Effect of Substrates During the Adaptation of Indigenous Bacteria in Bioleaching of Sulphide Ores

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Abstract

It is well known that thermophile bacteria (40-60°C) are indicated for leaching chalcopyrite. However, it is a long bioleaching process and this kind of bacteria is not available in indigenous areas. On the other hand, mesophilic bacteria (20-40°C) are easily found in many acidic drainage mines, which could be an opportunity to decrease investment costs as well as operation cost during the treatment. However, to achieve that mesophilic bacteria are able to leach chalcopyrite, it is necessary to select the appropriate conditions such as the choice of an adequate substrate, the pulp density, the pH, and oxidation reduction potential. In addition, a decrease in the latency period during bacterial adaptation and bioleaching process is necessary. Accordingly, the objective of this research is to study the bacterial adaptation of stocks in three culture media containing various sources coming from local sites of acidic drainage mines (Kipushi, Kisenda, Kamoto) at Katanga copperbelt (Democratic Republic of Congo, D.R.C.) in order to develop bacterial species that are able to leach a sulphide refractory ore such as chalcopyrite. The three media consisted of a media containing gray sulphur, another with yellow sulphur and a third one with pyrite. At the end of several tests, the pyrite media was retained as the best culture medium, with an optimum pulp density of 2% w/v, since a more rapid oxidation was observed, and the pyrite removed the lag phase. In addition, it had positive influence on the growth of cells, mostly when the pulp density is low, allowing availability of oxygen for bacterial action. Furthermore, a sharp loading of solid or again the coarse particle size during bioleaching process would lead to acid consumption by the gangue, which inhibits the growth of cells by probably damaging the cells.

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For this reason, bioleaching tests were performed with particle size (-75µm). The operating conditions of pH 1.8, 150 rpm and 30°C with a density of pulp 2 % w/v showed an optimum of 4.45 g/l obtained. Thus, it is demonstrated that the bacteria can be cultivated in a medium having pyrite-like substrate and it is expected that these mesophile species can leach also refractory sulphide ores such as chalcopyrite.

**Keywords:** Bioleaching; bacterial adaptation; copper; substrate; bacteria; chalcopyrite; D.R.Congo.

1. Introduction

Copper and cobalt are abundant metals and constitute an important part of Earth’s crust in terms of composition. Their use is increasing with the development of the world general infrastructure. The copper found in nature is generally coming from sulphide and oxidised ores [39]. Currently, oxidised ores are more and more rare; however, sulphide ores are abundant and exploited for extraction at industrial scale, being very complex ores, mostly when they have low grade (<3% and< 0,5%) [6, 12]. Hence, the processing pertaining to this kind of ores is too expensive by conventional methods in which the challenges are the high energy requirement, environmental problems, the need of complex machinery, the large number of workers, reagent costs, etc. Nowadays, bioleaching come out as an alternative with lower energy consumption, lower capital cost, fewer workers, and environmentally friendly [1] that could solve those problems concerning traditional techniques. In addition to this, thermophile bacteria are appropriate to leach chalcopyrite but this kind of bacteria is less available than the mesophilic group. However, bioleaching of sulphide minerals is catalysed by certain micro-organisms which obtain their energy from the oxidation of inorganic substances [21].

These micro-organisms, taken from water in underground mines or the sites of acid mine drainages, can be cultivated in a synthetic medium for reproduction before using them for the leaching of sulphides ores [9]. The major advantage of bioleaching refers to the possibility of dealing and extracting metals with economic interests [7] starting from mining waste as well as from mineral resources that the traditional techniques (i.e., foundry, pyrometallurgy, hydrometallurgy) are not able to revalorize. However, around 20 % of copper metals exported to the world market come from an extraction by bioleaching with thermophile bacteria. Nevertheless, using the extreme thermophile bacteria could present many difficulties such as a slight availability of oxygen. In addition, this kind of bacteria is not very robust towards high concentrations of metal and they present less mechanical resistance than mesophile bacteria because of their membrane. Furthermore, during bioleaching, exothermic reactions are reported for moderate thermophile bacteria, reaching a temperature between 60–80°C. In many cases, higher base metal concentrations are more bearable for moderate thermophile than extreme thermophile[27].This intolerance to high concentration for certain types of thermophile is why common bacteria easily found in our environment are mainly the mesophile bacteria, which application in industrial scale would be an attractive alternative. Recent research in copper flotation bioleaching concentrate with mesophile bacteria shows that the process is promising [32].

The present work attempts to provide further information on the influence of specific characteristics of substrates during bacterial adaptation in order to leach chalcopyrite using mesophile microorganisms.
2. Materials and Methods

2.1. Particulates

The sample of copper ore was collected from an exploration site of Kipushi deposit. It consists of chalcopyrite from Katanga in D.R.C. with a particle size of -75µm. A chemical analysis carried out with a ICP spectrometer showed a composition of: 28.79 wt% of sulphur, 40.82 wt% of copper and 18.81 wt% of iron. Regarding the bacteria strains used, the sampling was performed from different sources of local sites (Kipushi, Kisenda, Kamoto acidic mine drainage) coming from the old deposits of mining companies situated in the town in D.R.Congo. The culture medium used in this work was the 9K medium composed of: 3g/l (NH₄)₂SO₄; 0.5 g/l MgSO₄ 7H₂O; 0.5 g/l K₂HPO₄; 0.1 g/l KCl; 0.01 g/l Ca(NO₃)₂ with pH 1.8,120rpm and at temperature of 32°C. With added iron 43.3g FeSO₄ 7H₂O g/l (Silverman.,1959). Pyrite coming from D.R.Congo, yellow sulphur and gray sulphur from South Africa. All reagents used in this study were analytically grade reagents and, all aqueous solutions were prepared by using distilled water.

2.2. Experimental study and procedure

The Sample (20kg) of chalcopyrite was used after wet sieving and dried at 60-80°C in an oven. The experimental procedure consisted of the T three stages: i) activation of the local mesophilic bacterial strains; ii) adaptation tests of those strains on various substrates for some tests of bioleaching on chalcopyrite; iii) the bioleaching test using the adapted mesophile bacteria.

All samples for adaptation of bacteria on substrate or bioleaching tests were weighed from 0.5-2g using an analytical balance.

i) Activation of the local mesophilic bacterial strains

In a flask on the bench, 90ml of the 9K medium were mix with iron and 10ml of inoculums were added after sterilisation. The flask was placed for shirking at 150 rpm. Thereafter, the temperature was kept at 300°C using an incubator (Thermo Scientific: Test which is carried out in south africa), and the pH and the oxidation reduction potential were measured every 48 hours. It was important to measure periodically the quantity of evaporated water to readjust the weight with distilled water. In addition, an aliquot of 2mL was taken for analysis of sulphate ion and iron (II). Next, the volume was readjusted with the same quantity taken from the 9K media made before it is indicated that for the bacterial activation several repetition was made, it means that during the first bacteria culturing, once the re-doxygenation potential is at around 600mV, exponential phase of bacterial growth takes place and the inoculums were taken, generally 10ml to put them in a fresh media 9K media for creating the new culturing from the first one. This action is called passaging of cells. It is performed in order to decrease the period of adaptation named Hey flirk’s phenomenon. The inoculums obtained were mended on various substrates for the adaptation.

ii) Bacterial adaptation on various substrates
The classical procedure of bioleaching was carried out with inoculums coming from log phase of pyrite adaptation experiment.

iii) Bioleaching process

Different quantities of chalcopyrite were used as substrate and the consortium used was which picked up during bacterial adaptation log phase of pyrite as substrate. Copper and total iron were analysed by atomic absorption spectroscopy (AAS) in an Ultima device. Fe (II) was determined by manganimetrical titration. For the determination of sulphate obtained during our bacterial adaptation on substrates and bioleaching process, the classical test was carried out by precipitation using barium chloride instead of instrumental analysis. The analysis of the pH variation over time was carried out during bacterial activity and also while the sulphate formation were among the main subjects of our attention in this work.

3. Results and Discussions

3.1. Bacterial activation

![Figure 1: Optimization of lag phase during bacterial activation](image)

Figure 1 shows the bacterial activation of the culture. The objective is to have the bacteria in full activity before adaptation in the different substrates. Because the strains are coming straightly from the conservation medium (low temperature at 0°C), this operation is necessary to decrease the lag phase during bacterial activation, which has been performed by measuring the concentration of iron over time. The bacteria strains that have been used are anchorage independent cells (acidithiobacillus) and Fe²⁺ are their nutrients. Thus, it is easier to monitor the bacterial activity from the solubilisation of Fe²⁺ into Fe³⁺. From Figure 1, it is observed that the lag phase took 180 hours for the first culturing and the same time for the second one. As well as the first fresh culturing was carried out from the first culturing made. Normally, it is often noted a decrease as concerns the first fresh culturing. However, with the second repeated culturing performed also from first repeated one of which the latency time has been decreased at 96 hours. However with the third and the fourth repeated culturing, a latency period of approximately 48 hours is observed. It means that the bacteria are activated since from fourth repeated culturing, the latency period decreases with time. Furthermore, the chemical analysis of ferrous ion in solution
also confirms this assumption: the cells division became as fast as possible while the oxidation action that was involved was also faster [32]. These results are in agreement with those of [31, 34]. Foregoing, it is important to note that the bacteria activation was effective and those indigenous strains used for bacteria adaptation were picked up during their growth log period in order to keep constant the growth the next operations.

3.2. Bacterial adaptation

The tests of bacterial adaptation were carried out on various substrates (pyrite, yellow sulphur and gray sulphur). After 15 days period, a new culturing was carried out from the first one. The tests of bacterial adaptation were performed in an interval of 0.5 % to 2 % w/v. Figure 2 shows the comparison of results of solubilisation of sulphate in the three culture media. Figure 3, 4 and 5 show the solubilisation of iron (conversion of iron II into iron III) at different pulp densities in the culture medium having pyrite as substrate.

![Figure 1: Solubilisation of sulphate in 0.5, 1g, 2g of pyrite, yellow sulphur and gray sulphur during the bacterial adaptation time.](image1)

![Figure 3: Evolution of iron as a function of time while adaptation with 0.5 % w/v of pyrite.](image3)
Thereby, the purpose of those series of tests is to reduce the lag period in order to develop the species which are able to leach chalcopyrite just during a bit of time. In this respect, Figure 2 shows that for 2% w/v of pyrite as substrate, for instance, the solubilisation of sulphur into sulphate is practically the same as concerns substrates. The increase of sulphate during bacterial adaptation shows that there would be a solubilisation of sulphur into sulphate in the studied substrates. In addition to this, Figure 3 shows that the solubilisation of iron in the culture medium having pyrite as substrate generates Fe$^{2+}$ and it would be straight oxidized to Fe$^{3+}$ by bacterial action, thus, the bacteria are adapted to this substrate, which is in agreement with the work of [19]. According to the variation of iron in Figure 5, it might be noted that during the first four days, the solubilisation of the iron was not significant, which could be a major asset to the micro-organisms that are developing the substances
necessaries for their metabolisms. Between the 5\textsuperscript{th} and 7\textsuperscript{th} day there is an increase of iron solubilisation, indicating the exponential growth phase. Beyond the 7\textsuperscript{th} day, the growth is almost constant (stationary period of growth) until the 22\textsuperscript{th} day in which a reduction of Fe\textsuperscript{3+} concentration is observed. In this moment, the microorganisms reach the period of mortality \cite{36}. However, for the culture on pyrite, a reduction is observed in the 6\textsuperscript{th} day. The same phenomenon is observed in Figure 3 which shows the same culture but with 0,5\% v/w of pyrite; about the iron solubilisation process, as indicated to the figure 3, the oxidation of Fe\textsuperscript{3+} decrease from 23\textsuperscript{th} day this could be explained by the precipitate formation during the adaptation phase, such as the jarosite, polysulphur and elemental sulphur \cite{40}. Our approach is almost in accordance with the trend analysis observed by Reference \cite{36}, who highlighted the fundamental role of the ferric ions during the bio-oxidation of pyrite with T. ferrooxidans. Our research has shown that iron solubilisation becomes higher beyond the 8\textsuperscript{th} day. According to this work the increase of iron solubilisation was performed at 8\textsuperscript{th} exactly with a pulp density of 1 \% w/v of substrate as indicated at the Figures 2 and 4. There is a slight difference on the number of days found in the work of \cite{36}. Comparatively to our work approach. That slight difference might be explained by the major nature of substrate used for the culture. Regarding the solubilisation rate of the different pulp densities, the 2\% w/v pulp density of substrates were the better and the inoculums obtained from the previous tests were mended on each respective substrate while carrying the pulp density. A reduction of lag phase for all substrates was observed, caused by the repeated culturing from the first one with 1\% w/v which was carried out while the micro-organisms were into full activity. With the pyrite as substrate, the solubilisation of iron started to be accentuated from the 4\textsuperscript{th} days and two one of sulphur at 5\textsuperscript{th} and 7\textsuperscript{th} days.

Figure 6: Evolution of pH as a function of time while bacterial adaptation

Figure 6 show that this pH has a sharp influence in the bacteria growth, after observation of pH evolution on different pulp density (0,5g; 1g; 2g) while adaptation to the substrate. The shape of the curves in Figure 6 shows a variation of pH during the bacterial growth which is supposed to be equivalent to a certain red-ox potential given as a function of bacterial activity. However, the pH at 0,5g of different substrate reveals that there is an average bacterial activity which might be explained by the availability of oxygen supported by the phase
ratio between the solid and liquid phase, however the lag phase is observed during the first 3 days; then at the 5th day, a phase of exponential bacterial growth starts; then a stationary increasing phase to the 22nd day and beyond the 22nd day the shape of the curve shows that bacteria would be in a lethal phase. The pH and red-ox potential are largely attached to the bacteria activity. The explanation could be found by the effect of availability of oxygen and the quantity of nutrient present in the media as a function of bacterial growth. As well as with 1g of different substrate, it is also observed in Figure 6 that the pH starts to decrease from the 6th day, time when the bacterial activity could start. The study shows that when using pyrite, a jump is observed during the first 4 days. These results would be explained by the reduction in the concentrations of sulphate and iron during the culture on the pyrite. On the other hand, it could be due to the consumption of acid by the gangue. Thus, it is observed more Fe$^{2+}$ than Fe$^{3+}$ in solution during the culture on the pyrite, beyond the 12th day. The dissolution of pyrite is monitored by the competitive chemisorptions between Fe$^{2+}$ and Fe$^{3+}$ forms on the ore surface. The formation of Fe$^{2+}$ on the surface results in a diffusion screen to the Fe$^{3+}$ attack, increasing the ferric ion concentration in the bulk solution that blocked the microbial oxidation capacity for concentrations above 16 g/L.

It is also reported that the bacterial growth and the behavior of iron-oxidizing microbial communities are influence by ferrous and ferric ions in term of concentration. shows that a low concentration of ferric iron influences the oxygen uptaken by the acidophilic bacteria, although at higher concentrations, ferric iron inhibits oxidation. In addition, increasing the ferrous iron until a certain concentration around 4g/l improves the oxidation rate during inhibiting higher Fe$^{2+}$ concentrations. According to the studies perform by reference [37]. Which was carried out on the conventional and microbial leaching of chalcopyrite at 37°C, high Fe$^{3+}$ concentration retards the dissolution rate during bioleaching of chalcopyrite. This confirmed the results obtained by Reference [17]. reported that the increase of Fe$^{3+}$ up to 0.5 M is able to decrease the chalcopyrite solubilisation rate. Regarding using 2g as pulp density (Figure 6) the pH drops in the two types of sulphur, while in the pyrite, it tends to increase during the first 4 days and then starts to decrease. Thus, there is there ist bacterial activity as the following points reveal:

- The lag phase is not existing because bacteria were taken when they were into full activity, which could also explain the fact that the rise of pH on the pyrite is not very accentuated compared to the preceding test;
- The sharpe of the curve observed at the 10th day in Figures 3, 4 and 5 would be due and explain to undesirable phenomena generated by the bacteria into full activity, generating precipitates, for example the formation of jarosite:

$$1/2O_2 + 2H^+ + 2e^- \rightarrow H_2O$$  \hspace{1cm} (1)

$$Fe_2(SO_4)_3 + 14H_2O \rightarrow Fe_3(SO_4)_3(OH)_6 + 5H_2SO_4$$  \hspace{1cm} (2)

The fall of the pH on the last part of the curve would be due to the production of the sulphuric acid, given by the following reactions:

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4$$  \hspace{1cm} (3)
Accordingly to the different tests above, it is possible to make conclusion that during the culture with 0.5% w/v of three substrates, the culture took 26 days and that it would have reached the death phase of microorganisms, which makes that at passaging time from 0.5 to 1% w/v, the lag period would have been affected comparatively.

By observing the data of the culturing with 2% w/v densities of pulps, it is noted that our bacteria adapted well on our three substrates. However, the pyrite is the most significant metal sulphide and is found almost in all the layers of Katanga, while sulphur (yellow and gray) is a product imported in D.R.Congo. It is noted that rapid oxidation of pyrite substrate occurs at 2% w/v of pulp densities. In addition to this, in our approach, the choice of pyrite will be also justify by the pivotal role of iron in the composition of pyrite. If the ferric iron has an important influence to the heterotrophic bacteria during the bioleaching process, it means that the iron present in pyrite for autotrophic bacteria will play an important role. This approach is in agreement with the work by Reference [18].

who indicated that many heterotrophic acidophilus are able to anaerobic ferric iron respiration and proposed that ferric iron might be an interesting electron acceptor under acidic media. In anaerobic conditions, ferric iron for the growth anchorage dependent autotrophic practically on sulphur compounds can play an amazing influence in the iron and sulphur cycles in acidic environment. For the bioleaching, the best recovery is generally obtained with the mixture than pure bacteria culture accordingly by the fact the acidophilic bacteria type took their more energy in iron and others compound else as reveal the literature. In this respect, the choice of the substrate for the bioleaching tests will be the pyrite.

**Bioleaching test of chalcopyrite after bacterial adaptation on pyrite as substrate**

![Figure 7: Evolution of pH as a function of bioleaching time](image-url)
The inoculums used in the test of bioleaching were those having been adapted on the pyrite chosen as substrate, which allowed leaching chalcopyrite. Figure 8 shows the change of pH as a function of time. The pH is an important parameter because it indicates the activities of microorganisms in the medium or solution in addition to the acidity of the media. However, microbial activities have a significant influence at the solubilisation action during bioleaching process [28]. Figure 7 shows that bacteria are not active during at the beginning during the leaching process. First of all, it is observed that the pH of solution using pyrite as substrate rise comparatively to the one using sulphur as substrates for all pulp densities, it could be due to the consumption of the acid by the gangue as there was low production of acid through bacteria oxidation of the substrates. However, in the case still of the pyrite substrate, the bacterial oxidation of Fe$^{2+}$ in the 9K medium to Fe$^{3+}$ leads to the subsequent pyrite oxidation to produce Fe$^{2+}$ and H$_2$SO$_4$. As outcome expected there is a slight balance between the acid
consuming and expected reactions thus the increase of pH was different than that for the sulphur substrate which will produce more H$_2$SO$_4$ than Fe$^{2+}$ avoiding thus any formation at this stage. In the subsequent leaching period, the pH drop was higher for the sulphur substrate than for pyrite at the same initial pH, involving that there was higher rate of acidity with the sulphur substrates which is an outcome involving the behavior somehow and somewhere of the bacterial activity (Figure 7). According to Figures 7, 8 and 9, using 0.5g of pyrite leads to a pH increase from 2$^{nd}$ day to 6$^{th}$ day and a pH decrease from 7$^{th}$ to 22$^{th}$ followed by an increase from 25$^{th}$ day on. Those stages are corresponding to the lag period, from the 2$^{nd}$ day to 6$^{th}$ and 6$^{th}$ day to 22$^{th}$ the log or exponential period, the stationary phase occur at 24$^{th}$ day around about close 28$^{th}$ or 30$^{th}$ day it occur the death period. The same trend was observed for gray sulphur and yellow sulphur with 0.5g, 1g, 2g as pulp densities. However, it is showed that the log period for pyrite with 2g of pulp density was reached more rapidly than the others. It is also due to the bacterial oxidation which was quicker than the other one in term of comparative approach. The zone of pH from 1.5 to 2 is classically used but usually with a pH lower than 1.4. According to the studies which were carried out by reference [5]. The pH 1.5 and below are recommended so that the jarosite precipitation which might inhibit the copper dissolution action being just about insignificant [23]. However, in spite of being a refractory ore, the output of leaching was not very high. According to Figures 8 and 9 the study shows more copper sulphate in the solution for a pulp density of 2 % w/v than for the other one. On the other hand, there is not only jarosite as inhibitor agents during bioleaching process; an increase in the concentration of the inhibiting agents of the bacterial action like arsenic and the ions chlorides which will inhibit the rate dissolution of copper for instance [2]. Added to this, as concerns the wastes produced, it may be noted that a proportion of basal gangue and silica presence have an effect to the acid of medium which might or consumed much acid during bioleaching time and the micro-organisms might have a strong difficult to reach the substrates necessary to their growth. [2]. Furthermore, the high concentration of Fe$^{3+}$ reduced the bacteria ability to oxidize Fe$^{2+}$. The inhibition influence had been showed by [22].

In their works, pyrite bioleaching using Acidithiobacillus Ferrooxidans and Leptospirillum ferriphilum bacteria, and the studies conducted by Nyavor and his colleagues (1996) [25]. Which reported that the Fe$^{3+}$ high concentration competitively inhibits Fe$^{2+}$ oxidation by At. Ferrooxidans. Those two outcome works showed that the high concentration of Fe$^{3+}$ had also an inhibitory influence on the yield leaching of bacterial oxidation. However, this work is in accordance with Reference [25]. Regarding the influence the Fe$^{3+}$ concentration which had been produced by the bacterial oxidation. It is indicated in our result approaches of bioleaching process that the increases of pH were attributed to the formation of ferric hydroxides such as jarosite (chemical reaction 2), element sulphur, polysulphur and others by-product causing thus, a significant decrease Fe$^{3+}$ in the medium which has in turn led to a decrease in the value of ox-red potential.

Normally the bacteria growth would be eventually affected and the copper dissolution will be also affected as consequence to those different phenomenons. Finally, those different values of pH and iron concentration in the chalcopryite bioleaching mechanism have the influence on copper extraction such as shown at the Figures7 and 8. Figure 9 shows that the copper extraction was obvious but somehow weak because of jarosite and elemental sulphur. In addition to that, it is also observed that the copper oxidation was increased during the log phase of bacterial activities. This means that the pH, and concentration of iron have a pivot role in the bacteria activity during bioleaching time.
4. Conclusions

At the end of several tests, the pyrite was retained like culture medium, with an optimal pulp density of 2 % w/v. Some tests of bioleaching of chalcopyrite were carried out with the bacteria which adapted on the pyrite; the copper solubilisation has showed that these bacteria can leach sulphide ores. However, a great yield recovery of leaching was not obtained because of the refractibility of chalcopyrite ores, which are solubilised by bacteria of the thermophile type. At the end of 30 days of bioleaching time, copper concentrations of 3.77 g/L for 0.5 % w/v; 4.45 g/L for 2 % w/v and 2.15 g/L for 0.5 % w/v were obtained. Thus, to the sight of the results, before the bioleaching of sulphide ore, the bacteria can be cultivated in a medium having pyrite as substrate, which will make sharp influence to the lag period during bioleaching time. However, we can say that mesophile species develop during our various cultures (adaptations) are able to leach chalcopyrite. In addition, in term of recommendation, it may be reported that the copper recovery could be enhance taking into account further studies regarding the kinetic parameters as pH, oxidation reduction potential, effects of particle size, temperature, shirking rate (rpm) and, on the other hand, some additives (salts) which might influence the extracellular polymeric substances (EPS) for microorganisms and during the bioleaching process, this work observations relative to the kinetic influence are in accordance with reference [19].

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