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Extraction of Copper from the Malachite of Madagascar by Biological Leaching using *Pseudomonas aeruginosa* PA1 strain

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Abstract : *In an effort to minimize the environmental impacts caused by metal extraction, this study demonstrates the extraction of copper from the malachite using biological leaching as extraction technique and Pseudomonas aeruginosa as biological extraction agent. The content of copper and other minerals in the malachite was evaluated by X-ray fluorescence method. In addition, the copper content of the malachite particles with different sizes was determined by atomic absorption spectrophotometry. Four parameters were optimized to control the bioleaching process : malachite particle size, glucose rate, culture medium volume, and bioleaching time. Copper content was analyzed by atomic absorption spectrophotometry during the optimization. The results showed that the copper content in the malachite sample was about 5.74%. Other high-grade minerals such as Fe, Ca, Ba and S were also detected. On the other hand, the copper content in the different malachite particles varied from 5.08% to 6.83%. Regarding the bioleaching of copper from the malachite with Pseudomonas aeruginosa strain (106 cfu/ml), it was optimal with malachite particle size of 45-80µm, glucose rate of 8% and culture medium volume of 120ml for 4 days. Under these optimal conditions, Pseudomonas aeruginosa was able to extract 45.7% of copper during 20 days of experimentation.*

Keywords : *copper, malachite, bioleaching, Pseudomonas aeruginosa*

1. INTRODUCTION

The innovation brought by certain bacteria led to the emergence of new biotechnological, economic and environmental perspectives [1] which improves the management of natural resources. The bioleaching is known in the mining exploitation as a technique using microorganisms for extracting metals, it was demonstrated in several works efficient for enhancing the recovery of high added value metals as gold, copper, cobalt, etc.… [2]. In addition, this biological technique presents as benefits the reduction of toxic discharges in the air, the water and the soil generated by classical and conventional methods including hydrometallurgy and pyrometallurgy.

In this study, the malachite of Ambatovarahina, the one of the most potential copper deposits of Madagascar was chosen for copper extraction. The malachite is an oxidized ore of copper, a hydrated copper carbonate with light to dark green color. For several years, the hydrometallurgy has adopted as extraction method in the site of Ambatovarahina. A low copper content (<5%) resulted from this method and the reaction products (residues, effluents) formed at the end of the extraction can impact considerably the human health and the environment.

For this reason, in order to enhance metals extraction yield by adopting environmentally friendly extraction method, the bioleaching method of the oxidized ore using *Pseudomonas aeruginosa* as a biological leaching agent is studied in the present work. *Pseudomonas aeruginosa* is a Gram negative and heterotrophic bacterium, indigenous of soils. This strain is often used in the depollution or the treatment of soil and water due to its ability to synthesize organic acids and siderophores. Produced

acids dissolve metals by forming soluble complexes between metal ions (copper ions) and chelating molecules (siderophores) [3].

Thus, this work aimed to reveal the ability of *Pseudomonas aeruginosa* as bioleaching agent to extract copper metal from the malachite of Ambatovarahina, Madagascar. The different parameters influencing bioleaching process were studied for its optimization.

2. MATERIALS

2.1. Malachite Samples

The malachite samples used for copper extraction in this study were collected from Ambatovarahina mining deposit, District of Ambatofinandrahana, Region Amoron'I Mania, Madagascar according to the rectangular coordinates X: 456.085, Y: 622.095.024 at an altitude of 1.527m. The samples were constituted by malachite impregnations with cipolin gangue and stromatholite. They were put in plastic bags and transported immediately to the laboratory for analysis.

2.2. Bioleaching Agent

The bacterium used as bioleaching agent in the present study was *Pseudomonas aeruginosa* PA1 obtained from the Laboratory of Biotechnology and Microbiology, Faculty of Sciences, University of Antananarivo, Madagascar. The strain was cultured on Columbia agar: Polypeptones (1.7%); Heart Pancreatic Peptone (0.3%); Corn starch (0.1%); Sodium chloride (0.5%); Yeast extract (0.3%); Agar (13.5%) for revivification.

3. METHODS

DETERMINATION OF COPPER CONTENT

From the malachite sample

3.1. X-Ray Fluorescence Analysis

Copper content in the malachite sample was determined by X-Ray Fluorescence method, this technique is used for elementary quantitative analysis of the samples [4]. A spectrometer was put on the plastic bag containing the malachite sample for 12 to 15 minutes. Thereafter, the spectrometer was connected to a computer which displayed the values of mineral elements contents in the analyzed malachite.

From the malachite particle with different sizes

3.2. Particle Size Analysis

The ores were grinded and the obtained powder was sieved with different dimensions sieves (AFNOR): +200µm, 200µm, 125µm, 80µm and 45µm.

3.3. Atomic Absorption Spectrophotometry

This technique is used to determine the concentration of metallic elements and metalloids in the ore sample.

3.3.1. Mineralization

This step was performed using Rodier's method [5]. Four milliliters (4ml) of ammonium nitrate (100 g/l) were poured into a capsule containing 1g of grinded ore; the mixture was dried in the oven at 110°C, then placed at progressive increasing temperatures until 450°C for which it was maintained for 2h. After cooling, the residue was transferred into a beaker containing water. The capsule was successively rinsed with concentrated HCl and boiling water**;** 5ml of HNO_3 were then added. The mixture was covered, boiled about 10 minutes and evaporated. The residue was taken up with 20ml of HCl 2N and boiled. The obtained solution was filtered and the filtrate was collected into a flask (100ml); the beaker and the filter were rinsed with 10ml of HCl 2N and boiling water, the final volume was adjusted at 100ml with distilled water. The concentration of Cu in the solution was evaluated by spectrophotometry.

3.3.2. Determination of Copper Concentration

Copper content was evaluated from a copper standard and expressed according to the following formula:

$$\frac{x.10^{-6}\times dil\times V\times 100}{IT\ (g)} = \text{g/100g}$$

$$\frac{x.10^{-6}\times dil\times V\times 1000}{IT\ (ml)} = \text{g/l}$$

With:

x: concentration of the solution (mg/l);

V : volume of the assay (ml) ;

IT: weight of the sample (g).

The results were classified according to the sample particle sizes (45 µm, 80 µm, 125 µm and 200 µm).

3.4. Bioleaching Experimentation

3.4.1. Preculture preparation

A preculture of *Pseudomonas aeruginosa* was prepared with a culture medium composed of glucose (2%); peptone (2%); yeast extract (0.2%); KH_2PO_4 (0.075%); $MgSO_4$, $7H_2O$ (0.03%) [6-7**]**. For that, a loopful of *Pseudomonas aeruginosa* colonies was taken with a sterile loop from the tube collection and introduced into the bioleaching medium. The culture was then incubated at 37°C for 24h.

3.4.2. Bioleaching process

Bioleaching experimentation was carried out according to the technique described by [8]. In an Erlenmeyer flask (250ml) containing 80ml of bioleaching medium was inoculated a culture of *Pseudomonas aeruginosa* (10^6 ufc/ml) representing 20% of the medium volume. The culture was incubated at 37°C, under shaking at 80 rpm for 24 h. After incubation, 1g of sterilized ore was added into the flask. The culture was then, re-incubated at 37° C for 5 days. Thereafter, the culture was centrifuged at 2500 rpm for 20min. The supernatant was recovered to be analyzed by AAS for copper content evaluation.

The percentage of extracted copper (C) at the end of the experimentation was calculated using the following formula (C):

$$C = \frac{copper\ content\ in\ solution}{\text{copper content in the sample}} * 100$$

3.4.3. Optimization of bioleaching process

Four parameters susceptible to influence bioleaching process were tested in order to optimize the bioleaching of copper with *Pseudomonas aeruginosa* PA1.

- **Particle size**

The size of the particles constitutes an important factor in the bioleaching experiment. In this study, four particle sizes were assayed (45µm, 80µm, 125µm and 200µm). The other parameters were maintained as shown in the table 1.

Table1. *Composition of bioleaching medium according to the particle size*

Particle size (µm)	**45**	**80**	**125**	**200**
Glucose rate (%)	2	2	2	2
Medium volume (ml)	80	80	80	80
Bioleaching time (days)	5	5	5	5
Inoculum volume (ml)	16	16	16	16

- **Glucose Rate**

Glucose plays an essential role in bacterial activity for organic acids production, necessary for metal recovery. Indeed, the amount of organic acids produced by the bioleaching agent depends on the quantity of available substrate (glucose) in the medium. Then, different glucose rates were tested: 2%, 4%, 6% and 8%.

- **Volume Medium**

The solid/liquid ratio of the culture medium is essential for the bioleaching agent growth. The volumes of the bioleaching medium tested in this experimentation were 60ml, 80ml, 100ml and 120ml.

- **Bioleaching Time**

Bacterial activity is closely related to bacterial growth on which depends consequently the bioleaching time [9]. Thus, a bioleaching process of copper from the malachite with *Pseudomonas aeruginosa* was performed in this study during 5 days.

During the optimization, the temperature was fixed at 37°C and the pH values were measured. Copper content was evaluated by AAS every 4 days during the bioleaching time test. It should be noted that the parameter exhibiting high copper content was retained for the test of other parameters.

In order to evaluate copper extraction yield, a bioleaching experiment was carried out under optimized parameters with a bioleaching time of 20 days. An aliquot of 10ml of bioleaching medium was taken every 4 days for AAS analysis.

4. Results And Discussion

4.1. Particle Size Analysis

This analysis allowed to estimate the distribution of the particles in the malachite sample. According to the grain sizes obtained after sieving with sieves of different dimensions, the sample can be classified as shown in the table 2.

Table2. *Particle size distribution*

Size fraction (µm)	% Sample
+200	14,73
200	15,56
125	13,83
80	21,22
45	34,03

From these results, it follows that the sample was composed of fine grains. The dominant particle size was 45µm which constituted 34.03% of the sample, the other particle sizes (80µm to +200µm) represented 14,73% to 21,22% of the sample and 0,7 % of loss was recorded during the handling of the machine.

4.2. X-Ray Fluorescence Analysis

The results of the XRF analysis showed that besides the copper, other minerals were also present in the malachite sample. Among them, the Fe, S, Ca and Ba showed high contents (table 3) with the copper (9.06%) while Mo, Zr, Sr, Pb, Cd, Th were in traces (data non shown). The presence of Fe and S could be explained by the combination of the malachite with the bornites or the chalcopyrite. On the other side, the presence of Ca and Ba could be due to its combination with the cipolin and the stromatolite (there are both calcareous).

Table3. *Mineral elements content in the malachite sample based on XRF analysis*

Mineral elements	Cu	Fe	Ca	Ba	S
Content (%)	9.06	6.59	3.8	9.91	1,3
Content (ppm)	90 657.8	65 919.4	38 006.7	99 762.8	1239.9

4.3. Atomic Absorption Spectrophotometry (AAS)

The results of AAS analysis according to the grain size showed that Cu contents were identical for the first three grain sizes (45µm, 80µm and 125µm) with 5.081%, 5.589% and 5.431%, respectively. The higher value corresponded to the 200 µm grain size with a Cu content of 6.834% (table 4).

Based on this SAA analysis, the sample contained an average copper content of 5.74%.

Table4. *Copper content in the malachite sample according to the grain size*

Particule size (µm)	Cu (g)	Cu (%)
45	1263,8	5.589
80	1093	5.081
125	1276	5.431
200	1658	6.834

4.4. Copper bioleaching optimization

- **Effect of the Particle size**

Through the results obtained, it could be deduced that the particle size influenced copper bioleaching process. Copper content increased gradually as the grain size was fine. The higher copper content value (31.52%) was obtained with a particle size of 80µm. Then, the particle size favorable for copper extraction with *Pseudomonas aeruginosa* was 45µm – 80µm (figure 1).

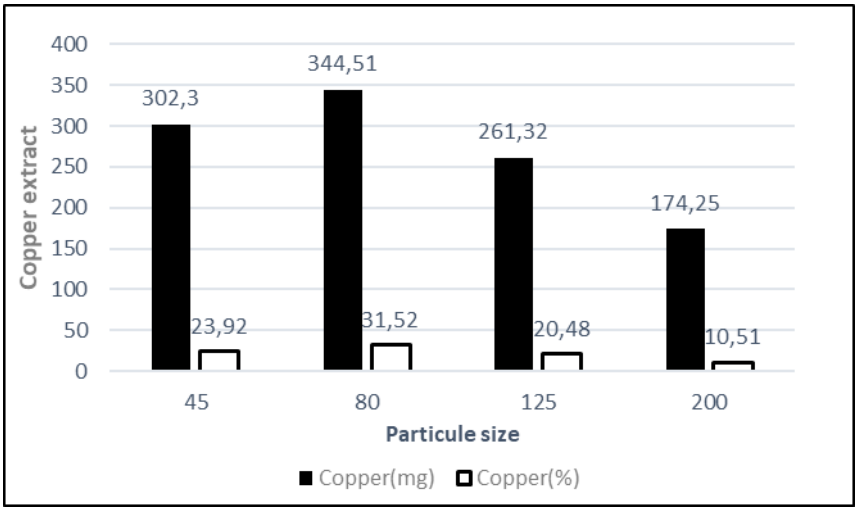

Figure 1. *Variation of extracted copper content according to the particle size*

Twenty-four hours (24 h) after the inoculation of the bacterium in the medium, the pH value of the medium varied from 5 to 5.9. However, the pH values were between 4 and 4.5 during the 5 days of experiment, this decrease of pH signifies an acidification of the medium (figure 2).

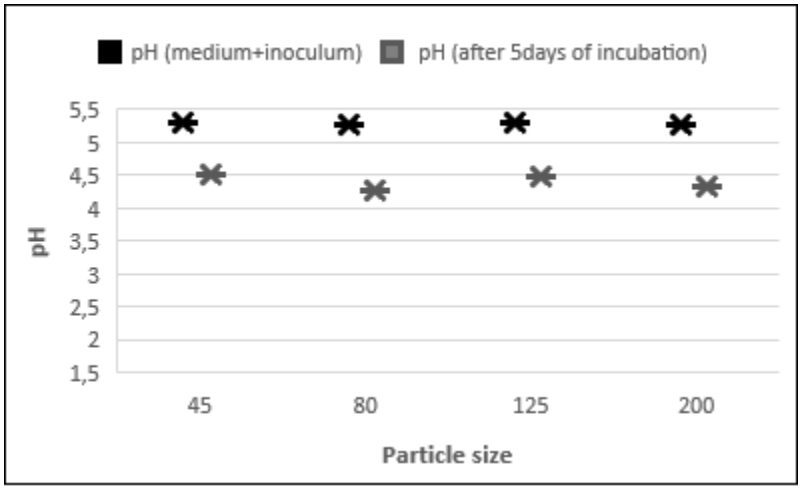

Figure 2. *Evolution of pH during particle size assay*

- **Effect of Glucose Rate**

The variation of the glucose rate in the medium gave different results. The evolution of the copper rate extracted according to the glucose rate in the medium is represented in the figure 3. The results showed that the content of extracted copper increased gradually as the rate of the glucose increased. In this experimentation, the high value was obtained (24.87%) with a glucose rate of 8%.

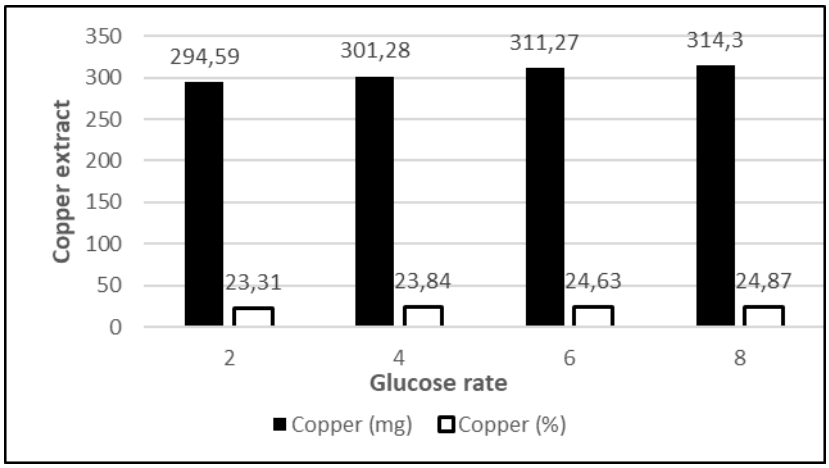

Figure 3. *Variation of extracted copper content according to the rate of glucose*

During the assay, a decrease of the pH until 3.9 was noted (figure 4). This resulted of the production of organic acids from the glucose by *Pseudomonas aeruginosa*.

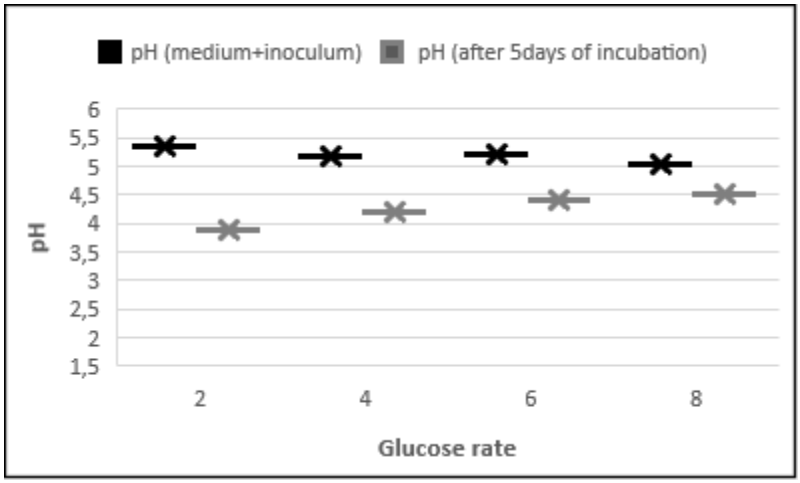

Figure 4. *Evolution of pH during the glucose assay*

- **Effect Of Medium Volume**

According to the results shown in the figure 5, the content of extracted copper increased also with the medium volume. In this assay, 32.45% of Cu were recovered with 120 ml of medium volume.

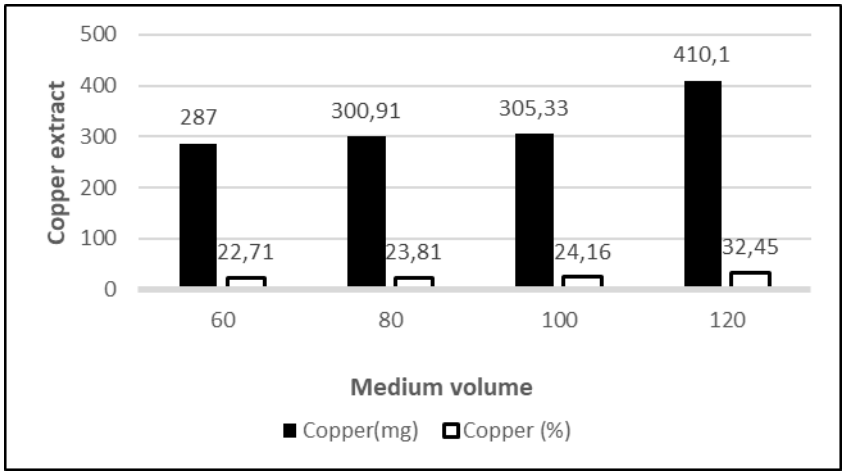

Figure 5. *Variation of extracted copper content according to the medium volume*

In fact, the water constitutes an important element for bacterial growth which impacts also bacterial activity. Hence, sufficient water allows a good bacterial growth [10] and an intense activity, represented in our case by organic acids production and copper extraction.

- **Effect of Bioleaching Time Under Optimal Bioleaching Conditions**

Regarding the results obtained with the bioleaching process under optimized parameters (figure 6), it should be deduced that in four (4) days, *Pseudomonas aeruginosa* can extract 43.8% of Cu from the malachite sample. After 20 days, extraction rate was 4.7%. Compared to other copper bioleaching process with *Pseudomonas aeruginosa* from other oxidized ore as chalcocite [4], this rate is low. These authors obtained 53% of Cu during 20 days of bioleaching test with the following parameters: particle size of 150-177µm, glucose percentage of 6%, bioleaching time of 8 d, and solid/liquid ratio of 1:80. In another study, a heterotrophic strain, *Providencia* sp. JAT-1 was able to extract 54.5% of copper with a pulp density of 1% of oxidized ores [11].

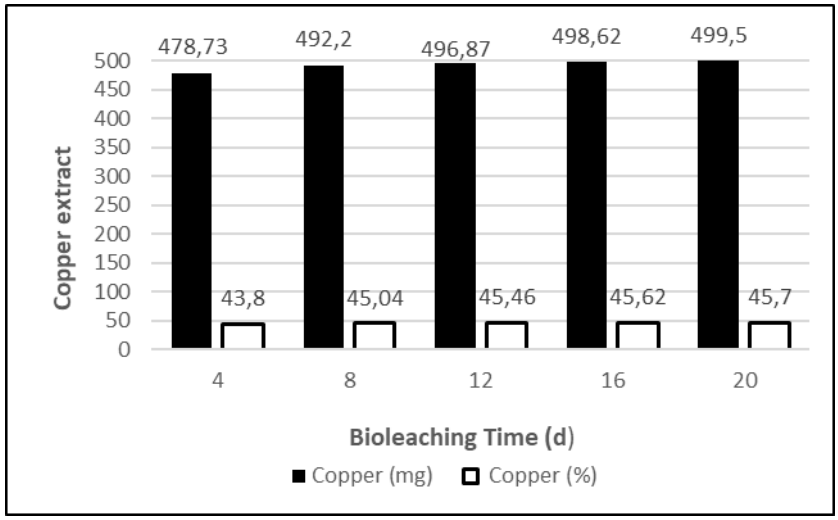

Figure 6. *Evolution of the copper extraction under optimal conditions*

This difference could be explained by the fact that the malachite contains also high Fe content and *Pseudomonas aeruginosa* is well-known as producers of siderophores which displays high affinity for iron [12-13]. Hence, the strain solubilized and chelated both Cu and Fe at the same time. In addition, several works demonstrated that bioleaching process was efficient using low grade deposits (<0,5 Cu per weight) [14].

5. CONCLUSION

Copper has always been considered as one of the base metals used in industrial sectors. However, the issue in the mining field remains the choice of the extraction method of this metal which depends on certain conditions. This study used various physico-chemical, mineralogical and bacteriological analyses to determine the appropriate application conditions for Cu extraction method allowing at the same time to recover valuable metals and to reduce environmental impacts, which is bioleaching.

Copper bioleaching with *Pseudomonas aeruginosa* is demonstrated in this work and according to the results obtained, this bacterium was able to extract up to 45.7% Cu from the malachite sample. Therefore, copper bioleaching from the malachite with *Pseudomonas aeruginosa* strain is technically feasible and beneficial.

Then, this method can be used as alternative not only for the extraction of metals, but also for the recovery of mining wastes and wastes of different origins with certain metallic values.

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REFERENCES

[1] Dreisinger, D., Lixiviation du cuivre à partir des sulfures primaires, Options pour l'extraction biologique et chimique du cuivre, Hydrometallurgy, 83(1-4), 10-20, (2006).

[2] Castro I.M., Fietto J.L.R., Vieira R.X., Tropia M.J.M., Campos, L.M.M. Paniago E.B. et Brandao R.L., (2000) : Bioleaching of zinc and nickel from silicates using Aspergillus Niger cultures, Hydrometallurgy, 57(1), 39-49, (2000).

[3] Drapeau A. J. ET Jankovic S. : Manuel de microbiologie de l'environnement. Ouvrage, p236, (1977).

[4] Valérie V. Thirion-Merle., Spectrométrie de fluorescence X. Circulation et provenance des matériaux dans les sociétés anciennes, Editions des archives contemporaines, Collection Sciences Archéologiques, 9782813001634, ffhal-01393984f, (2014).

[5] RODIER J., L'analyse de l'eau: eaux naturelles, eaux résiduaires, eaux de mer, chimie, physicochimie, microbiologie, biologie, interprétation des résultats, Paris (France), Dunod, p1384, (1996).

[6] Shabani A., Irannajad M., Azadmehr A.R. et Meshkini M., Bioleaching of copper oxide ore by Pseudomonas aeruginosa, International Journal of Minerals, Metallurgy and Materials, 20(12), 1130–1133 (2013).

[7] Mulligan C.N. et Kamali M., Bioleaching of copper and other metals from low-grade oxidized mining ores by Aspergillus Niger, J. Chem. Technol. Biotechnol., 78(5), 497-503, (2003).

[8] Rafalimanana L.J., Extraction du cuivre de la roche malachite par voie hydrometallurgique cas de la mine Pachoud d'Ambatovarahina, la zone de Daraina et la zone de Vohibory; mémoire de master II; Parcours Matériaux Métalliques et Céramiques, Mention Science et Ingénierie des Matériaux, ESPA, Université d'Antananarivo, p84, (2017).

[9] Nkulu wa ngoie G., Biolixiviation de la carrolite–Applications aux minerais sulfurés polymétalliques de l'Arc Cuprifère du Katanga en République Démocratique du Congo (RDC) (Cas des minerais de la mine de Kamoya/Kambove), Thèse de Doctorat, Université de Liège, p222, (2012).

[10] Singleton P., Bactériologie. Dunod 4ème édition, p415, (1999).

[11] Kaijian Hu and al., A New Heterotrophic Strain for Bioleaching of Low-Grade Complex Copper Ore, Minerals 6(1), 12, (2016).

[12] Ferret C., Rôle des Pseudomonas fluorescents dans la biodisponibilité des métaux contaminant les minéraux du sol : application à la phytoremédiation. Thèse de doctorat. Ecole Doctorale de la vie et de la santé, Université de Strasbourg, p333, (2012).

[13] Hider RC, Kong X. Chemistry and biology of siderophores. Nat Prod Rep, 27: 637–657, (2010).

[14] Kordosky's G.A.**,** Copper recovery using leach/solvent extraction/electrowinning technology : Forty years of innovation, 2.2 million tonnes of copper annually**,** J.- South African Institute of Mining and Metallurgy 102(8) :445-450, (2002).

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