drum biohydrometallurgical reactor for continuous operation

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Abstract

Reactor bioleaching of metal sulphide minerals, although much more environment-friendly than pyrometallurgical processing, has until now only replaced the latter in the industrial practice of pretreatment of refractory gold-bearing complex metal sulphide concentrates. One of the main reasons for this situation are the investment and operating costs deriving from some limitations intrinsic of the bioreactors currently employed in biohydrometallurgy. In view of overcoming this drawback, a prototype of revolving drum bioreactor for batch operation was designed and developed by the Biohydrometallurgy Laboratory of the Geoengineering and Environmental Technologies Department of the University of Cagliari in the last decade of past century. The encouraging performance of this machine justified a programme aimed at the development of a multi-stage continuously operating machine. This paper reports on its construction and operation details and on its performance in the continuous bioleaching of a gold-bearing arsenopyrite/pyrite flotation concentrate [specific gravity about 5 g.cm⁻³] that has been bioleached in the conventional stirred tank reactors of a commercial plant during the past 20 years. This concentrate was selected in order to make a comparison of the performance of the revolving drum bioreactor with that of the stirred tank reactors employed in the commercial plant, where, however, only a partial leaching is required. The machine can completely bioleach as much as 4 grams of concentrate per cubic decimeter per hour out of a 40% solids pulp hence its performance is better than that of the STR’s where the highest acceptable solids concentration of the pulp is 20%. The power requirement for mixing, and keeping homogeneous with the required atmospheric oxygen transfer, a 40% solids suspension of the above-mentioned concentrate in the Biorotor is considerably lower than that required in the STR’s and the microflora is subjected to practically no shear stresses. The operation of the machine is very simple, considerably insensitive to throughput fluctuations and can be easily adapted to changing feed conditions.

Keywords: bioleaching, revolving drum bioreactor, complex sulfides concentrates, Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans

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1. INTRODUCTION

Bioleaching of metal sulphides flotation concentrates has not, up to now, met with much success as an alternative to pyrometallurgical metal extraction processes except in some specific cases, like the pre-treatment of complex gold-bearing metal sulphides such as pyrite and arsenic-bearing sulphides. Among the main reasons for this are the relatively slow biooxidation and bioleaching kinetics and the limitations of the bioreactors where the process is carried out.

The development of bioreactors that fully exploit the potential of biohydrometallurgical processing is one research field that deserves attention.

This paper reports on the latest developments in research pursued over the last decade at the Biohydrometallurgy Laboratory of the Geoengineering and Environmental Technologies Department of the University of Cagliari (DIGITALB).

2. REACTORS USED IN CURRENT BIOLEACHING PRACTICE

The relative density of mineral sulphides, ranging from 4 to 6 g/cc, seriously impairs the performance of the two types of bioreactors currently adopted for mineral flotation concentrates bioleaching, i.e. the Stirred Tank Reactor (STR) and the Air Lift Reactor (ALR) represented by the so-called Pachuca Tank [Rossi, 1990]. Due to the hydrodynamics of these reactors energetic agitation is required in order to ensure the homogeneous suspension and mixing of such relatively high-density particulate solids that is generated by the stirrer in the STR and by the air flow and suspension circulation in the Pachuca tank.

In addition, the oxidation process is catalyzed by aerobic chemolithoautotrophic microorganisms, implying adequate aeration of the mineral suspension.

In the STR’s, aeration is achieved by injecting an air flow through spargers located beneath the stirrer. When the air flow rate exceeds a certain limit, depending on tank and stirrer geometry as well as stirrer rotation speed, part of the injected air is no longer dissolved in the suspension and simply rises as a bubble column escaping through the top. This phenomenon, called "flooding", [Rushton, and Bimbinet, 1960; Warmoeskerken and Smith, 1985] sets a limit on the flow rate of air that can be dissolved in the suspension.

A similar phenomenon occurs in the Pachuca’s [Chisti, 1989].

One parameter that typically characterizes the ability of any reactor to dissolve atmospheric oxygen into the water filling the tank is the \( k_{La} \) (oxygen mass transfer coefficient). The higher the value of this parameter the greater the amount of oxygen transferred from the atmospheric air to the water. This parameter may be affected by the physico-chemical conditions of the fluid contained in the reactor tank. The influence of the ions of several elements, particulate solids, dissolved chemical compounds, some of which lower its value significantly, is well documented [Liu et al., 1989; Lee et al., 1982; Ogut and Hatch, 1988; Cieszkovski and Dylag, 1988]. In reactor bioleaching mediated by aerobic microorganisms the \( k_{La} \) plays a particularly important role, oxidation and leaching process kinetics depending directly on the size of the microbial population which, in turn, is a function of its growth kinetics.

Among the factors that, for a given throughput, significantly affect the profitability of a commercial biohydrometallurgical operation the following should be taken into account: (i) the number and size of reactors; (ii) power requirements for mixing the solids suspension; (iii) power requirements for aeration.
The number of reactors is related to the residence time in each one. The size of the reactors depends on the solids concentration in the suspension and the power requirements are related to the particle size and density of the particulate solids as well as the solids concentration and atmospheric oxygen requirements. For the STR’s and the Pachuca tanks the interrelationships between the above factors have been exhaustively investigated for a variety of practical operating conditions. Several mathematical models, developed by different workers are utilized for tank and plant design [Bailey and Ollis, 1986; Chisti, 1989; Rossi, 2001]. However, the STR’s and Pachuca tanks, that biohydrometallurgy has borrowed from chemical engineering and hydrometallurgy, probably do not represent the best option for the specificity of bioleaching processes, where the systems consist of three phase suspensions and the microbial population plays a fundamental role.

One of the limitations of the STR’s, well documented by the reports on bioleaching plant practice, is the solids concentration of the suspension, defined as the percent ratio of the mass of solids contained in a given suspension volume to the mass of the latter. All the reports available in the literature indicate that this solids concentration never exceeds 20% as shown by Table 1.

Evidence has been provided that solids concentration imposes a limit on the $k_{La}$ [Liu et al., 1989; Lee et al., 1982; Ogut and Hatch, 1988; Cieszkovski and Dylag, 1988; Chisti, 1989], and this fact in itself raises some doubts as to the suitability of STR’s. However, a likely more significant drawback of the STR concerns the effect of the energetic agitation required for mixing and aeration and associated interparticle abrasion [Nienow and Conti, 1978] on the microbial population. Already in the early nineties Ragusa [1990] had demonstrated that strong agitation could have a detrimental effect on the microorganisms insofar as they appear to loose their bioleaching ability. The results of recent investigations [Arredondo, Garcia and Jerez, 1994; Crundwell, 1996; Escobar, Huerta and Rubio, 1997; Fowler, Holmes and Crundwell, 1999; Holmes, Fowler and Crundwell, 1999; Crundwell, Holmes and Harvey, 1996; Kinzler, et al., 2001; Crundwell, 2001] seem to provide a rational explanation for these findings. In effect, if, due to forces acting upon the microorganism - like shear stress or the abrasion caused by solids particles - the cell envelope is partially or completely torn away, it appears that the microorganism can no longer support the electron transfer required by the oxidation process, as it is unable to adhere to the mineral surface.

In STR’s and in Pachuca’s the agitation required for mixing and keeping the solids suspended very likely produces such high shear stresses or strong abrasion within the suspension as to either directly damage some of the microbial cells or traumatically detach them from the capsule if they are adhering to the solid surface.

### 3. DEVELOPMENT OF A NEW BIOREACTOR

The above considerations, together with the experience gained during batch bioleaching tests on several minerals carried out in the laboratory, justified investigating the features of a reactor more suited to biohydrometallurgical processing. The device should (i) ensure thorough mixing of the solids suspension, irrespective of the specific gravity of the solids, minimizing shear stresses; (ii) ensure the complete and homogeneous suspension of the particulate solids; (iii) provide an adequate and readily adjustable $k_{La}$; (iv) be supplied by an easily adjustable atmospheric air flow rate.

Several prototypes of a device complying with these requirements were designed and built in the 1990’s and finally a rotating drum batch bioreactor, called "Biorotor", was tested with encouraging results [Loi, Trois and Rossi, 1995]. However, batch testing did
<table>
<thead>
<tr>
<th>Plant and Location</th>
<th>Ore minerals</th>
<th>Reactor</th>
<th>% Solids concentration</th>
<th>Total useful bioreactor volume, m³</th>
<th>Daily throughput per bioreactor unit useful volume, tonn/m³ day</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairview South Africa</td>
<td>P, A</td>
<td>STR</td>
<td>20</td>
<td>90</td>
<td>0.444</td>
<td>[van Answgen and Marais, 2001]</td>
</tr>
<tr>
<td>Sao Bento Brazil</td>
<td>A, P, Pr</td>
<td>STR</td>
<td>20</td>
<td>580</td>
<td>0.138</td>
<td>[van Answfen and Marais, 2001; Dew et al. 1997, 2]</td>
</tr>
<tr>
<td>Olympia Greece</td>
<td>C</td>
<td>STR</td>
<td>20</td>
<td>15,936 (3 moduli of 41,328 m³ each)</td>
<td>0.048</td>
<td>[van Answgen and Marais, 2001]</td>
</tr>
<tr>
<td>Amantaytau Uzbekistan</td>
<td>Complex sulphides</td>
<td>STR</td>
<td>20</td>
<td>23,376 (4 moduli of 6x974 m³ each)</td>
<td>0.047</td>
<td>[van Answgen and Marais, 2001]</td>
</tr>
<tr>
<td>Wiluna Australia</td>
<td>P, A, Stb</td>
<td>STR</td>
<td>20</td>
<td>6x470 = 2,820 m³</td>
<td>0.045</td>
<td>[van Answgen and Marais, 2001 ; Dew et al., 1997 ; Brown et al., 1994]</td>
</tr>
<tr>
<td>Ashanti Sansu Ghana</td>
<td>A, P, Pr, Mrc</td>
<td>STR</td>
<td>20</td>
<td>16,200 (3 moduli of 6x900 m³ each)</td>
<td>0.0444</td>
<td>[van Answgen and Marais; 2001; Dew et al., 1997; Nicholson et al., 1994]</td>
</tr>
<tr>
<td>Cagliari Italy</td>
<td>P, A</td>
<td>Three stage Biorotor</td>
<td>40</td>
<td>0.045 m³ (3 moduli of 0.015 m³ each)</td>
<td>0.051</td>
<td></td>
</tr>
</tbody>
</table>

P = Pyrite; A = Arsenopyrite; Pr = Pyrrhotite; Mrc = Marcasite; Stb = Stibine; C = complex Cu, Zn, Pb, As, Fe Sulphides
not provide all the information about bioleaching kinetics that could be gleaned from continuous operation. Thus with the aim of identifying all the factors affecting bioleaching kinetics, a continuously operating device was designed and repeatedly tested with a view to carrying out bioleaching tests on a pilot scale.

Preliminary testing demonstrated that with a three-stage system consisting of three identical cylindrical barrels the flowsheets shown in Figures 1 and 2 were suitable for the most common mineral sulfide concentrates. Configuration (a), shown in detail in Figure 1, is the most convenient for difficult to leach ores (f. i. pyrite); of the flowsheets sketched in Figure 2, (b) and (c) are suitable for ores more amenable to leaching, like sphalerite; (d) has been satisfactorily tested for minerals such as chalcopryte, that require an intermediate grinding step for complete bioleaching.

Basically, the biorotor modules are similar to those described in detail in earlier papers. A module consists of a cylindrical barrel fitted, on its inner surface, with lifters and filled with the suspension practically up to the horizontal cylinder axis; as the barrel rotates the suspension is lifted and discharged by each lifter. The continuous sequence of laminar flowing films thus produced favours a gentle, though thorough, mixing and an effective atmospheric oxygen transfer to the suspension. Mixing and \( k_{L,a} \) depend on rotation speed, as shown by Figure 3, and on the angle formed by the lifters with the barrel radius. The design features of the new prototype used for continuous operation differ in certain respects from the batch device described in earlier papers [Loi, Trois and Rossi, 1995 and 1997]: the angle of the lifters has been modified and each barrel has been provided with a spiral scoop type feeder. This type of feeder ensures that the suspension flows by gravity from one unit to the next, doing away with the need for pumping which, apart from the associated power costs, is highly detrimental to the microbial cells.

Figure 1. Configuration (a) of flowsheet of the three-stage biorotor with three drums arranged in series. 1 = concentrate bin; 2 = stirred tank for culture medium; 3 = screw feeder; 4 = peristaltic pump; 5 = spiral scoop-type feeder; 6 = flowmeters; 7 = valves; 8 = air compressor; 9 = air inlets; 10 = stage No. 1; 11 = stage No. 2; 12 = stage No. 3; 13 = final thickener
Figure 2. Alternative flowsheets. (b): one final module fed by the outputs of two modules operated in parallel; (c): two parallel modules fed by one initial module; (d): initial bioleaching stage followed by a regrinding stage of the thickened output solids and by two modules arranged in series. B = module; T = thickener; M = grinding mill

Figure 3. Variation of $k_La$ versus barrel rotation speed

4. MATERIALS AND METHODS

4.1 The concentrate

The case history of the Fairview Plant, located in South Africa, is well documented [van Answegen and Marais, 2001]. This plant was the first in the world to introduce, in 1974, biohydrometallurgical processing for pre-treating complex gold-bearing arsenic sulphide minerals and has continued to operate successfully up to the present time. For this reason, it was decided to purchase 100 kilos of the concentrate processed at the Fairview Plant. The mineralogical components, as determined by X-ray diffractometry, of the concentrate are, in order of abundance, quartz, pyrite, arsenopyrite and illite.
concentrate arrived in Cagliari in polythene bags and was very moist. It was dried in a thermostated oven at 30°C and dry ground in a ceramic ball mill to –75 µm. Although in the form of a dry powder, the concentrate was very sticky and flowed with great difficulty.

For this reason, the continuous feed of the first stage posed some problems and two alternative feeding devices were tested: (i) a small silo with a screw feeder that delivered the dry concentrate to the scoop feeder box and a peristaltic pump that delivered to the same box the 9K medium in the ratio for the required solids concentration; or (ii) a 25 dm³ stirred tank reactor with no air injection, where the suspension was prepared in the required percent solids concentration and a peristaltic pump transferred the suspension from the reactor tank to the scoop feeder box. Both systems are sketched in Figure 1, but proved unsatisfactory, owing to the difficulty of adjusting the small flow rates required (in the order of a few grams of solids per minute). It was necessary to manually adjust the feeders quite frequently, so, at this stage of testing, the device can be better defined as a fed-batch reactor.

4.2 The inoculum

A mixed microflora, consisting of Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans strains, isolated from the drainage of the complex sulphide ores mine of Fenice Capanne, Tuscany, Italy, and bearing the conventional name "FC", was adapted to the Fairview concentrate in an STR; adaptation was very slow, requiring fifteen transfers.

4.3 The drums

The geometry was the same for the three drums: 300 mm inner diameter and 540 mm in length. In each drum 12 equally spaced lifters were installed. The rotation speed was in the range from 1.05 rad s⁻¹ to 1.36 rad s⁻¹. The temperature of the suspension ranged from 32°C to 35°C.

4.4 Monitoring

pH and Eh of pulp samples were determined daily using a conventional potentiometer. The solids were separated from the liquor by centrifugation. Ferrous and ferric iron in the leach liquors were determined by the 2,2'-dipyridil method; the solid phases (feed and bioleaching residues) were investigated by X-ray diffractometry and their composition was determined by quantitative chemical analysis. Power consumption was also recorded daily.

5. RESULTS OF CONTINUOUS OPERATION TESTS

Although the performance of the batch device was found to be quite encouraging, continuous operation at a suitable pilot scale, using a mineral concentrate, possibly well characterized and currently used in a commercial operation of proven performance, was considered the best procedure for properly evaluating the advantages of this bioreactor with respect to the STR.

Notwithstanding the difficulties in regular feeding mentioned above, the results obtained were quite encouraging and considered worthy of reporting. The solids concentration was easily maintained at 40%, and, for a total useful volume of 45 dm³ and a feed rate of 4 grams per dm³ per hour, complete bioleaching was achieved, the solids in the output of the final stage consisting of quartz and illite with only traces of iron and arsenic.
The last row of Table 1 shows that the performance parameters of the three-stage Biorotor are apparently poorer than those of only two plants out of seven. It should be borne in mind, however, that no published data specifying the extent of the partial bioleaching the minerals had undergone in those plants were available.

The composition of the liquor effluent from the final stage, once the steady state had been achieved, was rather unusual and particularly interesting: total iron concentrations were about 50 g dm\(^{-3}\), pH around 0.9 and Eh usually lower than 550 mV. Iron seems to be associated to an organic compound possibly an EPS. Considerable difficulties are being encountered in the chemical analysis of the liquors flowing out of the drums. Analytical determinations carried out on the same samples with different techniques exhibit systematic deviations. Therefore the existence of competing equilibria in the solutions, such as, for instance, the formation of high stability constants complexes with organic compounds, cannot be excluded. The results reported here should be considered valid insofar as they indicate a trend but their absolute values have to be considered with caution. Further investigations are under way and the results will be reported in a forthcoming paper. Figure 4 shows the pH and Eh plots of the liquors flowing out of the three drums arranged in series and in steady state:

![Figure 4. Plots of pH and Eh values of liquors flowing out of the three drums arranged in series in steady state. Each couple of lines refers to one day sampling](image)

In agreement with the observations reported by other researchers [Breed, Dempers and Hansford, 1999], disruption of the process caused by irregular or inadequate aeration, without interruption of mixing, slows down kinetics considerably, to the extent that the level of microbial growth is reduced. It may be expedient to empty the device and recommence the process with a fresh suspension and a new inoculum of suitably adapted microorganisms.

Therefore it is recommended to maintain a well-adapted and uncontaminated microbial population as standby in a conventional STR, to be used when necessary as new inoculum.

The total power requirement was about 1000 kWh per tonne of feed.
6. COMMENTS AND CONCLUSIONS

The three-stage biorotor was found to perform quite satisfactorily and its operating characteristics appear to be competitive with the conventional reactors currently used in commercial biohydrometallurgical operations. Tests showed the Achille’s heel of the process to be the regularity of the feed: with a regular feed the already very good performance is likely to improve significantly, widening the gap between the Biorotor and conventional reactors. The fact that even though complete bioleaching of the mineral had been achieved ferrous iron is still present in the third stage, thus confirming the redox potential of the liquor, is worth mentioning and can probably be explained by the presence of arsenic.

The high iron concentration in the effluent liquor and the absence of iron oxyhydroxides in the solid residue may appear to contradict the well-established principles concerning the physical chemistry of aqueous ferric and ferrous iron. However, it may be hypothesized that, owing to the almost shearless mixing that occurs in revolving drums, the EPS of the microbial cells is not damaged during their activity and can fully perform its function [Gehrke et al., 1997; 1998; Tributsch, 2001]. The iron ions may remain entrapped by the EPS free floating in the liquor after cell lysis. Analytical chemistry investigations, currently being conducted, are expected to elucidate this point thus enabling the authors to provide a more complete chemical description of the processes occurring in the different flowsheets proposed.

The figure of total power requirement may be misleading: in effect, it should be taken into account that the mechanical losses become very significant in pilot scale models; no power data but only percent cost analyses have been found in the papers reporting on commercial plants and this did not enable the authors to make any comparison. However, power seems to account for more than 60% of overall operating costs of STR’s and consists of the power required for mixing in the STR’s and for the blowers that provide the required aeration [Brown, Irvine and Odd, 1994]. Calculations, carried out with the well-documented design procedures [Rossi, 2001] and applied to the above-mentioned plants, seem to provide slightly higher values.

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Use of biosurfactants for the mineral surfaces modification

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Abstract

Modification of surface properties of various minerals can be a key for the mineral separation. Biomodification of the mineral properties was realised by the adsorption of biosurfactants, which were produced by Pseudomonas aeruginosa. In this study, it is shown that Pseudomonas can grow in the presence of minerals and produce a biosurfactant with substantially changes of the surface tension of supernatant. Measurements confirmed that an interaction of all used minerals with the supernatant caused a decrease of zeta potential. As expected, the ieps of mineral particles were shifted to lower pH values after the interaction with biosurfactant. Bio-pretreatment of the minerals has affected on the settling properties of mineral suspension. The settling results showed a strong stability for hematite and kaolin suspensions at the present of the high biosurfactant concentration (10% v/v).

Keywords: biosurfactant, adsorption, mineral suspension, stability, settling, zeta potential, Pseudomonas aeruginosa, kaolin, hematite, dolomite, chalk

1. INTRODUCTION

The problem of solid surface bio-modification is still an open issue despite some attempts to solve it. During the past decade the mineral beneficiation realised by chemoautotrophic bacteria is the most widely applied process for copper and gold recovery (Somasundaran et al., 2000 and Sharma et al., 2001).

Biosurfactants are mainly produced in aqueous media from the carbon sources by growing microorganisms. Their use has been restricted to specific application. Commercially they are used as emulsifier reagent for hydrocarbon (Bognolo, 1999). Many microorganisms produce effective biosurfactants which reduce the interface tension between oil and brine to less than 0.01 mN/m. Biosurfactants are easily biodegradable and are particularly suited for bioremediation of oil dispersion. The biosurfactants affect the rate of hydrocarbon biodegradation in two ways: by increasing solubilization and by changing the affinity between microbial cells and hydrocarbon (Zang and Miller, 1995).

Removal of entrapped organic liquid (hexadecane) can be enhanced by the use of biosurfactants. The in situ biodegradation of entrapped contaminants by rhamnolipid biosurfactant was investigated (Bai et al., 1997 and Herman et al., 1997).

A detailed understanding of the biosurfactant role in modification of the mineral surface is currently lacking. The aim of the work described in this paper is to investigate
how biosurfactants produced by *Pseudomonas aeruginosa* affect the behaviour of mineral suspensions.

## 2. MATERIALS AND METHODS

### 2.1 Microorganism

The bacterial strain used in our experiments was *Pseudomonas aeruginosa* isolated from the soil samples. Cells were grown in 250 ml Erlenmeyer flasks containing 50 ml of liquid medium. Growth experiments were carried out with the medium consisted: 20 g/l mannitole; 0.05 M NH$_4$NO$_3$; 0.03 M KH$_2$PO$_4$; 0.04 M Na$_2$HPO$_4$$\cdot$7H$_2$O; 8 $10^{-4}$ M MgSO$_4$; 7 $10^{-6}$ M CaCl$_2$; 4 $10^{-6}$ M Na$_2$EDTA; 10 mg/l FeSO$_4$$\cdot$7 H$_2$O. The chemicals were used as received without further purification. The strain was cultured in a rotary shaker (100 rpm) at the room temperature. The samples were taken every day, centrifuged (4000rpm at 10 min) and the supernatants were used for surface tension measurements. The bio-surfactants synthesized by *P. aeruginosa* are most probably a mixture of rhamnolipids and glycolipids. The mineral sample (2 g) was added to the medium before sterilisation. The number of living cells in the cultures was determined by the standard colony counting method.

### 2.2 Minerals

In this study, the pure mineral samples of hematite, kaolin, dolomite and chalk (calcite) were used. Kaolin was supplied by Surmin-Kaolin mine (AKW Group) (Poland). The average particle diameter was 1.1 µm. Hematite was purchased from Ward’s Natural Science Rochester, NY. (USA) and was ground to the size ~40µm in a laboratory mill. Dolomite and chalk powders with the particle size specifications given as 90 w% < 40 µm were supplied by the Department of Geology University of Wroclaw (Poland).

### 2.3 Surface tension measurements

Surface tension measurements were carried out by the ring method with a K10T tensiometer (Kruss, Germany). Surface tension measurements are a common tool to monitor the growth of microbial culture. Each value represents the mean of five measurements. All glassware was cleaned in chromic acid and washed in Mili-Q water.

### 2.4 Adsorption measurements

In the experiment for the biosurfactant adsorption, 2 g of mineral was added to the biosurfactant solution. The concentration of biosurfactant solution was changed from 1 to 0.1 of an initial concentration. After 12 hours equilibration, the surface tension of supernatant was measured. From the difference of the surface tensions between the initial solution and the equilibrium solution, the adsorption has been calculated.

### 2.5 Zeta potential measurements

Electrophoretic measurements were carried out with a particle micro-electrophoresis Nicomp™ 380 ZLS apparatus (Santa Barbara, California, USA). Measurements were made for diluted suspensions, obtained by adding a small quantity of mineral particles to the solution. The ionic strength of dilute suspensions was maintained at 10$^{-3}$ M using NaCl. The samples were ultrasonicated for 2 min before measurements.
2.6 Sedimentation experiments

The mineral suspensions were prepared by adding 2-gram mineral samples to the Andreasen pipette. Agitation and pH conditions were the same as for the biosurfactant free suspensions. Sedimentation measurements were performed using an Andreasen pipette. The stability of mineral suspension was calculated from the relationship:

\[ Stability(\%) = \frac{M_i - M_f}{M_i} \times 100 \]

\( M_i \) - initial concentration of solid (t=0)
\( M_f \) - concentration of solid after 3, 5, 10 and 15 min.

3. RESULTS AND DISCUSSION

The production of the biosurfactants was associated with the cell growth. For biomodification purposes, the mineral particles were inoculated with the bacterium. The growth curves were obtained for *Pseudomonas aeruginosa* with or without the presence of various minerals. The relationships between the cell quantity and time are presented in Figure 1 for four minerals.

![Figure 1. Growth curves of *Pseudomonas aeruginosa* with and without of mineral particles](image)

The surface tension changes of supernatants during the microbial growth for various minerals are shown in Figure 2.

Mineral particles may attain an electrical charge, depending upon the pH of aqueous suspensions and the concentration of ions. In the presence of biosurfactant molecules some changes in the electrical double layer should be expected. Figure 3 presents the zeta potential data, which were collected during the growth of microorganism cells for the investigated minerals.
Figure 2. The surface tension changes of supernatants during the microbial growth

Figure 3. Zeta potentials of mineral particles as a function of microbial growth time

As it can be seen, the mineral particles started with positive potential. Then, the positive potential steadily decreased. The zeta potential reversal was observed at the 9th day.

Zeta potential provides an effective measurement of the potential at the solid-solution interface. The zeta potential of mineral particles was measured to determine the effect of the biosurfactant on the mineral surface charge density. The isoelectric point (iep) of the mineral is determined as the condition under, which the zeta potential is equal zero. The isoelectric point is an important characteristic of a solid-liquid interface.
Table 1. Isoelectric points of minerals

<table>
<thead>
<tr>
<th>Mineral</th>
<th>pH of isoelectric point with bacteria</th>
<th>pH of isoelectric point without bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematite</td>
<td>4.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Kaolin</td>
<td>5.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Chalk</td>
<td>8.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Dolomite</td>
<td>9.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

The effect of biosurfactant addition on the zeta potential of the minerals was different among the examined minerals. After an interaction with the supernatant, the iep of the minerals was shifted to a lower pH value. For hematite the iep was shifted to pH 4.5 after interaction with biosurfactant. A small shift of iep was observed for the chalk particles. Similar behaviour of minerals has been observed by Deo and Natarajan (Deo et al., 1998).

Figure 4. The adsorption isotherm of biosurfactant on the four mineral samples

To observe the variation in adsorbed amounts with the biosurfactant concentration, the adsorption experiments were carried out. The results shown in Figure 4 reveal that the adsorption (the surface tension different, $\Delta \gamma = \gamma_i - \gamma_{eq}$) with increasing the biosurfactant concentration ($\gamma_{aq} - \gamma_{eq}$). The sequence of adsorbed amount in all four minerals is given below:

Kaolin > Hematite > Chalk > Dolomite.

The effect of the biosurfactant addition on the stability of fines is shown in Figure 5. Generally, a number of coagulation mechanisms including charge neutralisation, bridging and hydrophobic interactions can be used to the explanation. These results suggest that bio-surfactants can be utilised as an effective reagent to stabilise as well as to destabilise of mineral suspension.

It can be seen that lower amount of biosurfactant are needed to reach the fast rate of destabilisation of chalk, kaolin and dolomite suspensions. At the higher dosage of biosurfactant (10% v/v), the mineral suspensions are become more stabile. It is observed that high stabilisation occurs for both hematite and kaolin suspensions.
The interaction energy between two identical particles depends on the zeta potential and retarded Hamaker constant. Zeta potential value of about (plus or minus) 40 mV assures an energy barrier that prevents fast coagulation (Kosmulski, 2001). As seen from Fig. 4 the adsorption of biosurfactant onto hematite and kaolin was bigger than the adsorption onto chalk and dolomite. The results of zeta potential measurements with minerals in broth solutions clearly indicated the value about -40 mV for both hematite and kaolin particles (Fig. 3).

4. CONCLUSION

In this study, the attention was focused on the stability of mineral particles suspended in the various biosurfactants concentration solutions. There are two primary conclusions that can be drawn from this work. Substantial changes occurred in the zeta potential of mineral particles after their long contact with the culture broth. Observations of the mineral suspension stability after the biopretreatment demonstrate that biosurfactants are able to stabilize of hematite and kaolin suspensions more or less effectively.
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