ADAPTATION OF *Acidithiobacillus ferrooxidans* LR TO PRINTED CIRCUIT BOARD FROM SCRAP COMPUTER

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ABSTRACT

The application of bioleaching process in the refractory ore treatment and waste is already known and also can be applied like a pre-treatment of electronic waste to leaching base metals for later precious metals recovery. The objective of this work was evaluating the influence of bacterial adaptation on bioleaching process of copper from non-magnetic fraction of printed circuit boards. For this, a study of bacterial adaptation to printed circuit boards from obsolete computers using the bacteria *Acidithiobacillus ferrooxidans*, strain LR, in experiments shake flasks to 180rpm and 30℃ aiming to obtain an optimal bacteri al growth under acid condition (ph=1,8). The adaptation process was conducted with gradual increase of comminuted printed circuit board concentration in the bacterial culture medium (T&K medium). The parameters evaluated were: concentration of Cu and Fe (ICP-OES), analysis of ferrous iron concentration and pH measurements. The results show that adapted bacteria presented higher copper extraction rate (63%) compared with unadapted bacteria.

Keywords: bacteria, bioleaching, comminution, recycling, printed circuit boards

1. Introduction

The electronic waste has been reported as an alternative source to recover base and precious metals. The bioleaching can be an alternative method to recover base metals such as copper inside the printed circuit boards (Yang et al.,2009).

The bacterial adaptation is the first step of bioleaching process in which the bacteria come in contact with the waste or ore through several subculturing.

The adaptation process can be done by gradual substitution of Fe^{+2} and gradual increase of substrate concentration, but there isn't a protocol or predefined conditions such adaptation periods, pulp densities and inhibitory metal ion concentrations (Haghshenas et al., 2009; Bevilaqua et al., 2002)

Haghshenas et al. (2009) considered serial cultures in medium containing increasing pulp density as an efficient strategy for adaptation of *A. ferrooxidans*.

The bacterial growth is defined by 4 phases: latency phase, exponential phase, stationary phase and death phase. The latency phase is the period in which the bacteria adapt to new environment. In the exponential phase, the bacteria use the Fe^{+2} as energy source which leads to the maximum growth rate of bacterial population. In the stationary phase the nutrient finish and the bacterial population stop growing, and in the death phase there is a decrease in the number of bacterial cells.

In bioleaching studies (Haghshenas et al., 2009; Horta et al., 2009; Xia et al., 2008; Ilyas et al., 2007) that carried out the previous microorganisms adaptation, the results indicate that recovery rates of metals were superior when compared with recovery rates achieved with non-adapted bacteria.

According to Horta et al. (2009) and Xia et al. (2008), the importance of bacterial adaptation to the conditions of leaching medium was attested by better efficiency on copper extraction obtained by adapted cells when compared to non-adapted cells in study of chalcopyrite bioleaching using *A. ferrooxidans*.

Xia et al. (2008) describe that the adapted bacteria *A. ferrooxidans* can adhere more easily to mineral surface due to more proteins of cell surface acquiring specific interaction with the mineral increasing the solubilization rate of metals.

Ilyas et al. (2007) showed that metals can be recovered from electronic scrap by bacterial leaching using thermophilic bacteria such as *S. thermosulfidooxidans* and observed that pre-adaptation of microorganisms to high concentrations of metallic ions increased the metals solubilization rates.

Other events, such as increase of suspended bacterial population, decrease of cells sensibility to solids and ruptures of cell membrane and increase of cells ability of sulfur layer degradation (Haghshenas et al., 2009), might be able to explain what happens during the adaptation process. The objective of this work was to evaluate the bacterial adaptation influence on bioleaching process of copper from non-magnetic fraction of printed circuit boards of obsolete computers.

2. Objective

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3. Materials and methods

3.1. Printed circuit boards

The printed circuit boards from obsolete computers were comminuted (<2mm) in a hammer mill and put in the magnetic cross-belt separator. The results are presented as mass balancing. Samples obtained through quartering of non-magnetic fraction from magnetic separator were used in bioleaching experiments and analysis of copper concentration.

To determine the initial copper concentration, the sample was dissolved in aqua regia in 1:20 (Park and Fray, 2009) (1g of printed circuit boards sample to 20ml of aqua regia solution). The contact between the printed circuit boards waste and aqua regia was 24 hours, followed by simple filtration with quantitative filter paper. The leached fraction was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

3.2. Bacterial adaptation process

The bacteria *Acidithiobacillus ferrooxidans* strain LR was isolated from uranium mine effluents (Garcia Jr., 1991).

The process of bacterial adaptation was conducted through several subcultures with gradual increase of printed circuit boards concentration (non-magnetic fraction) for 2 months.

3.3. Culture conditions

The bacteria were cultured in the T&K medium (Tuovinen and Kelly, 1973) composed of two solutions (A and B), being:

Solution A: $(NH_4)_2SO_4$: 0,625g/l; $MgSO_4.7H_2O$: 0,625g/l e K_2HPO_4: 0,625g/l.

Solução B: 166,5g/l $FeSO_4.7H_2O$

For the preparation of T&K medium, the solutions A and B were prepared, acidified with H_2SO_4 10N to pH 1,8 and sterilized separately.

The solution A was sterilized by autoclaving at 120ºC for 30min and 1atm. The solution B was sterilized by filter-sterilized (0,45µm). Finally, the solutions A and B were mixed at proportion 4:1 respectively.

3.4. Bioleaching experiments

Bioleaching experiments were carried out in 200ml T&K medium in 250ml shake flasks containing printed circuit boards samples (15g/l) and inoculated with 5% (v/v) *A. ferrooxidans* LR. The cultures were incubated at 180 rpm and 30℃.

The water evaporation was replenished at each sampling with sterile acidic water (pH=1,8) and the medium pH was adjusted with H_2SO_4 10N to 1,8-2,0. All conditions were performed in triplicate. For measurements of pH, a bench pH meter was used (an Ag^0/AgCl reference).

Adapted bacteria and unadapted bacteria were used as inoculum. The nomenclature was:

- MP: T&K medium + Printed circuit boards´ sample (control)
- MPBA: T&K medium + Printed circuit boards´ sample + adapted bacteria
- MPBNA: T&K medium + Printed circuit boards´ sample + unadapted bacteria

The aim of these experiments was to evaluate the influence of bacterial adaptation on copper bioleaching from non-magnetic fraction of printed circuit boards.

3.5. Analytical methods

Leach samples were periodically withdrawn for chemical analyses (1, 2, 3, 4, 5, 6, 7, 10, 15, 20, 30, 40° days) and centrifuged dur ing 20min at 5000rpm.

10ml from supernatants were used to determine Fe^{+2} and 5ml were preserved with 1m HNO_3 concentrate at 4℃ for metal analyses (Cu and tota l Fe).

The determination of Fe^{+2} concentration was made by titration with potassium dichromate ($K_2Cr_2O_7$) and metals concentration (Cu and Fe_{total}) by inductively coupled plasma optical emission spectroscopy (ICP-OES).

The results of copper analyses were used to determine the extraction rate through of difference between initial concentrations of copper in the non-magnetic fraction of printed circuit board and leach concentration taken from bioleaching experiments.

4. Results and discussion

4.1. Printed circuit boards characterization

The results obtained in the comminution, magnetic separation and ICP-OES initial analyses are showed in Fig. 1.

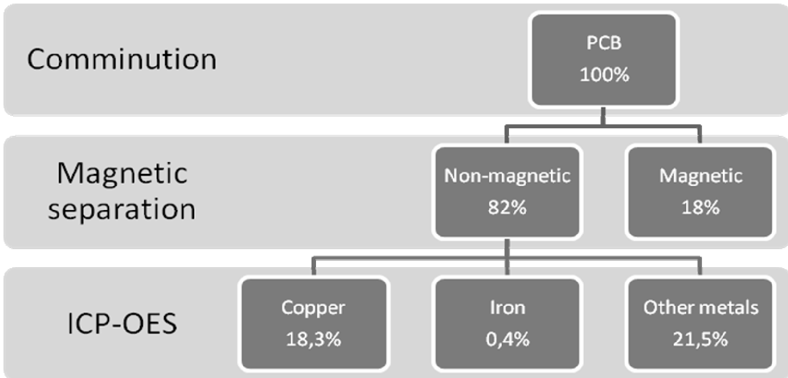

Fig. 1 – Flowchart of characterization operations of printed circuit boards with mass balancing

The non-magnetic fraction of printed circuit boards consists of 40% metals and 42% other materials (ceramics and polymers). Copper is the metal of highest percentage in the printed circuit board (18,3% w/w); this is because the printed circuit boards of computers are type FR2, which is a fiberglass layer or cellulose paper and phenolic resin. The surface has copper layer to connect electrical contacts.

Studies by Park & Fray (2009), Tenório et al. (1997) and Veit et al. (2006) demonstrate that the printed circuit boards' composition changes probably due to different methodologies applied in the works or because the composition has changed over time. Ilyas et al.(2007) also suggested that analytical methods and origin of the material can be attributed to this difference.

4.2. Bioleaching experiments

The results obtained in copper extraction are presented in Fig. 2.

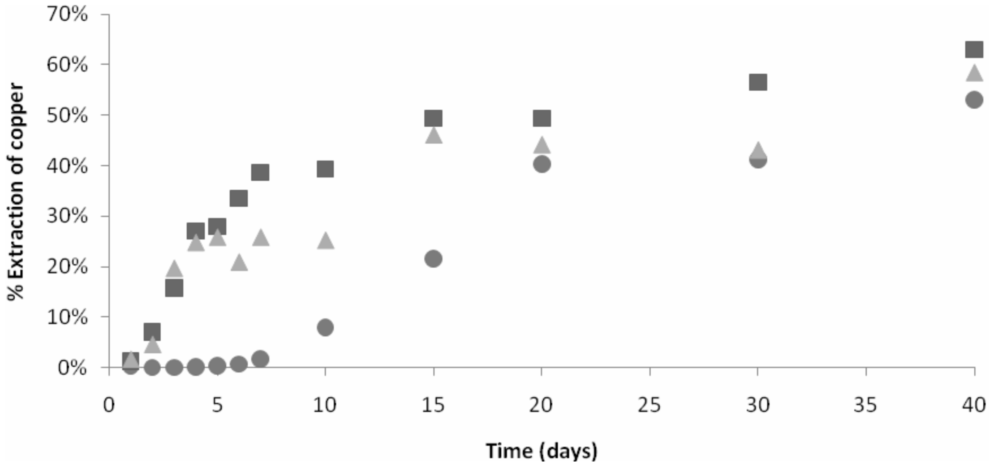

Fig. 2 – Cu^{+2} extraction vs time. Symbols: (●) abiotic control, (■) *A. ferrooxidans* LR adapted to 15g/l printed circuit boards, (▲) *A. ferrooxidans* LR unadapted growing with printed circuit boards.

In the inoculated flasks (Fig. 2) the extraction was higher in the first 7 days probably due to the bacterial growth to be in exponential phase, consequently, the exponential increase of bacterial cells caused an increase of bacterial metabolic activity involving the ferrous iron oxidation to Fe^{+3} and copper solubilization.

After 8^{th} day, the bacterial growth entered the stationary phase and the copper extraction rate decreased because, at this stage, the number of bacterial cells remained constant until the death phase.

The bacterial adaptation increased the copper bioleaching by *A. ferrooxidans* LR, reaching rate of copper extraction of 63% with adapted bacteria while the unadapted bacteria reached 58% copper extraction. This probably occurred due to the adaptation that have decreased the bacteria sensitivity to printed circuit boards and the presence of ions generated during the oxidation of metals contained in the printed circuit boards, such as Zn^{+2}.

Xia et al. (2008) also observed that the adapted bacteria had dissolution rate of copper increased compared to unadapted bacteria in a study of chalcopyrite bioleaching using *A. ferrooxidans* adapted and unadapted suggesting that there are significant differences in bacterial attach and tolerance to shearing stress of adapted cells and unadapted cells due to changes in components and structure of cell wall.

Fig. 3 present changes in pH before adjustment for the range of 1,8 to 2,0.

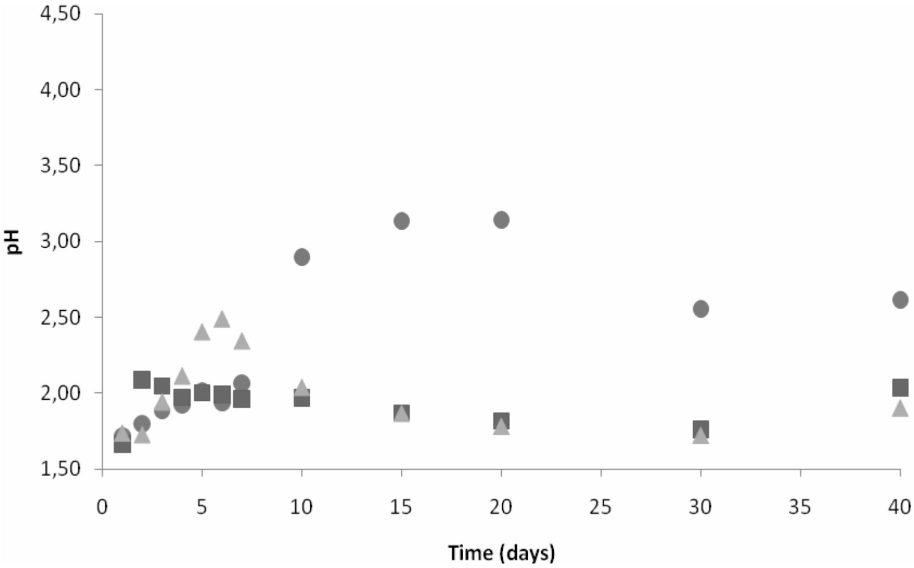

Fig. 3 – Changes in pH vs time. Symbols: (●) abiotic control, (■) *A. ferrooxidans* LR adapted to 15g/l printed circuit boards, (▲) *A. ferrooxidans* LR unadapted growing with printed circuit boards.

The pH increased in all conditions studied at the beginning of the experiment (Fig. 3), similar behavior was observed by Brandl et al. (2001) in electronic scrap bioleaching study, suggesting that the initial increase of pH also occurs due to alkalinity of electronic waste.

The pH changes presented similar behavior between adapted and unadapted bacteria there at the beginning of the experiment a marked elevation of pH. This behavior occurs due the exponential phase of bacterial growth, because with the increase in the number of bacterial cells occur the Fe^{+2} oxidation in Fe^{+3} consuming H^+, as show in reaction (1) below.

$$2Fe^{+2} + ½\ H_2O + H^+ \leftrightarrow 2Fe^{+3} + H_2O \qquad (1)$$

After 8 days, the inoculate conditions remained within the range 1,8 to 2,0 until the end of the experiment, possibly due to the combinations of reactions that consume acid, such as chemical and microbiological oxidation of Fe^{+2} and the reactions that produce acid, as the hydrolysis of ferric ion (Brandl et al., 2001; Wang et al., 2009).

In the control condition there was an increase of pH showing the consumption of acid during the oxidation process of printed circuit boards. Another factor that could explain the higher pH values of control even with the adjustments is the H^+ consumption from metals oxidation which was not reset through the Fe^{+2}/Fe^{+3} cycle.

Fig. 4 show the results of Fe^{+2} concentration analyses.

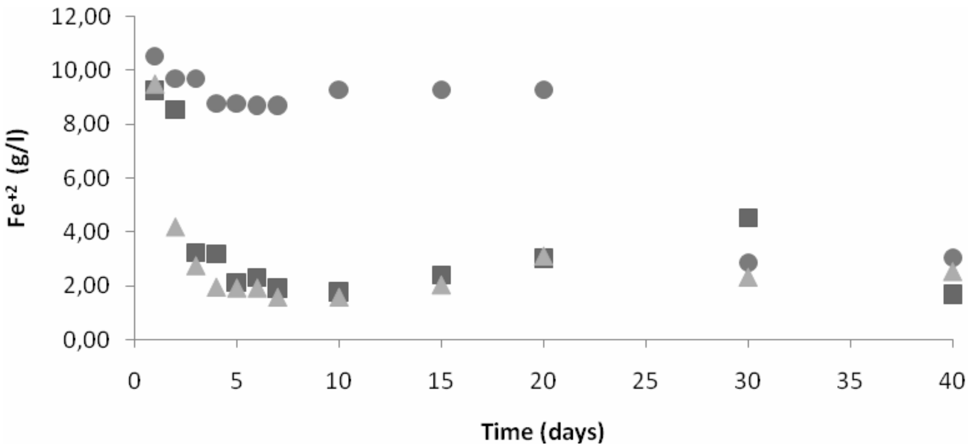

Fig. 4 – Changes in Fe^{+2} vs time. Symbols: (●) abiotic control, (■) *A. ferrooxidans* LR adapted to 15g/l printed circuit boards, (▲) *A. ferrooxidans* LR unadapted growing with printed circuit boards.

In the early days of the experiment there was a decrease in the Fe^{+2} concentration due to the increase in the bacterial activity (Fig. 4), thereby increasing the Fe^{+3} concentration in the inoculated conditions.

The Fe^{+3} produced acts as an oxidant, oxidizing metals such as copper in their corresponding ions, being reduced to Fe^{+2} as shown in the reaction (2) below:

$$2Fe^{+3} + Cu_{(s)} \leftrightarrow 2Fe^{+2} + Cu^{+2} \qquad (2)$$

In the control, the Fe^{+2} concentration starts to decline after the 20th day (Fig. 4). Similar behavior was observed by Francisco Jr. (2007) in bacterial leaching of complex mineral sample containing pyrite, pyrrotite and molybdenite, which suggested that this fact may have occurred by natural oxidation of Fe^{+2}.

5. Conclusion

The bacterial adaptation increases the percentage of copper extraction in the bioleaching process of non-magnetic fraction of printed circuit boards from obsolete computers.

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