E-waste valorisation by recovering valuable metals with microorganisms

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Abstract

The effectiveness of bioleaching in copper recovery from printed circuit boards by *Acidithiobacillus ferrooxidans* has been evaluated under a wide range of conditions demonstrating the suitability of the technology and their limits. The process has been tested using a column reactor simulating conditions found at industrial scale and operating in continuous mode. Moreover, new strategies have been adapted to increase the efficiency of the operation and to reduce the time required for this purpose. Taking into account the complex composition of electronic waste, the limitations of applicability, for instance due to the accumulation of toxic metals in the solution, have been also identified by microrespirometric measurements. Experiments carried out at laboratory scale verifies the proof of concept of the biotechnology for this application, recovering 37% of copper using flasks with a concentration of 7.5 g/L of e-waste in 6 hours. This efficiency was improved in the case of the packed column, where 50% of copper was recovered using the same amount of e-waste at the same period time. New strategy was developed to increase the kinetic of reaction and overcome transport limitations for the leaching solution, achieving copper recoveries up to 80% in the same period than previously reported. Regarding toxicity assays, the methodology used allows to identify that, depending on the concentration and the time exposed, nickel, copper and aluminium affected the microorganisms' activity, inactivating them. It was concluded that aluminium resulted more toxic than copper, which in turn was more toxic than nickel, at the conditions tested.

1. INTRODUCTION

During the last years, factors like rapid technology development and ever-shorting lifespans have been contributing to the growing of electronic waste disposal (e-waste). This is the kind of waste that is increasing at a higher rate in comparison with others, which is estimated around 3-5% per year [1]. However, the percentage of the material recycled is still very low, about 11%. In this sense, the United Nations has warned that millions of tons of old electronic goods are illegally exported to developing countries. This fact has an important impact on the environment and the health of people exposed. The recycling process of this type of waste is not trivial because of its complex composition, with more than 60 different chemical elements [2]. In a common printed circuit board (PCB) it is found 30% of plastics, 30% of refractory materials and up to 40% of metals [3], some of them are valuable metals such as copper, silver or gold, among others, which could be retrieved to new use. Two main processes to recover these metals exist at industrial scale, the pyrometallurgy and the hydrometallurgy. However, they are characterized by the use of high temperatures and aggressive chemicals to melt or to dissolve the matrix containing the metals, giving place to an important economic cost and large environmental impact that make them only profitable centralizing very high volumes. Bioleaching has been tested as an alternative to those methods, especially in the mining field for low-grade ores. The bioleaching technique is based on the activity of microorganisms that are able to regenerate the leaching agent responsible for the extraction of the metal. For copper extraction, this process is based on the action of iron (III), which oxidizes the metallic copper in the electronic scrap to soluble copper (II) and in turn it is reduced into iron (II). The role of the microorganisms is reoxidise the iron (II), to close the loop without the needing to add new reagents in a cyclic process (Fig. 1). Therefore, bioleaching presents important advantages over traditional methods such as low cost, simple operation and environmental friendliness. However, until now, the time required for the bioleaching technique (around several days) limits its application at industrial scale.

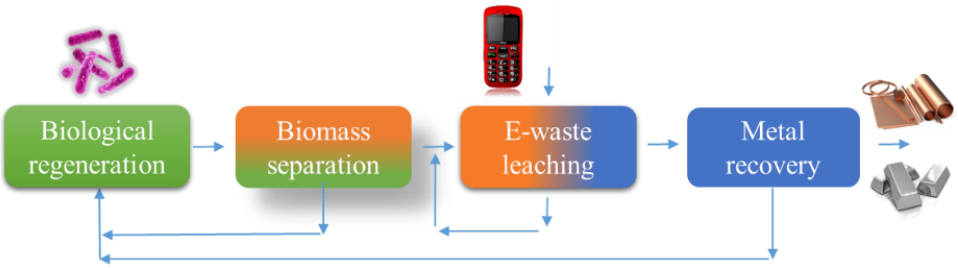

Fig. 1 Diagram of the bioleaching process

In addition, although metal recovery from electronic scarp by bioleaching has been previously studied, almost all of them were performed in batch conditions using stirring flasks [4, 5]. However, reported studies performed in column reactor are scarce in the literature and just some authors have focused on it [6–8]. Nevertheless, the column reactor are more suitable to scale-up the process to adapt it at industrial scale, allowing the continuous mode operation.

In a common bioleaching operation, not only the metal of interest are extracted since many other metals could be also bioleached during the process, including toxic metals. Moreover, although promising microorganisms for bioleaching applications have been used [9], the metal presence in the solution at some conditions could affect biological activity. In the case of *Acidithiobacillus ferrooxidans*, one of the important microorganism for this application [10], some studies have focused on their tolerances to different metals, evaluating its effect on the iron oxidation rate by measuring iron concentrations along time [11]. For this purpose, *o*-phenantroline and ferrozine are the commonly applied colorimetric methods [12]. However, colorimetric measurements could be affected by the precipitates formed during the oxidation process. Giebner et al. [13] suggested that another obvious approach was to quantify the consumption of the electron acceptor oxygen. In this regard, an optode-based technique was adapted to measure oxygen consumption of *Acidithiobacillus ferrooxidans* to determine their metabolic activity.

The aim of the present work was to investigate the copper recovery from printed circuit boards, evaluating the bioleaching process at different conditions to demonstrate the suitability of the technology. In addition, new strategy was adapted in column reactor to increase the efficiency of the operation as well as to reduce the time required. Moreover, this work also investigated the potential toxicity of copper, nickel and aluminium for *Acidithiobacillus ferrooxidans* by means of microrespirometric measurements in terms of concentration and exposed time. The assays were performed in order to delimit the maximum concentrations tolerated by the microorganisms without negative effects on their metabolism.

2. MATERIALS AND METHODS

2.1. Electronic scrap

The printed circuit boards (PCB) used in this work comes from end-of-life mobile phones. The PCB was removed manually from the phone structure and the main electronic components as resistors, capacitors and chips were also separated. The particle size were reduced, and then, they were crushed and sieved, collecting the particles between 0.2 and 1.0 mm of diameter.

The copper content of the scrap was determined by analysing it after acid digestion. In particular, 0.15 g of e-waste were digested with 10 mL of HNO_3: HCl (3:1) at 150 ºC for 15 minutes in a microwave (*Microwave System, Millestone, Italy*). After the digestion, samples were diluted and analysed by atomic absorption spectroscopy (*Solar S2, Thermo Scientific, United States*). The assays were measured in triplicate. The average content of copper in the PCB was (44.0 ± 0.6)%.

2.2. Microorganisms and mineral medium

The bacterial strain *Acidithiobacillus ferrooxidans* (ATCC 23270) was used. It was kindly provided by the Department of Chemical Engineering from the University of País Basco (Spain). The mineral medium used in the experiments, named 6K, has been prepared as follows: $(NH_4)_2SO_4$ 3.00; K_2HPO_4 0.50; $MgSO_4 \cdot 7 H_2O$ 0.50; KCl 0.10; $Ca(NO_3)_2 \cdot 4 H_2O$ 0.014 grams were dissolved in 900 mL of distillate water. The pH

was adjusted with H_2SO_4 10 N to pH 1.7. Then, 30 grams of $FeSO_4 \cdot 7\ H_2O$ were dissolved in 100 mL of distillate water and the pH was also adjusted with H_2SO_4 10 N to pH 1.7. After that, both solutions were mixed and the pH was readjusted to pH 1.7 if it was necessary.

The growth of the *Acidithiobacillus ferrooxidans* was performed in a stirrer-tank bioreactor equipped. It was filled with 2 L of 6K mineral medium, containing 10% of inoculum. The pH was controlled at pH 1.75 by the dropwise addition of H_2SO_4 10 N and they were stirred at 200 rpm. Moreover, the temperature was maintained at 30 ºC with the reactor jacket, by a thermostatic bath. Aeration was achieved by compressed air at 0.5 L/min. When the solution turned red and the Oxidation-Reduction potential was up to 600 mV it was considered that all the iron has been oxidized. Then, 20% of the total medium was renewed with new 6K medium in order to promote the growth of the microorganisms.

2.3. Bioleaching experiments and column reactor

Batch bioleaching tests were performed in 500 mL baffled Erlenmeyer flasks, containing 350 mL of iron (III) solution from the growth reactor. The solution was previously sedimented in order to separate the biomass from the solution and also to remove the possible iron precipitates that have been formed. Then, 7.5 g/L of crushed PCB were added. The flasks were kept in an incubator at 30 ºC and shaken by an orbital agitation at 130 rpm, measuring the pH and the Oxidation-Reduction potential periodically. In addition, the pH was controlled at pH 1.75 by the dropwise addition of H_2SO_4 10 N. For iron and copper analysis, samples were taken every hour, being filtered and diluted before their analysis.

For continuous bioleaching, a column reactor was used (Fig. 2). It consists on a cylinder tube of 10 cm height with an internal diameter of 3 cm. The column was filled with 7.5 g/L of electronic scrap mixed with plastic particles between 1 and 3 mm of diameter as a structuring in order to provide better contact among particles. Moreover, a reservoir was incorporated to easy the sampling and also the measurements of pH and ORP during the experiment. Hence, the reservoir were filled with 400 mL of the iron (III) solution from the growth bioreactor. As it was carried out in batch conditions, the iron solution was previously sedimented, and the supernatant was used. The leaching solution was pumped at a rate of 54 mL/min by a peristaltic pump. Samples for iron and copper measurements were taken every hour from the reservoir as well as pH and ORP measurements were done. As it was carried out in batch conditions, the pH was controlled at pH 1.75 by the dropwise addition of H_2SO_4 10 N.

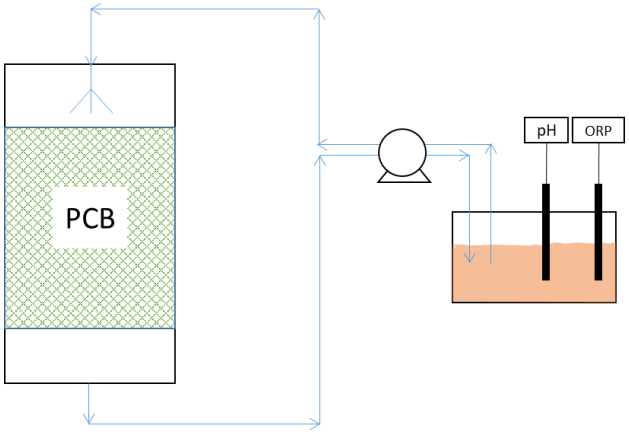

Fig. 2 Diagram of the column reactor for copper bioleaching

2.4. Toxicity tests development

Toxicity tests were evaluated with three different metals which are copper, nickel and aluminium at different concentrations. The metals to evaluate were added as $NiSO_4 \cdot 7\ H_2O$, $CuSO_4 \cdot 5\ H_2O$ and $Al_2(SO_4)_3 \cdot 18\ H_2O$ for nickel, copper and aluminium, respectively. For each metal, six different concentrations were also evaluated at different molarity. For the experiment development, two 500 mL baffled Erlenmeyer flasks were prepared for each metal and each concentration, so 18 flasks in total were needed for the study. From the two flasks, one was used as a control without the toxic addition whereas in the other the toxic metal concentration was added. In this sense, it was possible to calculate the effect of the metal toxicity to the microorganism, taking into account the possible loss of activity due to starvation.

This loss of activity was measured by respirometric assays with the optode system. In particular, it was calculated the relative loss of activity as a percentage between the toxic and its control. Samples for respirometric measurements were taken every two hours, whenever possible, or during 48 hours.

2.5. Optode system for oxygen consumption measurements

The optical oxygen system (*FireStingO2, PyroScience GmbH, Germany*) are composed by a combined excitation and detection module, connected to a sensor spot by a fibre-optic cable. Sensor spot is coated with an oxygen-sensitive fluorophore. This sensor is located opposite to the fibre-optic at the one side and the other of the glass cuvette. The operating principle is based on the red light excited REDFLASH indicators, which show luminescence in the near infrared (NIR). The light emitted depends on the oxygen concentration, being higher when there is less oxygen concentration detected. The optode signal was evaluated with a PC using the software Pyro Oxygen Logger v.3.213. Since the fluorescence depends on the temperature, an automatic compensation was performed by an integrated temperature sensor from the optode system. Even so, all the oxygen measurements were taken in a thermostat cabinet at 30 ºC to compensate temperature fluctuations. According to the manufacture's indications, the sensor spots were calibrated using air-saturated water (100% O_2) and 2% w/v sodium sulphite (0% O_2) as references.

For respirometric measurements, 2 mL of biological sample was taken and, after the addition of 2 mL of 6K medium, the mixture were vortexed. Then it was transferred to the cuvette of the optode system in which the spot sensor was previously incorporated. The signal was recorded for 30 minutes, and to express the respiration activity, the oxygen decreases rate was determined.

2.6. Analytical methods

Copper ions concentration was analysed by an atomic absorption spectroscopy (*Solar S2, Thermo Scientific, United States*) whereas total iron and iron (II) determinations were carried out by the 1,10-phenantroline method [14] with an UV/VIS spectrophotometer (*Lambda 25, PerkinElmer, United States*). Oxidation-reduction potential and pH were measured with a multimeter (*MultiLine Multi3620 IDS, WTW, Germany*).

3. RESULTS AND DISCUSSION

3.1. Copper recovery in batch and column

In the present study bioleaching was applied to recover copper from mobile phones' printed circuit boards. The process was carried out using two different methodologies. On the one hand, stirring flasks were used whereas on the other hand, column reactor was performed, as it has been described in section 2.3. Results of both processes are shown in Fig. 3. It is observed that it was possible to recover 34% of copper using flasks with a concentration of 7.5 g/L of e-waste in 6 hours, verifying the proof of concept of the technology for this application. Although the extraction was not so high, it was noticing that it was done in just 6 hours, whereas Yang et al. [15] recovered less than 20% in the same period of time. In addition, general methodologies to recover copper by bioleaching using flasks spent more than 6 hours to recover this amount of metal from the scrap [16–18]. Moreover, the efficiency obtained was improved in the case of the packet column, where 50% of copper was recovered using the same amount of e-waste at the same period time. No similar results were found in the literature since column reactor studies in e-waste field are scarce in the literature, as it was mentioned before. The most similar study was performed by Chen et al. [19], who also carried out the experiments with a pure culture of *Acidithiobacillus ferrooxidans*, but the column used was twice the size of the column used in the present study. They concluded that this system are feasible, obtaining 95% of copper contained in the scrap. Nevertheless, they need 30 days to achieve this recovery. Hence, although the bioleaching in the present work was not totally completed, the time was significantly reduced from several days to just some hours.

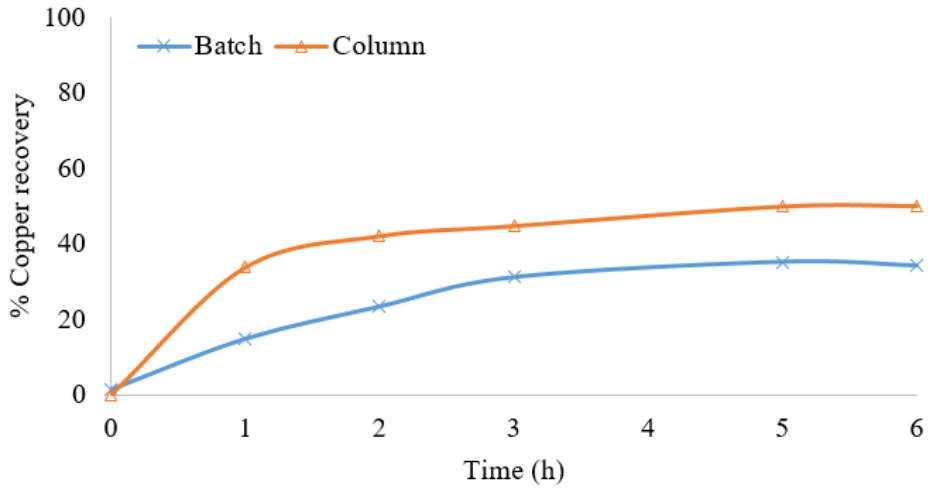

Fig. 3 Copper recovery along time in batch and column experiments

3.2. Column bioleaching in cycles

As it has observed in Fig. 3, column reactor allowed to recover copper but at the conditions tested it remains almost half of the copper present initially in the scrap. It is associated to the mass transfer and accessibility limitations detected during the operation. For this reason, a new strategy was developed to increase the kinetic of reaction and overcomer problems of accessibility for the leaching solution. In this regard, during the column reactor bioleaching the waste inside it was stirred every two hours to avoid dead zones and stagnant regions. Moreover, the iron (III) solution was also renewed to avoid limiting reagent concentrations during the metal extraction. After 3 cycles, as can be seen in Fig. 4, the copper recovered increased up to 80% in the same period than previously reported. Therefore, the new strategy proposed in this work improves significantly the process since the extraction of copper with this methodology was 1.6 times the extraction obtained by the column reactor previously experimented.

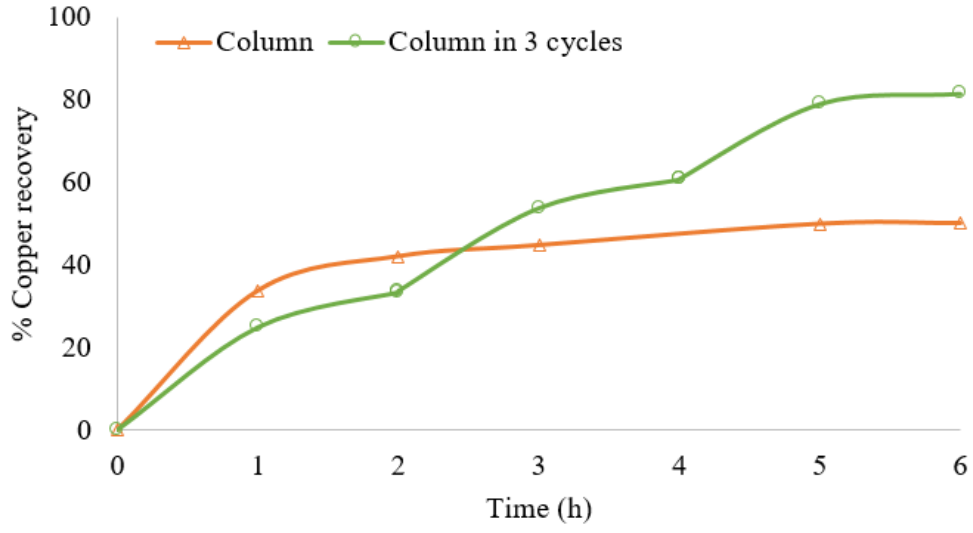

Fig. 4 Recovery of copper during column bioleaching experiments

3.3. Toxicity assays by microrespirometries

Although different studies has focused on the resistance of *Acidithiobacillus ferrooxidans* to high heavy metal concentrations [9, 11, 20]. It has been reported that e-waste contain toxic materials that can affect biological activity in bioleaching studies [21]. In the present work it was studied the toxicity effect of three

common metals presented in PCBs (copper, nickel and aluminium) at different ion concentrations each one, as it was described in section 2.4.

Copper was the first metal evaluated in this work, since this is the most studied metal in bioleaching studies [22–26]. Results for six different concentrations of copper (0.010, 0.050, 0.60, 0.800, 1.000 and 1.200 M) are shown in Fig. 5. In general, it was observed that the relative loss of activity increased when copper concentration also increased. It means that the higher the copper concentration, the more toxic for microorganisms is after 48 hours. Moreover, it is noteworthy that the toxic effect was immediate at metal concentrations over 0.6 M whereas at concentrations below 0.05 M there was no effect on biological activity. Taking into account that the lowest concentration tested (0.010 M) was the copper concentration obtained in the previous bioleaching experiments, it was confirmed that this concentration had not negative effect on microbial metabolism after 48 hours. Nevertheless, it should be considered for those cases when the biological and leaching steps are taking place in the same place, since in this cases the copper concentration will be accumulated being possible to reach concentrations that affect negatively the microbial activity. Despite the fact that Leduc et al. [11] concluded that the inhibitory concentrations depend on the specific strain of *Thiobacillus ferrooxidans*, Cho et al. [9], who based their study on the inhibition effect of copper on the rate of iron oxidation of *Acidithiobacillus ferrooxidans*, found that the maximum tolerance concentration for copper was 0.142 M, which is in agreement with the results obtained herein.

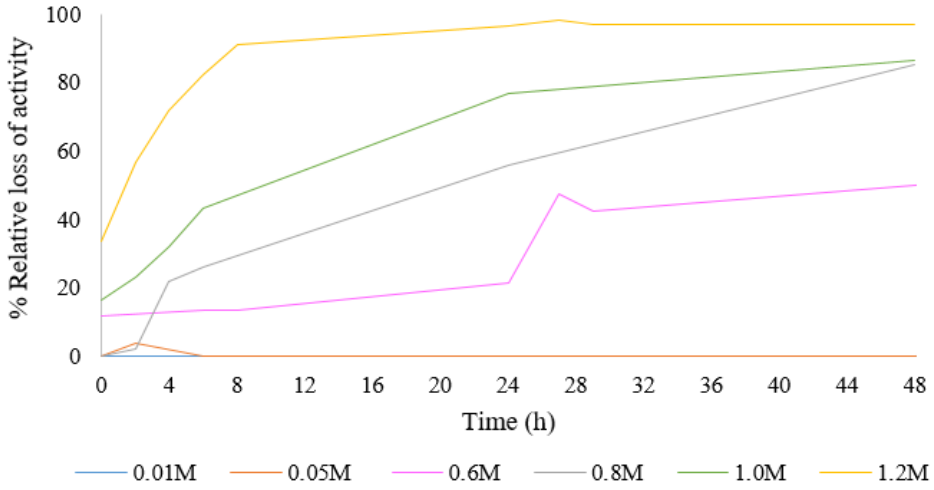

Fig. 5 Toxicity evaluation of copper at six different metal concentrations on *Ac. ferrooxidans* activity by microrespirometries

Similar assays were performed to evaluate the potential toxic effect of nickel on the activity of the iron-oxidizing microorganisms at six different concentrations (0.0005, 0.050, 0.100, 0.300, 1.000 and 1.500 M). In Fig. 6 it is shown that nickel was also toxic to microorganisms after 48 hours, especially at concentrations over 0.3 M, at which conditions, the effect was immediate. Moreover the results demonstrated that those concentrations of nickel described in the literature as natural tolerance (0.3 M) are not completely innocuous for microorganism when the contact with the solution is prolonged for several hours. Although microorganism metabolism has not been disrupted after one cycle of leaching, operation strategies for reducing time on continuous mode, as the strategy developed in previous column experiments, should take into account these evidences avoiding long contact times by previous separation operations. The toxicity effect of nickel observed is in agreement with Cho et al. [9] who reported that the inhibitory effect of the metal was produced on concentration over 1.02 M, which is the concentration when the toxic effect was visible from the beginning of the contact. However, it was demonstrated that depending on the total time contact, the negative effect could be also effective over 0.3 M of nickel instead of 1.02 M as it was reported.

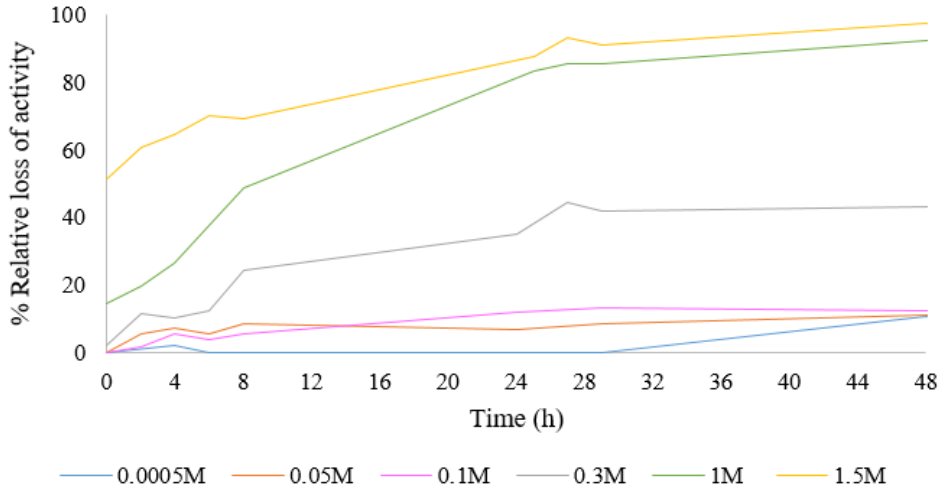

Fig. 6 Toxicity evaluation of nickel at six different metal concentrations on *Ac. ferrooxidans* activity by microrespirometries

Finally, the toxic study was completed with the effect of aluminium ion for different concentrations (0.001, 0.050, 0.100, 0.200, 0.350 and 0.500 M). It is observed that the aluminium resulted toxic at all the concentrations tested (Fig. 7). However, this toxicity increased as the metal concentration also increased. In the particular case of this metal, the toxicity was observed from the beginning of the experiments in all the assays, including the bioleaching concentration (0.001 M). This revealed that this metal was potentially toxic despite not being included in toxicity studies [9, 20]. In this sense, results confirmed the speculated toxicity of aluminium reported by Brandl et al. [21]. Therefore, aluminium represents an important metal to take into account if it is bioleached from the scrap due to its toxic effect to microbial activity over 0.001 M, being more noticeable over 0.05 M.

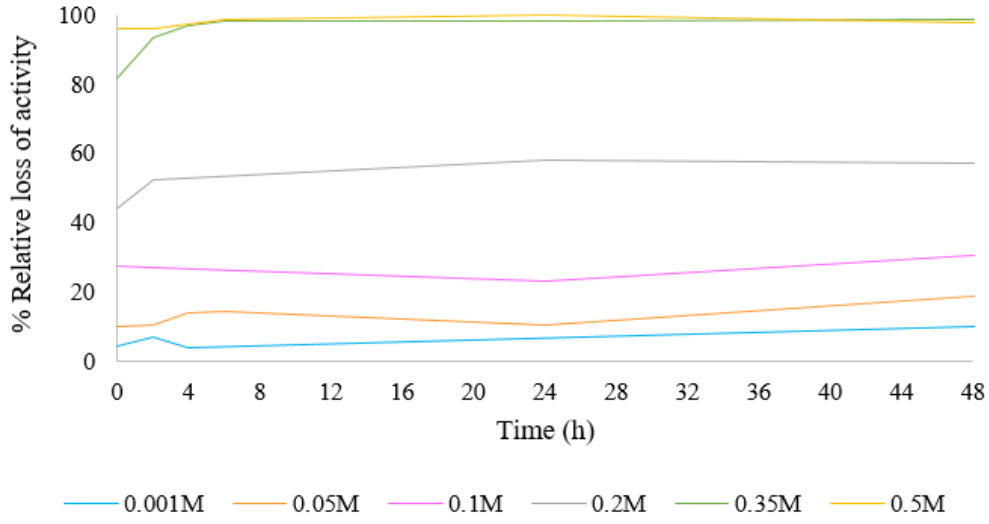

Fig. 7 Toxicity evaluation of aluminium at six different metal concentrations on *Ac. ferrooxidans* activity by microrespirometries

In order to compare the toxicity effect of the three metals studied, it was considered to compare them at three different ion concentrations. On one hand, the bioleaching concentration, which was the average concentration of the metal obtained after the bioleaching process (0.05, 0.0005 and 0.001 M for Cu, Ni and Al, respectively). On the other hand, the concentration referring to the natural and limit concentrations were also evaluated. These tolerances were described by Magnin et al. [27] for copper and nickel whereas the resistance of aluminium was studied by Fischer et al. [28]. The three metals comparison are shown in Fig. 8.

It can be seen that there are noticeable differences between the three metals. Regarding the natural tolerances, although it was described that they did not affect microorganisms' activity, it was observed that they produced negative effect on them in the case of nickel and aluminium, especially after 48 hours of contact when the microorganisms loss 43% and 19% of their activity, respectively. Nevertheless, copper natural tolerance concentration did not reduce their activity which means that they can tolerate it during their growth. In the case of limit tolerances, they have negative effect on the bacteria in all metals, losing 50, 57 and 93% of the activity for Cu, Al and Ni, respectively, after 48 hours. It means that nickel was more toxic than aluminium to the microorganisms, which in turn was more toxic than copper at this concentration. However, it is noticeable that aluminium was the most toxic metal during the first 10 hours at the limit tolerance concentration, although negative effect was visible from the beginning of the experiment in all cases. Therefore, it is observed that the time contact between the metals and the microorganisms are crucial during their growth due to the higher the time, the more toxic the metal. For this reason, reducing the experimental time of the bioleaching process, as well as the development of the process in two different steps are key considerations to maintain the activity of microorganisms at their optimal conditions. Regarding bioleaching concentrations, results demonstrated that they were not toxic for the microorganisms since the relative loss of activity was less than 10% in 48 hours in all cases. However, it should take into account that, as stated previously, the accumulation of the metals could cause inactivity to the microorganisms for longer time operations.

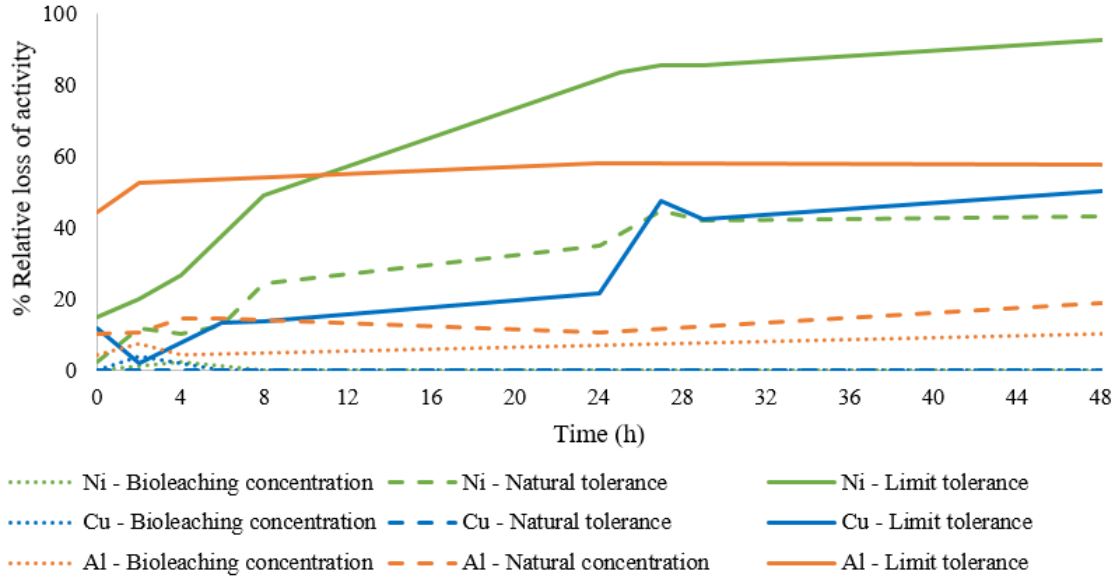

Fig. 8 Toxicity measurements of Cu, Ni and Al for *Ac. ferrooxidans* by microrespirometries

4. CONCLUIONS

From these results it was possible to conclude that the bioleaching process to recover copper from the electronic scrap is effective, verifying the proof of concept of the technology by flasks performance. However, the use of column reactor improved the metal extraction in comparison to the flask bioleaching although the extraction was not completed (50%). Moreover, the new strategy developed in the present work allow reaching attractive amounts of copper (80%) at competitive period time (less than 6 hours).

Regarding toxicity assays, it was concluded that the presence of copper, nickel or aluminium in the biological solution can affect their activity, depending on the concentration of those metals as well as the time contact. In particular, aluminium resulted more toxic than copper since their toxicity was higher in less time and lower concentration. Copper in turn was also more toxic than nickel by the same reason. Moreover, their toxicity was clearly observed after 48 hours although the most toxic concentrations were noticed from the beginning, especially for the aluminium which all the concentrations were toxic from the first instant of contact.

This led us to the conclusion than the strategies developed in the present work, combined with the methodology applied for the monitoring activity of the biological process, make very promising this technology as an alternative to conventional processes at industrial scale.

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