

Research Paper

## Bacterial consortium for copper extraction from sulphide ore consisting mainly of chalcopyrite

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### Abstract

The mining industry is looking forward for bacterial consortia for economic extraction of copper from low-grade ores. The main objective was to determine an optimal bacterial consortium from several bacterial strains to obtain copper from the leach of chalcopyrite. The major native bacterial species involved in the bioleaching of sulphide ore (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum*) were isolated and the assays were performed with individual bacteria and in combination with *At. thiooxidans*. In conclusion, it was found that the consortium integrated by *At. ferrooxidans* and *At. thiooxidans* removed 70% of copper in 35 days from the selected ore, showing significant differences with the other consortia, which removed only 35% of copper in 35 days. To validate the assays was done an escalation in columns, where the bacterial consortium achieved a higher percentage of copper extraction regarding to control.

**Key words:** bioleaching, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*.

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### Introduction

While world demand for copper is growing, the minerals industry is increasingly faced with the need to process low-grade ores, overburden and waste from current mining operations (Watling, 2006). Biotechnology is an area that has produced a considerable increase in recent years, mainly because occurrence of microbiological techniques and molecular biology, which help to isolate and identify the species that are related to the dissolution of minerals (Rawlings and Johnson, 2007). The economic extraction of copper from low-grade ores requires low-cost processing methods such as *in situ*, dump and heap leaching. Bacterially assisted heap leaching of low-grade copper sulphides is a developing technology that has been applied successfully for the extraction of copper from secondary sulphide minerals such as chalcocite at a number of operations worldwide. However, heap bioleaching of the refractory

primary copper sulphide chalcopyrite, has yet to be implemented at commercial scale (Watling, 2006).

The predominant metal-sulfide-dissolving microorganisms are extremely acidophilic bacteria (meaning organisms thriving at pH values below 3) that are able to oxidize either inorganic sulfur compounds and/or iron (II) ions (Rohwerder *et al.*, 2003). The first isolates of extremely acidophilic sulfur- and/or iron (II)-oxidizing bacteria are the mesophilic *At. thiooxidans* and *At. Ferrooxidans* (Rohwerder *et al.*, 2003). Most microbiological researches were developed utilizing known iron- and sulphur-oxidizing bacteria, either as single strains or in mixed cultures. However, in many instances, endogenous bacteria in the ore are not excluded and may thus contribute to bioleaching. Indeed, it is often noted that endogenous bacteria, being acclimatized to high levels of selected metals in their environment, are more effective as bioleaching catalysts. The acclimatization of bacteria to a particular mineral system, by subjecting them to progressively greater amounts

of the major elements, is common practice in test work (Watling, 2006). The combination of different bioleaching bacterial species is known as “bacterial consortium”. It is known that bacterial consortia are modified according to specific type of mineral and its environmental conditions (Rawlings and Johnson, 2007). Each kind of ore has unique characteristics, either a particular strain or a consortium of bacterial strains, which serves very well for a given mineral, but it is useless against another type of ore (Okibe and Johnson, 2004). That is why despite that already they have isolated and patented a large number of bioleaching strains; the industry continues investing in a variety of research to find new strains that achieve optimize the bacterial bioleaching (Rawlings and Johnson, 2007).

In copper industry, Chalcopyrite ( $\text{CuFeS}_2$ ) is the most abundant and refractory copper-bearing mineral worldwide and also the principal mineral source from which copper is recovered commercially (Xia *et al.*, 2012). That also converts it in the main copper sulphide mineral mined at bioleaching operations. Some of the chalcocite heap operations began as oxide (chemical) leach operations and were converted to bioleach (oxidative) by heap aeration and/or inoculation, when the oxidized ore was depleted. However, even if bacterial activity is not facilitated, microbial assisted air oxidation of iron (II) and sulphur will contribute to copper extraction if sulphide minerals are present in a heap (Watling, 2006). Thus, is important to know whether, for a given process and mineral, if there is one ideal combination of micro-organisms or whether combinations of different microbial isolates (or species) are likely to be as efficient as each other once they are equally adapted to the mineral in synergic activity. The question arises, therefore, as to whether the microbial populations in commercial operations are the most suitable and efficient consortia (and the most effective strains and species) that could be used for processing different minerals (Rawlings and Johnson, 2007). The objective of this study was isolate a bacterial consortium present in mineral composed mainly of chalcopyrite-bornite and determine which of this isolated consortia was best suited for the extraction of copper from sulphide ore samples.

## Materials and Methods

### Characteristics of the mineral

A single sample of sulphide ore was collected from the region of Atacama, Chile, specifically from the Mining Company El Abra. This mineral presented a mineralogy of 0.64% from the total Cu (of which 70% corresponded to chalcopyrite and bornite 30%), 0.8% pyrite, 43.7% quartz, 14.6% K-feldspar, 11% plagioclase, 16.1% of muscovite/sericite, 2.41% biotite, 2.1% chlorite, 3% smectite/kaolinite, 1.3% iron and 4.35% of diverse compounds in very small amounts (less than 0.5%).

### Enrichment of different bacterial consortia

For the enrichment of the different bacterial species from the sulphide ore studied, were used 250 mL Erlenmeyer flasks with 100 mL of the culture medium for each bacterial species described by the following authors: *Leptospirillum ferrooxidans* (Battaglia *et al.*, 1994); *Acidithiobacillus ferrooxidans*, (Tuovinen and Kelly, 1973); *Leptospirillum ferriphilum* at a temperature of 45 °C (Battaglia *et al.*, 1994); *Acidithiobacillus thiooxidans* (Nakamura *et al.*, 1997). Then, the flasks with enrichment media for the various species were incubated in a thermo regulated shaker (Lab Line brand model No. 3528-5), at a temperature of 30 °C (except for the enrichment of *Leptospirillum ferriphilum*, where the temperature for growth was 45 °C). All cultures were grown at a stirring speed of 3.13 g. It was performed a cell count in the Neubauer chamber (Brand Braun) with a phase contrast microscope (Nikkon brand model E-3200), an operation that was performed with a frequency of 48 hours; after the bacterial concentration reached its plate ( $1 \times 10^9$  cells/mL), we proceeded to draw 10 mL of bacterial culture and inoculated into 90 mL of media-specific bacterial species. The above step was repeated for at least 10 times to achieve isolation of the different bacterial consortia (Battaglia *et al.*, 1994).

### Measurement of chemical parameters

The pH and the electrochemical potential of the Bioleaching test in flasks were checked by a portable pH/mV/Temperature Meter (WTW model 340i). The test started with different values of electrochemical potential due to the different culture media for the enrichment of each bacterium, which were inoculated with a concentration of 10% of the total volume of the test according to the method of Xia *et al.* (2008). For the measurement of copper extraction was performed a chemical analysis with an atomic absorption equipment (Perkin Elmer model 3110).

### Bioleaching tests in flasks

Once enriched the bacterial consortia present in the ore, we performed the method described by Xia *et al.* (2008). It was used 250 mL Erlenmeyer flasks, to which were administered an amount of 5 g (5% w/v) copper sulphide ore (Bulk-2) with a particle size distribution between 1180 to 1700  $\mu\text{m}$  in diameter, 90 mL of medium (3 g/L  $(\text{NH}_4)_2\text{SO}_4$ , 0.1 g/L KCl, 0.5  $\text{K}_2\text{HPO}_4$ , 0.5  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ , 0.01  $\text{Ca}(\text{NO}_3)_2$ ) and 10 mL of bacterial inoculum with a viability above 95% for each species through the use of the kit LIVE/DEAD® from Invitrogen. Then the flasks of all tests (in triplicate) were incubated for 35 days in a thermo regulated shaker (model MRC brand TU-454) at a constant temperature of 30 °C and constant agitation of 170 rpm.

## Bioleaching tests in columns

Finally, it was performed a scaling on columns of 1.5 m, with an inoculation of 5% of the total volume of solution that passed through the column vs. a control column without inoculation in a period of 55 days of operation.

## Statistic Analysis

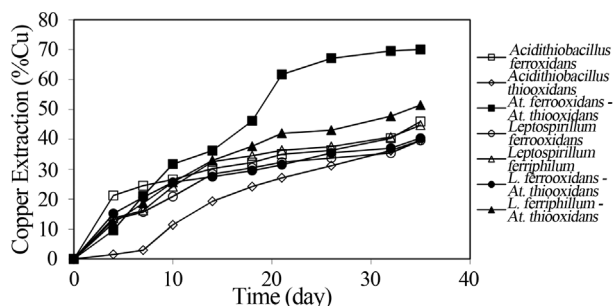
For the statistic analysis it was used ANOVA and the Tukey's Post Hoc Test. It was considered a  $p < 0.05$ . Data were analyzed by Software GraphPad Prism v. 5.0 (San Diego, 189 California).

## Results and Discussion

### Copper extraction level

In these tests it was studied the ability of copper extraction by different bacterial consortia and their interaction with sulphide ore of a mining company. The species used have a viability of over 95% for the development of all tests (data not shown); which ensures that the inoculated bacteria were viable for the development of tests in flasks. Figure 1 shows that the bacterial consortium that achieve a greater extraction of copper since day 10 until the end, was composed by *At. ferrooxidans* with *At. thiooxidans*. This is in line with the reported by Norris *et al.* (2000), who notes that for the dissolution of chalcopyrite usually requires iron- and sulfur-oxidizing bacteria. However, this does not apply by combining the iron oxidizing bacteria *L. ferrooxidans* with the sulfur oxidizing bacteria *At. thiooxidans*, since the combination does not produced an increase in the extraction of copper. In fact, the amount of copper extracted at the end of the test from each bacterium is the same compared to when they are combined.

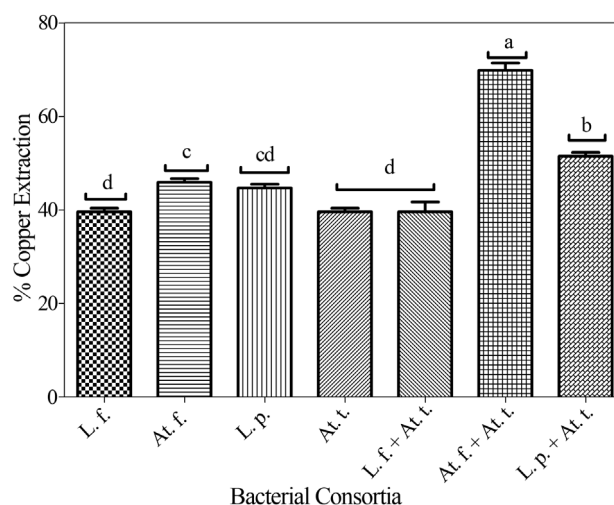
The consortium enriched for *At. thiooxidans* started with the extraction of small amounts of copper, but this amount increased as time went on. This may be due to the appearance of iron oxidizing bacteria during the development of the test, which were in the mineral surface and began to grow. It must be taken into account that the industrial process does not require sterilization and will always exist native bacterial species on the mineral surface that may affect the behavior of the inoculated bacteria. In this case, the



**Figure 1** - Level of copper extraction through time. Percentage of copper extraction by different bacterial consortia through time.

concentration of every consortium isolated was over 90% (data not shown). However, not being 100% pure cultures is likely that the bacteria present in low amounts affects the results. Also, by combining both bacterial consortia (*L. ferriphilum* and *At. thiooxidans*), it's possible to increment the extraction percentage of copper in ore. From this two bacteria, the most important is *L. ferriphilum*, a bacterium moderately thermophilic and, as Coram and Rawlings (2002), is found primarily in the operation of stirred tanks of arsenopyrite at 40 °C temperature. The test of bioleaching in shake flasks was carried out at a controlled temperature of 30 °C, therefore temperature was not optimal for this bacterial consortium. However, at the end of the test in Figure 2, the consortium achieved a good extraction of copper, being in second place. There is a difficulty in the extraction of copper from different bacterial consortium, due to the large amount of chalcopyrite present in the mineral studied (70% total Cu).

Of this aspect, Klauber *et al.* (2001) notes that in the case of chalcopyrite, both sulfur and iron compounds of this mineral ( $\text{CuFeS}_2$ ) contain products that, when the dissolution occurs, is responsible for the low speed in the leaching reaction. Subsequently, Tshilombo *et al.* (2002) indicated the formation of so-called "passivation of chalcopyrite", in which is deposited a layer of elemental sulfur in a stage of dissolution of this. The layer would create a strong inhibition on the transfer of ferrous ions on the surface of this mineral polarized. According to Dreisinger *et al.* (1995), this proceeds by the formation of intermediates polysulphuretted on the surface of chalcopyrite. Regarding this, the bacterial consortium that achieved the greater extraction of copper from sulphuretted ore (Figure 2), was the one that consisted of consortia *At. ferrooxidans* with *At. thiooxidans*, making a 70% extraction of copper in the final



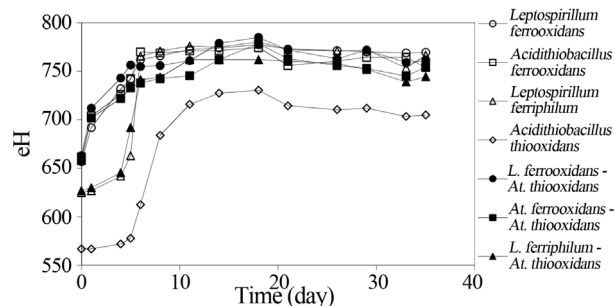
**Figure 2** - Final level of copper extraction. Represents the percentage of copper extraction at the end of the test (day 35) by different bacterial consortia; Lf: *Leptospirillum ferrooxidans*; At.f.: *Acidthiobacillus ferrooxidans*; Lp: *Leptospirillum ferriphilum*; At.t.: *Acidthiobacillus thiooxidans*. (E.E.:  $p < 0.05$ ).

test. The consortium presented a statistically significant difference from the rest of the tests. This, as described by Meyer *et al.* (2002), who notes that *At. ferrooxidans* clearly possesses a chemosensor called chemotaxis system, reacts positively to the gradients of iron ions (II)/(III) and sulfur compounds, and can help *At. ferrooxidans* to prevent the formation of passivation of chalcopyrite and having both bacteria in combination (*At. ferrooxidans* and *At. thiooxidans*), optimizing the ability to oxidize elemental sulfur to sulfuric acid allowing the ferric ion to attack the mineral for getting the release of copper.

### Electrochemical potential

For the electrochemical potential values, it shows a similar value for the tests that had an enrichment of iron oxidizing bacteria and in those in combination with sulfur oxidizing consortium (*At. thiooxidans*), but when *At. thiooxidans* is alone, there was a decrease in the values of electrochemical potential. This result, as Rawlings and Johnson (2007) indicated, is due to the non-generation of ferric iron by sulfur oxidizing bacteria, but when *At. thiooxidans* is accompanied with an iron oxidizing bacteria we observe an increase in the electrochemical potential of the test. This is because the mineral studied was not autoclaved (and thus sterilized), since the manufacturing process does not present any sterilization, and for not to change the physical-chemical composition or galvanic interactions after the sterilization process. *Leptospirillum ferrooxidans* showed the greatest levels of electrochemical potential (Figure 3), but not the highest extraction of copper (Figure 2). This, according to Stott *et al.* (2000), is due to *Leptospirillum ferrooxidans*, which is particularly efficient in the oxidation of ferrous ions to ferric ions and passing them to generate high levels of electrochemical potential, which generates a lower conductivity on the surface of chalcopyrite, generating in turn a lower copper extraction.

Although the values of the electrochemical potential of the tests are very similar, there is a significant difference in the extraction of copper (Figure 2) by different consortia, this contrasts with results reported by Hiroyoshi *et al.* (2004), who notes that the reaction of leaching of chalcopyrite is known to be very sensitive to the redox potential values, in fact, higher dissolution of chalcopyrite ranges have



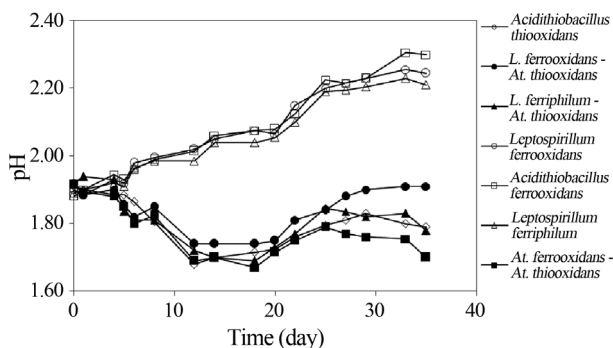
**Figure 3** - Level of electrochemical potential. Measurement of variations of electrochemical potential among days in bioleaching tests on flasks.

been reported in lower electrochemical potential values at levels of between 450 to 650 mV; however testing for major consortia *At. ferrooxidans* with *At. thiooxidans* shows the opposite, removing 70% of copper at the end of the test. This result validates the proposal that *At. ferrooxidans* has double ability to oxidize iron and sulfur, helping to prevent the formation of the “passivation of chalcopyrite” on the mineral, allowing the spreading of ferric ion on the ore and copper’s release

### Measurement of pH in time

We observe a difference in pH values over time (Figure 4), showing that by combining the iron oxidizing consortia type with the consortium of *At. Thiooxidans*, the pH tends to be lower than when the iron oxidizing consortia are alone (*L. ferrooxidans*, *At. ferrooxidans* and *L. ferriphilum*). This, according to Norris *et al.* (2000) is because the bacteria of the sort *Leptospirillum*, unable to oxidize sulfur in sulfuric acid, cannot maintain the pH values in the ranges suitable for leaching. However, while maintaining acidity in the middle, the pH values for the test with *L. ferrooxidans* and *L. ferriphilum* alone, did not rise above of 2.3. This may be because the presence of sulfur oxidizing bacteria in the mineral helped the maintenance of pH levels.

With regard to the change in pH, the tests are consistent as described by Rawlings and Johnson (2007), who reported that most microbiological applications in the ore are dependent on the presence of microorganisms that oxidize iron (*L. ferrooxidans* and *L. ferriphilum*) or iron and sulfur (*At. ferrooxidans*). The presence of only sulfur oxidizing bacteria for reducing sulfur compounds formed during the dissolution of the mineral ensure its presence in most bioleaching operations, where they play an important role generating acid in processes, which ensures the solubility of ferric ion in the operation. This continues validating that *At. ferrooxidans* helps prevent formation of the “passivation of chalcopyrite”, introducing the combined pools (*At. ferrooxidans* with *At. thiooxidans*). Hence lower pH values increased oxidation of sulfur to sulfuric acid. This result is associated with increased extraction of copper from this test (Figure 2).



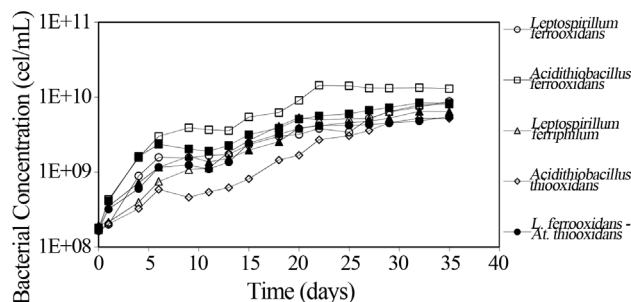
**Figure 4** - Level of pH. Change of pH among days in the bacterial consortia, in bioleaching tests in flasks.

## Changes in bacterial concentration

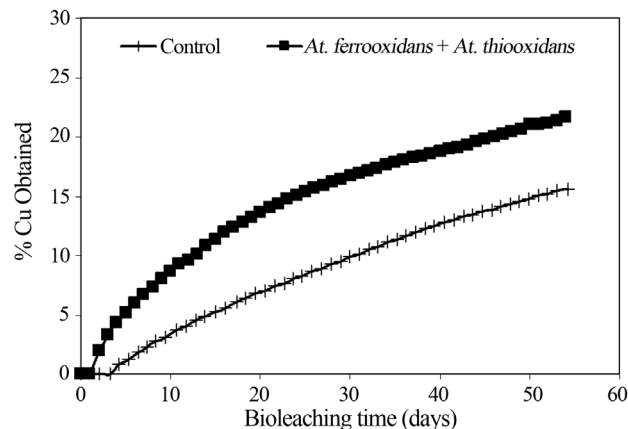
The values for the bacterial concentration (Figure 5) were similar in all tests, nevertheless there is a higher bacterial concentration when the consortium *At. ferrooxidans* is alone than when it is combined with *At. thiooxidans*. However, there was a greater extraction of copper by the combination of both bacterial consortia.

## Scaling in bioleaching columns

To validate the study that was performed in flasks, it has been done an escalation of Bioleaching columns (Figure 6) with the consortium that achieved a greater extraction of copper in the test flasks (*At. ferrooxidans* with *At. thiooxidans*). There is clearly a difference between this consortium and the control test in the extraction of copper. The overall results of this research are consistent with those described by Parker *et al.* (2004), who notes that each bioleaching operation presents unique physical and chemical properties due to their mineralogical composition, therefore it can not be extrapolated results of previous deposits, since it is very difficult for two sites present the same mineralogy in full composition of the mineral and can not be performed a generalization.



**Figure 5** - Level of bacterial concentration. Variation of bacterial number (concentration cel/mL) among days in bioleaching tests on flasks.



**Figure 6** - An escalation in Bioleaching columns. Percentages of extraction of copper columns 1.5 m by biolixiviation, until 55 days duration and an inoculated amount of 5% of the solution passed through the column.

## Conclusion

The group found an optimal bacterial consortium for sulphide ore, which is composed mainly with the bacterial species *At. ferrooxidans* with *At. thiooxidans*. This consortium achieved a 70% removal of copper in 35 days compared to the consortium composed of *L. ferrooxidans* with *At. thiooxidans* (35% in 35 days). The escalation in bioleaching columns with the bacterias *At. ferrooxidans* with *At. thiooxidans* shows the increase in the extraction of copper from the ore, which demonstrates the ability of the consortium chosen and its industrial relevance.

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