CAUSES OF INHIBITION OF BIOLEACHING BY Cu ARE ALSO THERMODYNAMIC

A. Mazuelos*, C. J. García-Tinajero, R. Romero, N. Iglesias-González, F. Carranza

Address:
Department of Chemical Engineering of the University of Seville
Faculty of Chemistry of the University of Seville
c/ Profesor García González, Edf. Química
41012 Seville
Spain

* Corresponding author: A. Mazuelos
e-mail: mazuelos@us.es
fax: +34 954 55 64 41

A.Mazuelos: Alfonso Mazuelos Rojas; e-mail: mazuelos@us.es
C.J. García-Tinajero: Carlos Jesús García-Tinajero; email: Carlosgarti@gmail.com
R. Romero: Rafael Romero Aleta: email: aleta@us.es
N. Iglesias: Nieves Iglesias-González: mnieves@us.es
F. Carranza: Fancisco Carranza Mora: fcarranza@us.es

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Abstract

BACKGROUND. Cu is an indispensable natural resource for society, as its use is widespread and essential for many technologies. Consequently, worldwide production is around $18 \cdot 10^6$ tn/year, and close to 25% is produced by bioleaching. Bioleaching enables Cu extraction from minerals in atmospheric conditions by virtue of microorganisms' catalytic activity. Microbial catalysis consists mainly in Fe^{3+} production as a leaching agent, a process known as Fe^{2+} bio-oxidation. However, bio-oxidation is inhibited by Cu^{2+} which limits the use of bioleaching with Cu mineral ores.

RESULTS. This paper, for the first time, examines the effect of Cu^{2+} on continuous Fe^{2+} bio-oxidation using a packed bed bioreactor with supported cells. Bio-oxidation was possible in the presence of 20 g/L Cu^{2+}, with a less than 25% reduction in rate. About 15% of this drop in rate is due to biological inhibition and the rest to a reduction in oxygen solubility because of salting-out. The biotic effect is reversed when Cu^{2+} is removed from the input, and the salting-out effect can be overcome by improving aeration conditions.

USEFULLNESS. These results, apart from having important ramifications for designing Cu ore bioleaching facilities, contribute to a more versatile and competitive idea of this clean technology.

Keywords:

Bioleaching, Bioreactor, Heavy Metals, Hydrometallurgy, Mass Transfer

Highlights:

- Influence of Cu^{2+} on continuous Fe^{2+} bio-oxidation is studied for the first time.
- Fe^{2+} bio-oxidation slowdown because of Cu^{2+} presence is caused both by biotic and physical phenomena.
- Adequately modifying bio-reactor aeration would reduce the negative effect associated with Cu^{2+} by 35%.
- Adaptation of cells to Cu^{2+} is possible in continuous bio-reactors.
- The inhibitory effect of Cu^{2+} is reversible.

Symbols:

a: gas-liquid specific interfacial area (m^{-1})

CT: complete oxidation time (h)

C_L: oxygen concentration in the liquid (mg/L)

C_L^*: saturation concentration for oxygen (mg/L)

C_L^o : saturation concentration for oxygen in water at working temperature and pressure (mg/L)

C_{LS}: concentration of oxygen on the surface of the packing material (mg/L)

$[Cu^{2+}]$: copper concentration (g/L)

E(t): residence time distribution function

F(t): cumulative residence time distribution function

$[Fe^{2+}]$: ferrous iron concentration (g/L)

$[Fe^{2+}]_0$: initial ferrous iron concentration (g/L)

$[Fe^{2+}]_t$: ferrous iron concentration at time t (g/L)

k_L: gas-liquid mass transfer coefficient for oxygen (min^{-1})

k_S: mass transfer coefficient for oxygen transfer across the boundary layer surrounding the packing material (min^{-1})

m: mass of ferrous iron inside the bioreactor (g)

MBR: maximum bio-oxidation rate (g/Lh)

Me: mass flow rate of ferrous iron entering the bioreactor (g/h)

MOTR: maximum oxygen transfer rate (g/Lh)

MOUR: maximum oxygen uptake rate (g/Lh)

Ms: mass flow rate of ferrous iron leaving the bioreactor (g/h)

Q_O: gas-liquid oxygen transfer rate (g/Lh)

Q_{Fe}: instantaneous bio-oxidation rate (g/Lh)

s: specific surface area of the packing material (m^{-1})

V: liquid volume inside the bioreactor (L)

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INTRODUCTION

The strategic importance Cu has in industrialised societies is well known; distribution of water, energy and communications are to a great extent influenced by the use we make of this natural resource. Currently, the main raw materials from which Cu is obtained are mineralised rocks containing mineralogical species in which Cu is part of sulphide-like chemical compounds, the most abundant being chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) and covellite (CuS).[1]

In recent decades, biotechnological applications used for producing commercially valuable metals, classified within the field of biohydrometallurgy, have garnered increasing approval in industrial contexts. This is owing to the technical simplicity, low operational costs and moderate environmental impact inherent to them.[2] As a result, between 20 and 25% of global Cu production is obtained by these methods; this amounts to over 4.5 million tons per year.[3] Considering that the price of Cu over the last five years averages at 6600 US$/tn,[4] this gives us an idea of the economic impact associated with Cu biohydrometallurgy.

Biohydrometallurgical technologies for producing metals from sulphide minerals are based on bioleaching.[2,3,5,6] Bioleaching is a process in which raw minerals are subjected to the action of microorganisms so as to free the metal from the sulphide crystal lattice by oxidising it.[7] This way, the metal dissolves as ions and can be purified by solvent extraction and electrolysis, reaching the specifications demanded by the market for its commercialisation. Various mechanisms have been described to explain bioleaching,[2,8,9] among which the most widely accepted — constituting the base for other models — is known as indirect bioleaching.[10] According to this mechanism, metal sulphides (MeS) are indirectly oxidised by O_2 in the air when they are in contact with microorganisms in an acidic medium.

$MeS + ½\ O_2 + 2H^+ \rightarrow S^0 + Me^{2+} + H_2O$ (1)

O_2 and MeS do not physically interact, and electron transfer between them happens through an electronic intermediary, the Fe^{2+}/Fe^{3+} electrochemical couple. This model postulates that the bioleaching process happens in two simultaneous stages:

- A chemical leaching stage, in which Fe^{3+} takes electrons from the sulphide, oxidising it to S^o, and modifies its oxidation state to Fe^{2+}.

$MeS + 2Fe^{3+} \rightarrow S^o + Me^{2+} + 2Fe^{2+}$ (2)

- A biological stage, in which the electrons taken by Fe^{2+} are finally transferred to O_2, and, in doing so, Fe^{3+} is regenerated so it is once more available as an oxidising agent. The cells use Fe^{2+} as an energy substrate and act as a catalyst to the process, which is known as bio-oxidation.[11] Cells capable of metabolising Fe^{2+} are known as iron-oxidising microorganisms.[12,13]

$2Fe^{2+} + ½\ O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$ (3)

Due to this mechanism, bioleaching applications happen in atmospheric conditions, and no reagents are consumed in the process. These facts, derived mainly from the microbial activity in Fe^{2+} bio-oxidation, place bioleaching at an advantage when compared with high pressure leaching technologies, which require far greater pressures and temperatures in order to regenerate the leaching agent.[14]

Among biohydrometallurgical technologies based on bioleaching, the most widely used for producing Cu are those known as heap bioleaching and dump bioleaching.[2,3,5,6] Conceptually, these technologies consist in wetting — with a leaching solution — mineral particles piled into heaps or dumps that act as a packed bed, the process of metal extraction being catalysed by cells adhered to the mineral surface.

It is possible to accelerate bioleaching by carrying out the chemical leaching and biological Fe^{2+} oxidation stages in separate locations, allowing to optimise each independently.[15-17] Bio-oxidation can be improved using reactors designed specifically for this purpose. Among the tested designs, the one which has shown the greatest Fe^{3+} productivities is the flooded packed bed reactor.[18,19] In these reactors, cells adhere to the particles of the packed bed forming a bio-film, and they are fed with liquors containing Fe^{2+} and air, which are introduced through the bottom of the reactor and flow upward.

A special trait of iron-oxidising microorganisms is their ability to tolerate high concentrations of heavy metals as a result of them almost always being present in their natural environment.[20-23] However, there is literature reporting bioleaching inhibition when cells interact with the heavy metal ions freed from the sulphide, the negative impact of which depends on the microbial species, the metal and its concentration.[11]

Regarding the interaction between Cu and iron-oxidising microorganisms, most of the consulted papers concur that cellular metabolism is inhibited when Cu concentration reaches 5 g/L, and that the biological tolerance limit is about 20 g/L.[24-31] However, when it comes to quantifying kinetic variations caused by Cu^{2+}, little has been published and with varying results, despite using the same system; that is batch culture trials. Difficulty in processing data and interpreting results is inherent to this methodology, partly because cells are amidst a medium the composition of which is ever changing.[32] On the other hand, literature always attributes the effect of Cu exclusively to biotic factors.

Another point cited authors have in common is the lack of attention payed to the supply of oxygen to cells, offering no guarantee that, under the tested conditions, there was no bio-oxidation limitation due to lack of oxygen, a necessary condition for $[Cu^{2+}]$ to have been the sensitive variable in these tests.

It is common in aerobic systems for the supply of oxygen to cells to limit their growth and functionality. The limiting stage of this transfer is located in the gas-liquid interphase. In such a case, the rate of transfer, Q_O, is given by the equation:

$$Q_O = k_L \cdot a \cdot (C_L^* - C_L) \qquad (4)$$

Where k_L is the gas-liquid mass transfer coefficient, a is the gas-liquid specific interfacial area, C_L^* is the saturation concentration for oxygen, and C_L is the concentration of oxygen in the liquid phase. It is possible to estimate C_L^* in mg/L for typical bioleaching liquors, if pH, and $[Fe^{2+}]$, $[Fe^{3+}]$ and $[Cu^{2+}]$ in g/L are known, using the equation from Mazuelos et al.[33]

$$C_L^* = C_L^o - 12.698 \cdot 10^{-pH} - 0.0555\,[Fe^{2+}] - 0.0290\,[Fe^{3+}] - 0.0265\,[Cu^{2+}] \qquad (5)$$

Where C_L^o is oxygen solubility in water at working temperature and pressure.

According to equation 5, the greater $[Cu^{2+}]$, the lower C_L^* will be, and the lower the driving force for oxygen transfer.

The consulted literature only mentions the negative effect of Cu^{2+} on microbial activity and overlooks the simultaneous — and synergic — effect Cu^{2+} has as a chemical catalyst on Fe^{2+} oxidation.[14]

Therefore, nowadays the available information about the influence of Cu^{2+} on bioleaching, which is necessary for the design of plants for the treatment of Cu ores, is very limited.

This paper shows the results obtained from studying the effect of Cu^{2+} on Fe^{2+} bio-oxidation, with the novelty of experiments being conducted in continuous operation of a packed bed bioreactor with immobilised cells. Using this type of bioreactor, as well as obtaining results about the influence of Cu^{2+} on bio-oxidation in a device closer to industrial reality, it is possible to attain steady state conditions for the microbial population that it holds.[18,19] Like this, it is possible to maintain cells' response to variations in $[Cu^{2+}]$ in

the feed through time. Special attention will be paid to cell oxygenation in the aim of testing whether the negative effect of Cu^{2+} on Fe^{2+} is exclusively biotic, as stated in the literature, or, on the contrary, it bears some relation to oxygen solubility reduction as a result of the salting-out effect. If the latter were true, a suitable modification of bioreactor aeration conditions would curb the negative effect associated with Cu^{2+}, allowing for a more versatile and competitive idea of Cu ore bioleaching.

MATERIALS AND METHODS

Microorganisms.

The culture used for this study was originally obtained from the Rio Tinto Mine in Huelva, Spain. This strain, designated FNN-9K, consists mainly of the extremophile iron-oxidising species *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. The culture is routinely maintained on a modified Silverman and Lungren 9K nutrient medium[10] at pH 1.25 (adjusted with concentrated H_2SO_4).

Batch bio-oxidation preliminary assays.

A number of batch Fe^{2+} bio-oxidation experiments were conducted in Erlenmeyer flasks containing varying concentrations of Cu^{2+}. The necessary amounts of Cu^{2+} were added to 80 mL of modified 9K medium[10] at pH 1.25 in order to obtain Cu^{2+} concentrations of 0.5, 10, 15 and 20 g/L, and then they were completed with 20 ml of FNN-9K. Flasks were stirred at 180 rpm, and temperature was maintained at 30ºC in a thermostatic chamber. Control tests were carried out at the same conditions without Cu.

Throughout the experiments, Fe^{2+} concentration was measured as a function of time. Maximum bio-oxidation rate (MBR) was calculated by plotting [Fe^{2+}] against time and determining the maximum slope.

O_2 transfer rate in batch cultures.

In order to ascertain the rate of oxygen transfer in batch cultures, the sulphite oxidation method was used.[32] This consists in determining the rate at which sulphite is oxidised in an aerated solution, assuming that the oxygen transferred to the liquid oxidises sulphite instantly.

$$SO_3^{2-} + 0.5\ O_2 \rightarrow SO_4^{2-} \qquad (6)$$

When Cu^{2+} or Co^{2+} are present, this reaction happens at such speed it can be assumed that the value of $C_L=0$. This means that, by knowing the saturation concentration for oxygen, it is possible to establish the value of $k_L \cdot a$ by means of equations (4) and (6).

Several experiments were carried out in Erlenmeyer flasks with 100 mL of a 0.25 M $NaSO_3$ solution stirred at 0, 120, 160, 180, 190 and 200 rpm. 1mL of a 0.5 M $CuSO_4$ solution was added at the beginning of each experiment. Sulphite concentration was measured over time by iodometry, and the slope of the resulting graph was taken as the rate of sulphite consumption.

Packed-bed continuous bioreactor.

Continuous bio-oxidation experiments were conducted in a packed bed bioreactor with an immobilised bacterial film.

The bioreactor was a column measuring 8.4 cm in diameter and 17.8 cm in height. A 5 cm segment in the lower end was left empty of packing material and fitted with inlets for liquid feed and air. The nozzle for air injection measured 1 mm in diameter. Atop this empty

space, held by a mesh with 4 mm openings, was the packing material, with a height of 10 cm. Liquid left the reactor by overflow through an orifice designed for this purpose.

Pseudo-spherical siliceous sand particles ranging between 6 and 7 mm in size were used as support for the biofilm. These particles were randomly placed in the bioreactor to form a packed bed with a porosity of 0.4.

The bioreactor was set up following the protocol described by Mazuelos et al [19] using an FNN-9K inoculum. Once the bioreactor was operational, it was fed with a 7 g/L Fe^{2+} solution at pH 1.5 containing an increasing concentration of Cu^{2+}, from 0 g/L at the start of the experiment to 20 g/L at the end. This solution was pumped into the bioreactor by means of a Heidolph peristaltic pump at a rate of 240±4 mL/h.

Air flow was kept at 750 mL/min throughout continuous operation. This was monitored with a Cole Palmer flowmeter (±15 mL/min).

Residence time distribution.

Residence time distribution was determined by negative step input response technique. Cu^{2+} was used as a tracer. Before the experiment ($t < 0$), $[Cu^{2+}]$ = 20.2 g/L in feed and output alike. Once the experiment had started ($t > 0$), $[Cu^{2+}]$ = 0 g/L in the feed. $[Cu^{2+}]$ was measured in the output throughout the experiment.

The cumulative distribution function F(t) is calculated using equation (7).

$$F(t) = \frac{[Cu(II)]_{t=0} - [Cu(II)]_t}{[Cu(II)]_{t=0}} \qquad (7)$$

F(t) is defined as the fraction of the output that has remained inside the reactor for a time between *0* and *t*. It is also the likelihood that an element of fluid which entered the reactor at *t = 0* will have left the reactor by time *t*. Also, *F(0) = 0,* meaning no fluid leaves the reactor

before *t = 0*, and *F(∞)=1*, meaning all fluid leaves the reactor between *t = 0* and infinity. *F(t)* and the residence time distribution function *E(t)* are related:

$$F(t) = \int_0^t E(t)dt$$

As $\mathrm{E(t)} = \frac{\mathrm{dF(t)}}{\mathrm{dt}}$, *E(t)* can be obtained by calculating the slope at each point of *F(t).*

Oxygen concentration.

The concentration of dissolved oxygen, C_L, was measured with an Orion 3 Star dissolved oxygen meter equipped with a semi-permeable to gas membrane electrode, with Thermo Scientific 081010MD temperature compensation. In order to establish the correction factor for salinity, a Radiometer CDM 210 conductivity meter was used. This device allows for continuous measuring of C_L with a resolution of 0.01 mg/L and relative accuracy of 1.25%.

In order to measure dissolved oxygen on line, the experimental device shown in the diagram in figure 1 was used.

Part of the liquor flowing inside the bioreactor is driven continuously, recirculating at high flow, to an external chamber. This is where the dissolved oxygen electrode and the conductivity electrode were placed. Any bubbles remaining in the liquor were eliminated on its way to the external chamber in a trap designed for this purpose; bubbles interfere with the reading for dissolved oxygen concentration. The total volume of liquor in the recirculation line was 36.71 mL, with a flow around 2900 mL/h.

Chemical analysis.

Fe^{2+} concentration was determined with $K_2Cr_2O_7$ 0.05 N (accuracy ±3 mg/L) by potentiometric titration[18] (Radiometer-Copenhagen). [Cu] and [Fe] were determined by atomic absorption spectroscopy (Perkin Elmer 2380) at 324.8 nm (Cu) and 248.3 nm (Fe) in air- acetylene flame.

RESULTS

Batch bio-oxidation preliminary assays.

With the aim to define the operational conditions and elements of control for this study, a series of batch cultures were carried out with Cu^{2+} present in concentrations of 0, 5, 10, 15 and 20 g/L using the culture FNN-9K as inoculum. Figure 2 shows the results obtained for Fe^{2+} conversion against time. The results for analogous experiments without cells (abiotic) are also shown, where [Cu^{2+}] was 0, 2.5, 10 and 20 g/L.

Figure 2 shows that, under abiotic conditions and with Cu^{2+} being present, barely 35% of Fe^{2+} is converted 400 hours after beginning the experiment, and conversion is scarcely affected by the presence of Cu^{2+}. With no Cu^{2+} present, not even 5% conversion was achieved (results not shown). Kinetic models found in literature[14] suggest an order of reaction between 1.84 and 2.7 for Fe^{2+} — the most frequent value being 2 — and between 0 and 0.5 for Cu^{2+}. In this regard, the results shown in figure 2 are consistent with the information found in literature.

Similar results were obtained with ferroxidant cells and [Cu^{2+}]=20g/L, indicating a severe impairment of microbial catalytic action, which is consistent with the information found in most of the consulted papers.[24-31] Among these, Brahmaprakash et al[26] reports that the impossibility of *Acidithiobacillus ferrooxidans* oxidising Fe^{2+} under these conditions can be overcome through successive re-cultivation, adapting the cells to this environment. Hong-

Mei Li and Jia-Jun Ke[30] describe Fe^{2+} bio-oxidation with 20 g/L of Cu^{2+} present in the medium after previously adapting the microorganisms by successive re-cultivation, a procedure which required over a year in order to be effective.

Fe^{2+} bio-oxidation was however achieved when the assayed Cu^{2+} concentration was equal to or less than 15 g/L (figure 2). In these cases, there are periods in which conversion rises exponentially, which is indicative of microbial catalysis. The times to reach them and their slopes suggest that the negative effect Cu^{2+} has on bio-oxidation is proportional to its own concentration, which is consistent with the consulted literature [24-31]. Nevertheless, the bio-oxidation curves that were obtained for 10 and 15 g/L of Cu^{2+} are unusual; during the time before the exponential phase, Fe^{2+} conversion is higher than expected, allowing that the biotic and abiotic curves may be superimposed. One characteristic effect of bio-oxidation inhibition caused by Cu^{2+} is a longer latency period, and one of the advantages of microorganism adaptation is that this period is shortened.[26,28,31] Taking this information into account, one can suppose the necessary time to reach the exponential phase was long enough for chemical catalysis of Fe^{2+} oxidation to be noticeable, achieving conversions close to 20%.

It is worth noting that the cited works concentrated their efforts on determining Cu^{2+} tolerance limits of ferrooxidant cells — and even tried to surpass them through adaptation for potential use in bioleaching processes — but kinetics were not an important goal; the main kinetic parameter used was complete oxidation time (CT). Despite the similar methods employed — batch cultures of the microbial species *Acidithiobacillus ferrooxidans* growing in 9K medium in stirred Erlenmeyer flasks — the results obtained for this parameter are very different and depend largely on whether adaptation processes were previously applied (table 1).

With these caveats in mind, there is some research with results similar to those reached in this paper. For example, in the paper by Hong-Mei Li and Jia-Jun Ke[30], time until complete Fe^{2+} bio-oxidation with 5 g/L of Cu^{2+} was 70 hours, compared to the 69 hours used for the same purpose in this research project. In the paper by Brahmaprakash et al[26], complete Fe^{2+} oxidation was reached after 84 hours with 5 g/L of Cu^{2+} and, with 10 g/L of Cu^{2+}, it took 8 days to fully oxidise the 9K medium, compared to the 7 days needed in this paper for the same purpose. However, other authors report very different findings to the ones in this paper, like those in the paper by Das et al[27], in which adaptation led to far more effective bio-oxidation. In this regard, other differences between the experiments carried out for this paper and those found in literature must be mentioned, namely initial pH and non-limiting oxygen conditions.

To use 9K medium, with a pH value above 2 — as do most cited papers[24-31] — means to accept that part of the Fe^{3+} generated during bio-oxidation will precipitate; Fe^{3+} precipitation is to be avoided in bioleaching applications as Fe^{3+} only acts as a leaching agent when it is dissolve. Modified 9K medium with lower pH by adding H_2SO_4 was chosen for this paper in order to avoid Fe^{3+} precipitation and obtain results that are closer to industrial situations.

The oxygen requirement of the cells is an essential operational variable to implement when the microbial population being studied is aerobic.[32] If limiting aeration conditions had occurred, the effect of Cu^{2+} on Fe^{2+} bio-oxidation could have been masked. In relation to this, it must be mentioned that oxygen solubility decreases as Cu^{2+} concentration increases because of the salting-out effect. [33]

Experiments were conducted to characterise oxygen transfer under conditions analogous to those used for the cultures. In addition, other stirring rates were assayed. Figure 3

shows measured transfer rates as a function of stirring rate across the full operational range of the orbital shaker employed (0-200 r.p.m.).

As figure 3 shows, oxygen transfer rate is directly proportional to stirring rate for most of the studied range. At the stirring rate assayed in the cultures, that is 180 rpm, oxygen transfer rate was 0.25 g/Lh. Taking into account that the average oxygen solubility under the studied conditions is 6.44 mg/L,[34] the value of $k_L{\cdot}a$ calculated with the equation (4) is 0.63 min^{-1}.

Using this value for $k_L{\cdot}a$, the maximum oxygen transfer rate MOTR was calculated for each of the bio-oxidation assays, having previously calculated C_L^* using the equation (5), and considering that MOTR implies that $C_L=0$. The results reached for C_L^* and MOTR are shown in table 2.

From the maximum Fe^{2+} bio-oxidation rates (MBR), the maximum oxygen uptake rate (MOUR) was determined using the reaction stoichiometry (equation 3). Table 2 shows the results calculated for MOTR and MOUR, revealing that MOTR was far greater than MOUR in all experiments. It can therefore be stated that in this preliminary study limiting oxygen conditions did not exist.

Continuous bio-oxidation.

Continuous bio-oxidation assays were carried out in a packed bed bioreactor with adhered biofilm.

Working volume and flow model.

Prior to continuous operation, the packed bed column was geometrically characterised. Its total volume was 824 mL, before adding the particles of packing material and air. This volume dropped 314 mL when the packing material was added. Finally, air was pumped at a flow of 750 mL/min; the bubbles occupied 31.7 mL. In other words, the volume taken up by liquid when operation started was 478.3 mL.

After continuous operation concluded, the bioreactor was drained, leaving a drained volume of 184.2 mL. It was then placed in an oven at 40ºC. After 600 hours its weight remained constant, leaving an evaporated volume of 178.5 mL. This means that, after continuous operation, the volume taken up by liquid was 362.7 mL.

The difference in volume occupied by liquid in the bioreactor between the start and the end of its continuous operation (115.6 mL) is attributed to the biofilm. The biofilm was made up of cells and accumulated ferric precipitates forming a continuous three dimensional structure.[35] (See in complementary data the evolution of the pH throughout the continuous operation)

Stirring of the liquid inside the bioreactor is caused by the bubbles of air, although the packing material limits this induced movement. In order to obtain information about the flow, assays were carried out to determine residence time distribution. Figure 4 shows the cumulative residence time distribution curve F(t). This curve's appearance suggests that flow inside the bioreactor might resemble the perfect mix model.[36] For this reason a least squares adjustment was performed. It can be seen in figure 4 that the experimental results fit the model, the calculated correlation coefficient being R^2 = 0.996, and mean residence time being 90.51 min.

Rate of bio-oxidation at different concentrations of Cu^{2+}.

After inoculation the bioreactor was operated by 700 hours. It was fed with an acidic solution of $FeSO_4$ and $CuSO_4$, the latter increasing its concentration stepwise[19]. Taking into account the results obtained in the batch bio-oxidation tests, the selected range of $[Cu^{2+}]$ was 0 to 20 g/L. Figure 5 shows the mean bio-oxidation rate plotted against $[Cu^{2+}]$. For each $[Cu^{2+}]$ studied steady state was reached; the operation was considered in steady state if the $[Fe^{2+}]$ in the outlet stream varies less than 5% with respect to the mean value for a time higher than mean residence time multiply by 50.

As can be seen in figure 5, Cu^{2+} also exerts a negative effect on continuous bio-oxidation. However, in relative terms, the influence of this effect is considerably smaller in continuous operation than in batch cultures (figure 5, table 2). Fe^{2+} bio-oxidation was not only possible when $[Cu^{2+}]$ = 20 g/L, but also the bio-oxidation rate suffered a less than 25% drop in this situation, while the maximum bio-oxidation rate dropped by about 50% in batch cultures with only 5 g/L of Cu^{2+}. It seems reasonable to state that cells inside the bioreactor have been adapted to Cu^{2+} concentration. Cell adaptation to Cu^{2+} is a phenomenon corroborated by several authors. [26, 27, 31]

Oxygen transfer

Figure 6 shows the values of C_L measured in the bioreactor and the values of C_L* calculated with equation 5.

It can be seen in figure 6 that C_L never was lower than 45% of C_L*. For this reason, it might be thought that the supply of oxygen to the bioreactor was sufficient to cover cellular demand. However, the cells are not suspended in the liquid medium, but rather adhered to the packing material, forming a biofilm. O_2 concentration on the surface of this biofilm could be lower than measured in the liquid phase.

After 526 and 651 hours of continuous operation, experiments were carried out in which the current of air was replaced with oxygen, [Cu^{2+}] being 15 and 20 g/L respectively. Figure 7 shows how Fe^{2+} concentration and instantaneous bio-oxidation rate Q_{Fe} evolved over time during the transient state, as calculated by the corresponding substrate continuity equation for Fe^{2+}:

$$M_e - M_s - Q_{Fe} = \frac{dm}{dt} \qquad (8)$$

Where:

$\frac{dm}{dt}$ is the rate of accumulation of Fe^{2+} inside the reactor (accumulation term) calculated with the following formula:

$$\frac{dm}{dt} = \frac{d[Fe(II)]_{salida} V}{dt} \qquad (9)$$

where V is the volume of liquid.

M_e and M_s are the mass flow rates of Fe^{2+} entering and leaving the bioreactor.

It can be observed in figure 7 that, at both the tested [Cu^{2+}], the instantaneous bio-oxidation rate rose suddenly when oxygen replaced air. Subsequently, the bio-oxidation rate gradually dropped, which can be attributed to gradual Fe^{2+} depletion as this substrate becomes limiting, dropping below 1 g/L.

According to Henry's law, the bioreactor's yield should have grown by a factor of 5 if there were sufficient active biomass inside it. For [Cu^{2+}] of 15 and 20 g/L in the feed, the average yield during continuous operation with air was 2.11 g/h and 1.96 g/h respectively, while the maximum instantaneous bio-oxidation rates reached with pure oxygen were 6.60 and 6.15 respectively. That is to say, bio-oxidation rates triple their value when air is replaced with O_2.

This response of the bioreactor reveals that during continuous operation, when air was injected, the oxygen transfer rate was insufficient to meet demand by the cells. It can therefore be stated that, during continuous operation, the oxygen concentration surrounding the biofilm must be lower than in the midst of the liquid phase.

It is possible to calculate the rate of oxygen demand by the cells if the bio-oxidation stoichiometry (equation 3) and the rate of Fe^{2+} bio-oxidation (figure 5) are known. In steady state conditions, this must necessarily be equal to the rate of oxygen transfer from the gaseous to the liquid phase. It is possible to solve (Eq. 4) for $k_L{\cdot}a$ if C_L and C_L* are known. Figure 8 shows the mean values of $k_L{\cdot}a$ calculated this way from the results shown in figures 5 and 6 during continuous operation.

It can be observed in figure 8 that the values calculated for $k_L{\cdot}a$ display no trend; the mean value is *0.028* s^{-1}, with a standard deviation of 0.0026 s^{-1}. This result is consistent with the assayed flow conditions, invariable throughout continuous operation.

Once O_2 is in the liquid phase, it must travel to the vicinity of the particles of packing material, on whose surface the biofilm is adhered. Akin to the mathematical treatment used for gas-liquid transfer, there can be considered to be a boundary layer surrounding the packing material, across which O_2 is transferred at a rate given by the integrated expression of Fick's law:

$$Q_O = k_S \cdot s \cdot (C_L - C_{LS}) \qquad (10)$$

Where k_S is the mass transfer coefficient for O_2 transfer across the boundary layer surrounding the packing material, *s* is the specific surface area of the packing material and C_{LS} is the concentration of O_2 on the surface of the packing material.

Oxygen transfer rate surrounding the biofilm will be highest when $C_{LS}=0$. Assuming O_2 is depleted, based on the limiting effect of oxygen discussed earlier, $k_S{\cdot}$ s can be estimated if

C_L (figure 6), the bio-oxidation rate (figure 5) and the bio-oxidation stoichiometry (equation 3) are known. As occurred with the results given for $k_L \cdot a$, values for $k_S \cdot$ s show no trend for similar reasons; the mean value is *0.023* s^{-1}, with a standard deviation of *0.0027* s^{-1}. The real mean value must necessarily be equal or lower, depending on the value of C_{LS}. However, given the similarity between the calculated average values of $k_L \cdot a$ and $k_S \cdot s$, it can be postulated that resistance to O_2 transfer from the gaseous to the liquid phase must be of the same order of magnitude as that surrounding the biofilm.

Under these limiting oxygen conditions, the saturation concentration for oxygen C_L^* critically affects the bio-oxidation rate. C_L^* decreases as $[Cu^{2+}]$ in feed rises because of the salting-out effect (figure 6). The average values for the percentage drop in C_L^* due to Cu^{2+} being present calculated from the results shown in figure 6, for $[Cu^{2+}]$ 0, 5, 10, 15 y 20 g/L respectively, are the following: 0, 1.73, 3.94, 6.30, 8.18. Therefore, assuming O_2 is depleted on the surface of the particles of packing material, the drop in C_L^* due to Cu^{2+} being present would account for a drop in the bio-oxidation rate of as much as 35% regarding the bio-oxidation rate achieved when Cu^{2+} is absent. In other words, it must be understood that, in addition to the biotic effect, part of the effect Cu^{2+} has on bio-oxidation is thermodynamic (salting out effect).

Reversibility of the inhibitory effect associated to Cu^{2+}.

Obviously, this physical effect on bio-oxidation can be reversed once $[Cu^{2+}] = 0$ in the feed, but it remains unknown whether the biological effect is equally reversible. So as to answer this question, an experiment consisting in suddenly removing Cu^{2+} from the feed was carried out; this was done at the end of the period when continuous bio-oxidation happened with 20 g/L of $[Cu^{2+}]$. Figure 9 shows the concentrations of Fe^{2+} measured in the effluent and the bio-oxidation rate during the transition stage.

In figure 9, it can be seen that removing Cu^{2+} from the feed is matched by an immediate and gradual drop in Fe^{2+} concentration that ends with an asymptote. As a result, the bio-oxidation rate rose and, in under 60 hours, even surpassed the initial bio-oxidation rate, when [Cu^{2+}] = 0 g/L. This result reveals that the inhibitory effect associated with Cu^{2+} is reversible, and that the biofilm was even capable of growth during this time.

CONCLUSIONS

It is possible to achieve the adaptation of ferrooxidant cells to tolerate as much as 20 g/L Cu^{2+} in continuous operation. In this situation, the bio-oxidation rate drops almost 25% with respect to when Cu^{2+} is absent. Close to 10% of this decrease can be attributed to lower O_2 solubility because of the salting-out effect. However, the bio-oxidation rate can be increased three fold if the feed of air is replaced with pure oxygen. The effect of Cu^{2+} on continuous Fe^{2+} bio-oxidation is reversible.

The fact that bio-oxidation inhibition by Cu^{2+} is not exclusively caused by biological factors, and that the kinetic delay is partly due to thermodynamic reasons offers the prospect of improving bioreactor design in the field of bioleaching applications, which contributes to a more versatile and competitive view of this clean technology.

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Table 1: Results found in literature for complete bio-oxidation time and those reached in this paper.

Reference	$[Fe^{2+}]_0$ (g/L)	microorganism	Stirring rates (rpm)	adaptation	$[Cu^{2+}]$ (g/L)	CT (h)
[31]	2	*A. ferrooxidans*	200	No	5	160
				Yes		75
[28]	5.6	*A. ferrooxidans* *L. ferrooxidans*	90	No	5	40
[26]	8.8	*A. ferrooxidans*	240	No	5	84
					10	192
[27]	8.8	*A. ferrooxidans*	240	Yes	5	44
					10	78
[30]	10	*A. ferrooxidans*	140	Yes	5	70
					10	120
This work	8.8	*A. ferrooxidans* *L. ferrooxidans*	180	No	5	69
					10	165

Table 2: Bio-oxidation assays with Cu^{2+} concentrations of 0, 5, 10 and 15 g/L. Modified 9K medium at pH 1.25. Agitation at 180 rpm. 30ºC temperature.

$[Cu^{2+}]$ (g/L)	MBR (g/Lh)	MOUR (g/Lh)	MOTR (g/Lh)	MOTR/MOUR	C_L^* (mg/L)
0	0.184	0.026	0.237	9	6.22
5	0.093	0.013	0.232	17	6.09
10	0.057	0.008	0.226	28	5.96
15	0.031	0.004	0.222	49	5.82

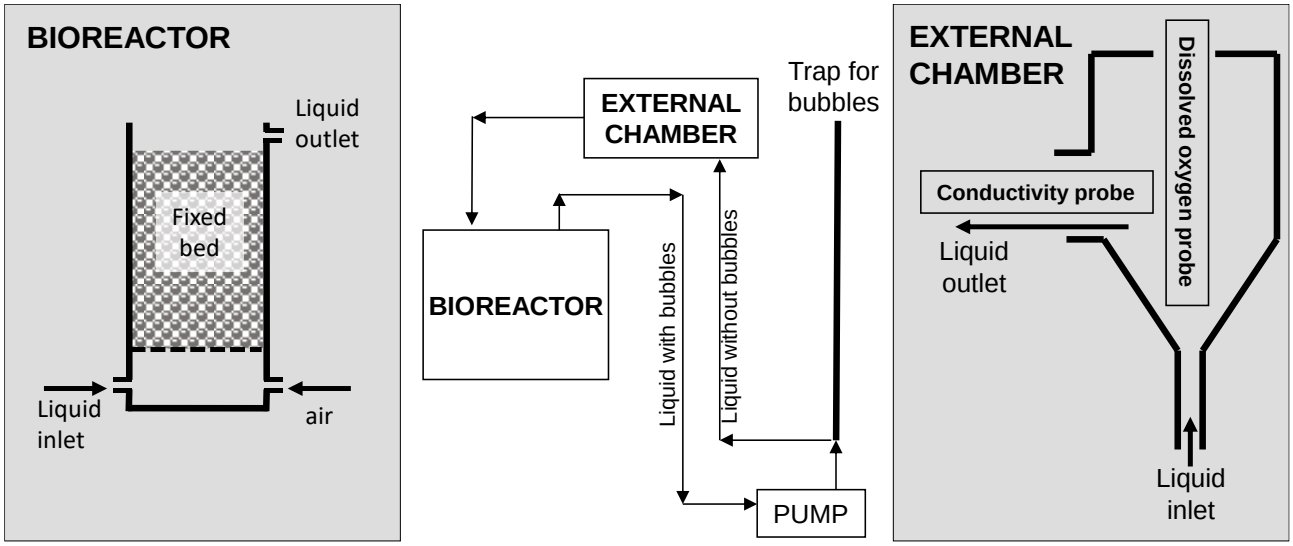

Figure 1: Experimental device for measuring dissolved oxygen concentration on line.

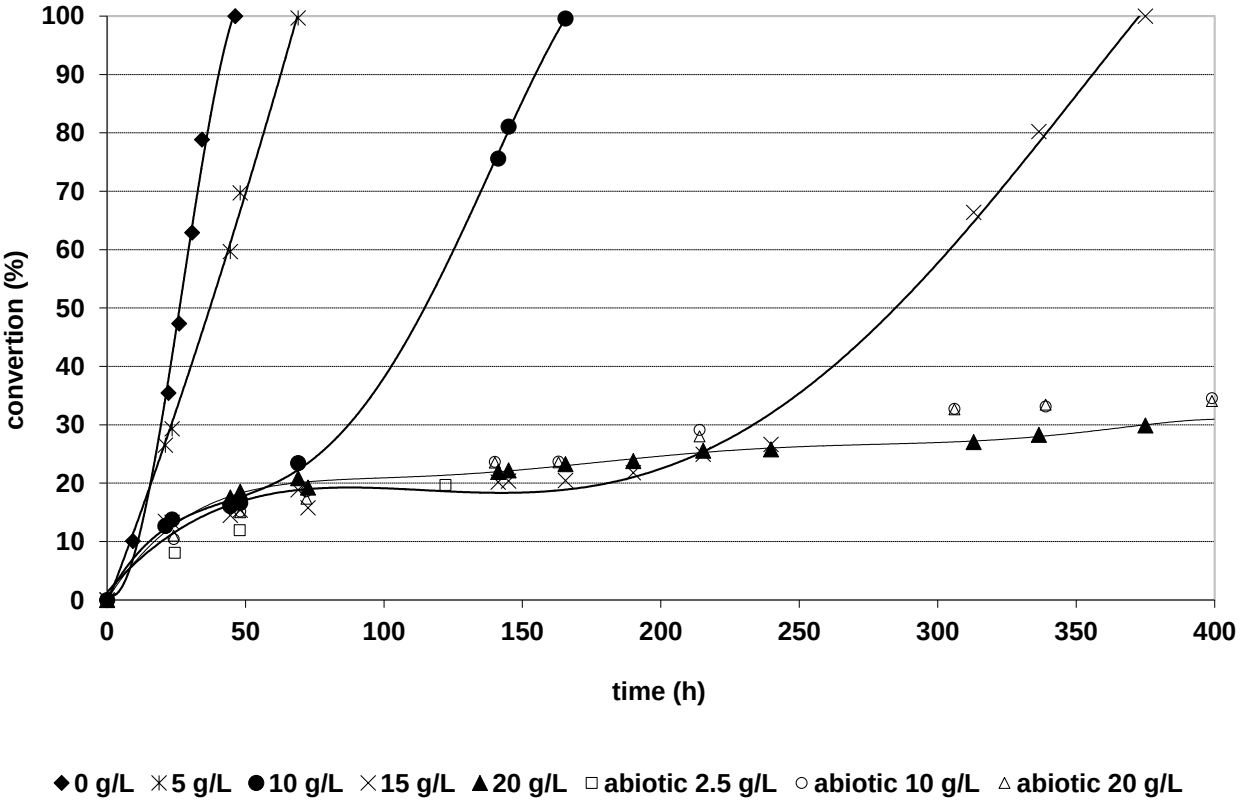

Figure 2: Batch Fe^{2+} bio-oxidation with 0, 5, 10, 15 and 20 g/L of Cu^{2+}, and abiotic Fe^{2+} oxidation with 0, 2.5, 10 and 20 g/L of Cu^{2+}. Modified 9K medium at pH 1.25. Agitation at 180 rpm. 30 ºC Temperature.

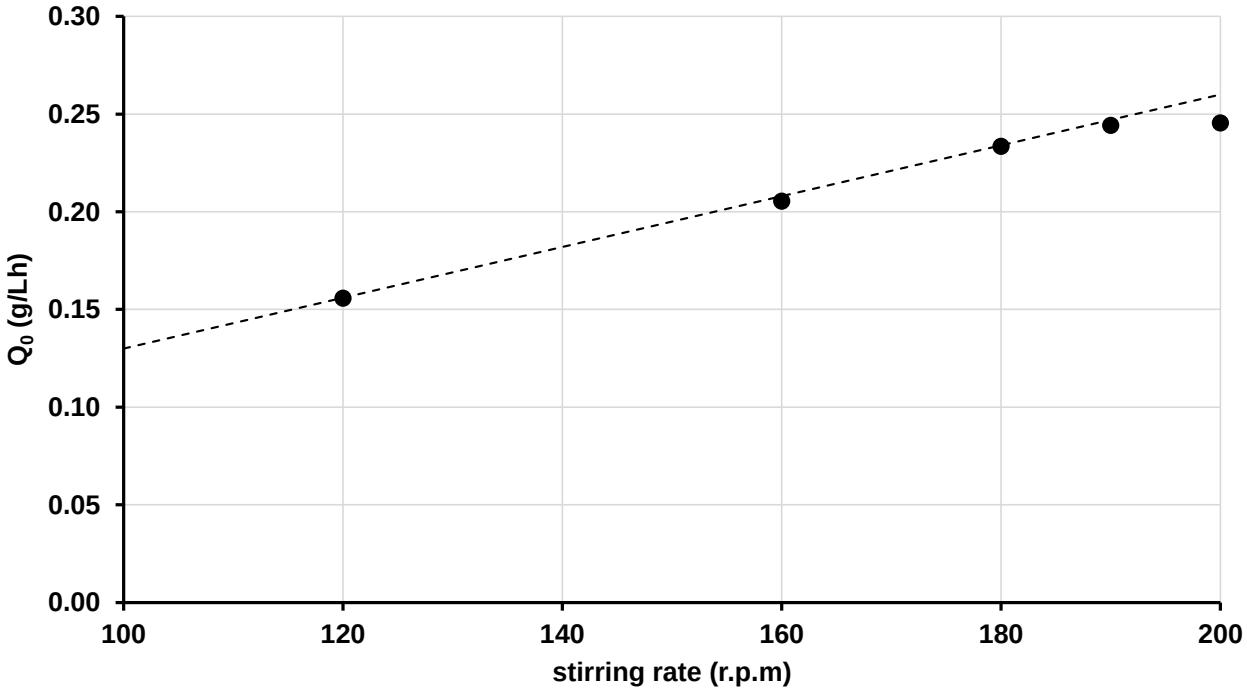

Figure 3: Oxygen transfer rate (Q_0) in 250 mL Erlenmeyer flasks at different stirring rates measured using the sulphite method; temperature: 30 ºC.

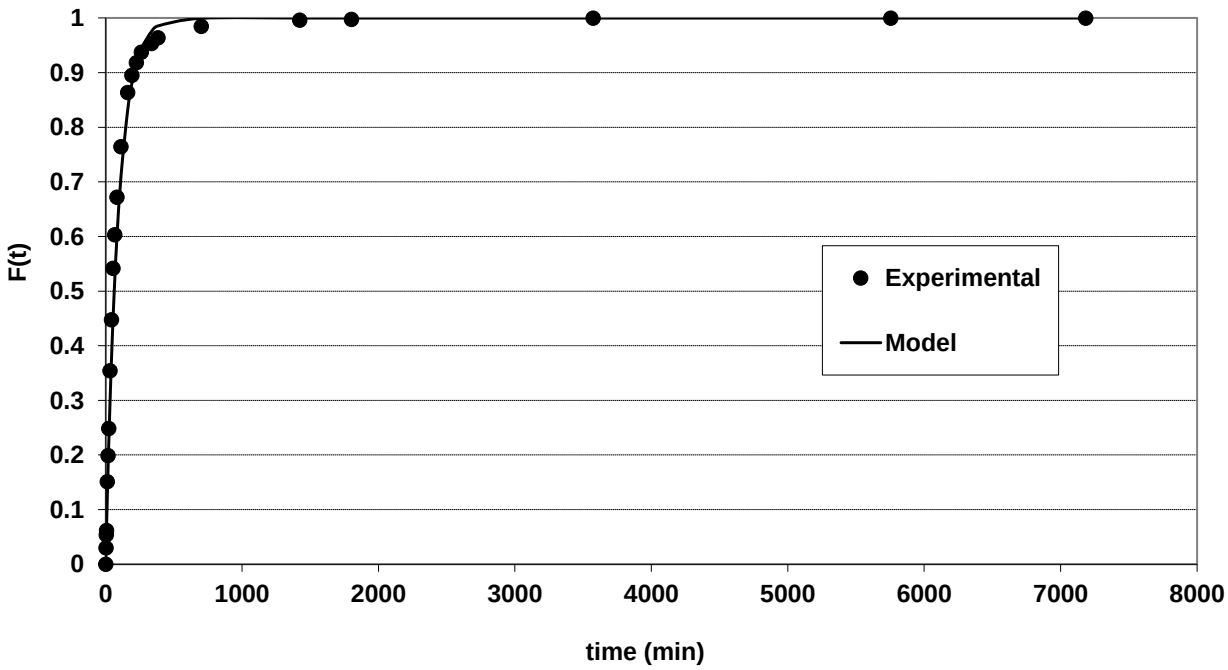

Figure 4: Cumulative residence time distribution function F(t). Liquid flow rate = 240 mL/h, air flow rate = 750 mL/h. Negative step input signal. Cu^{2+} as tracer, at a concentration of 20.2 g/L. Perfect Mix Model.[36]

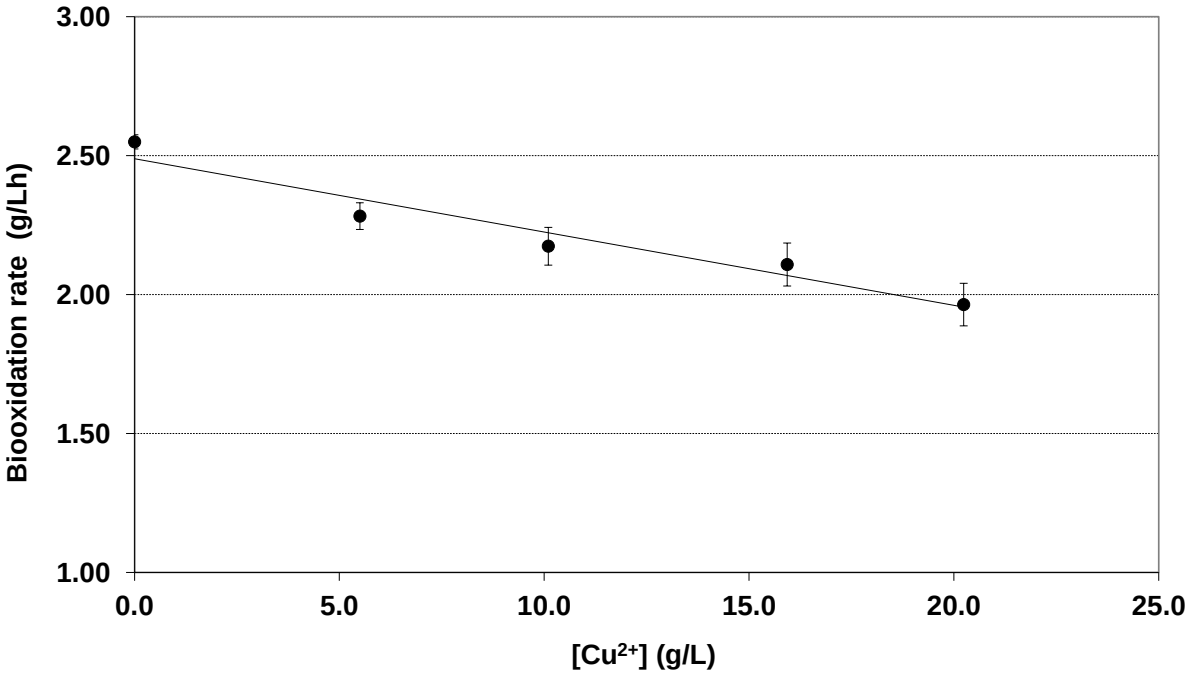

Figure 5: Continuous operation. Mean Fe^{2+} bio-oxidation rate against $[Cu^{2+}]$ in feed, within the range 0 to 20 g/L. Feed: $[Fe^{2+}]$ = 7 g/L pH = 1.5. Average liquid feed flow: 240 mL/h. Air flow: 750 mL/h. Temperature: 30ºC. Operating time: 700 h. Error bars represent the standard deviation.

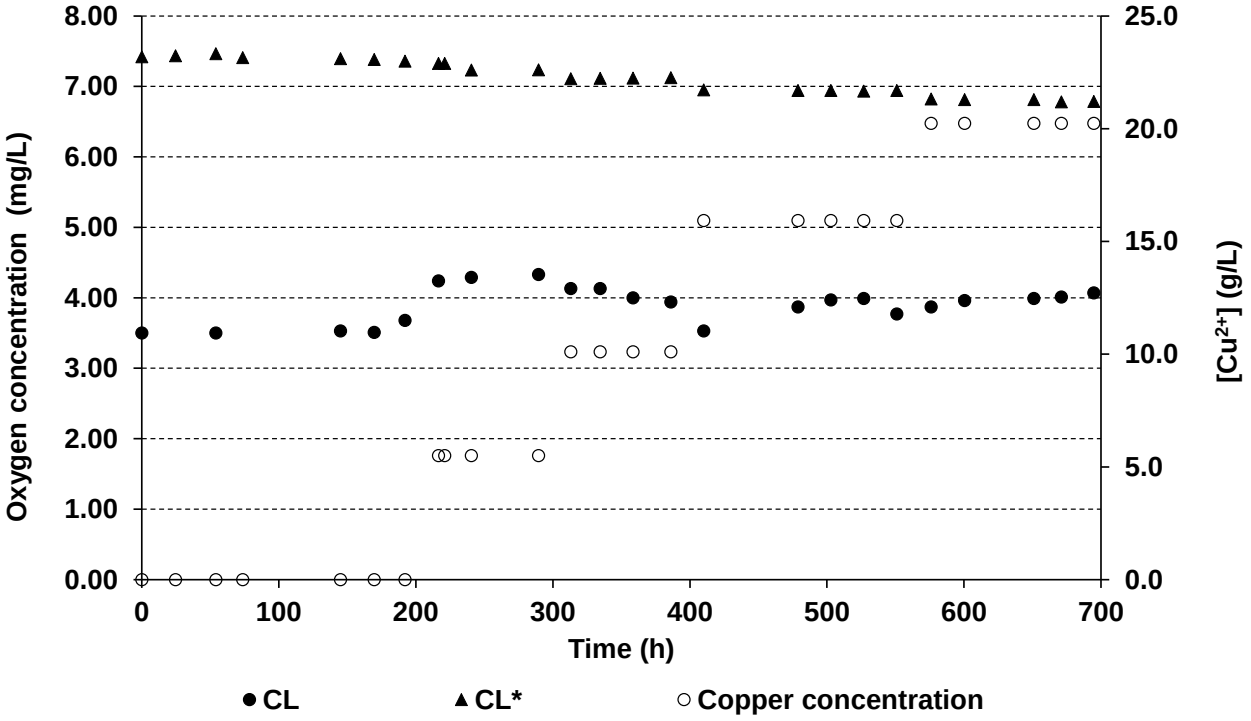

Figure 6: Continuous operation. C_L y C_L* against time for different [Cu^{2+}] in feed, within the range 0 to 20 g/L. Feed: [Fe^{2+}] = 7 g/L pH = 1.5. Average liquid feed flow: 240 mL/h. Air flow: 750 mL/h. Temperature: 30ºC.

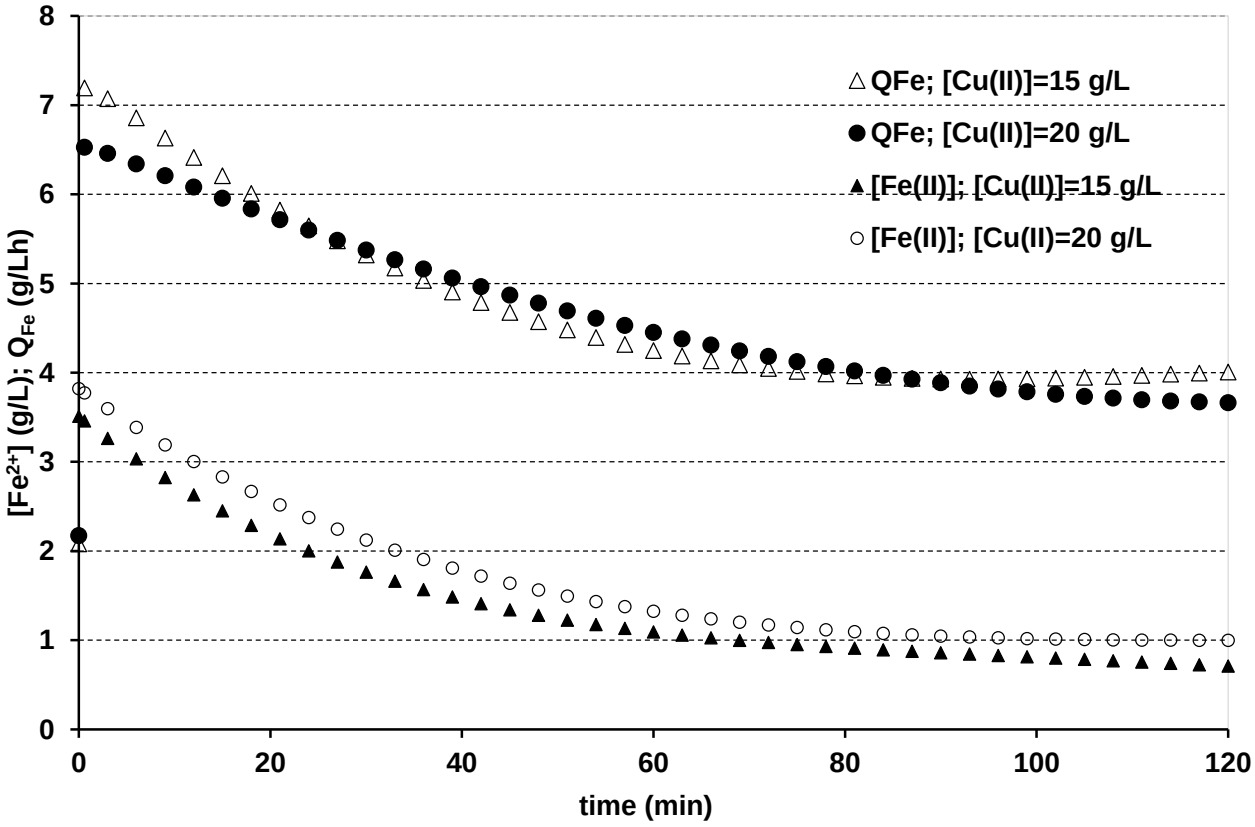

Figure 7: Results for $[Fe^{2+}]$ and bio-oxidation rate Q_{Fe} when the current of air was replaced with a current of oxygen, $[Cu^{2+}]$ in feed being 15 and 20 g/L.

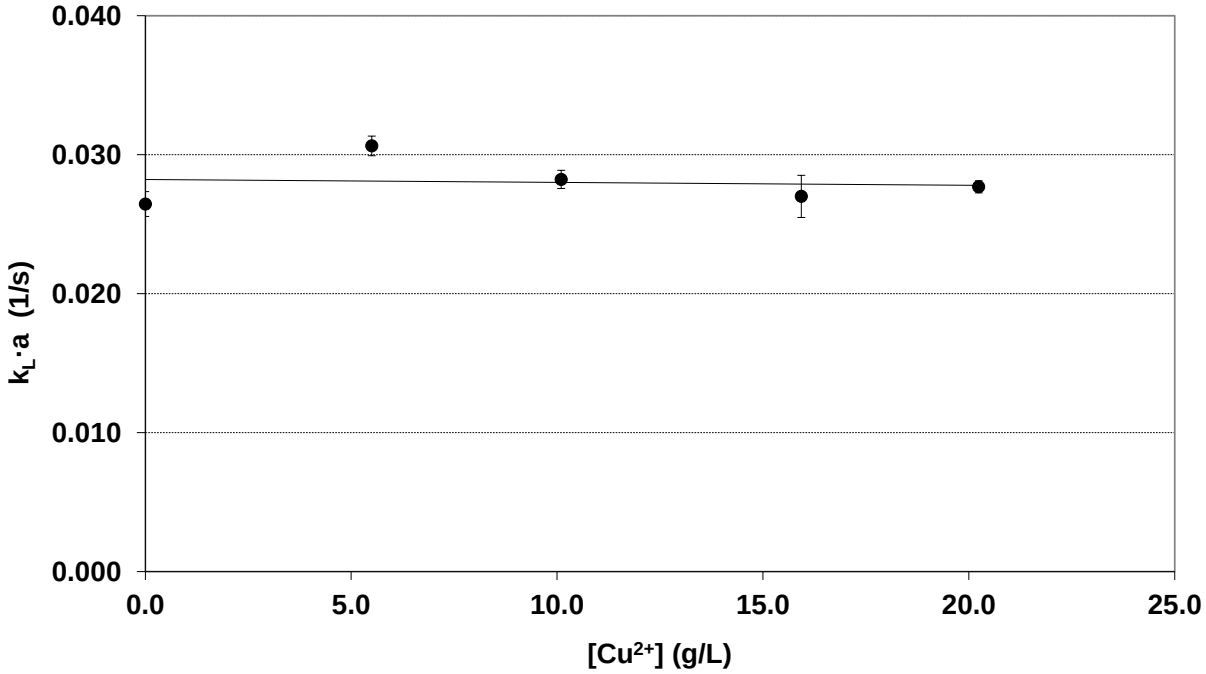

Figure 8: $k_L \cdot a$ versus [Cu^{2+}] in feed within the range 0 a 20 g/L. Feed: [Fe^{2+}] = 7 g/L pH = 1.5. Average liquid feed flow: 240 mL/h. Air flow: 750 mL/h. Temperature: 30ºC. Error bars represent the standard deviation.

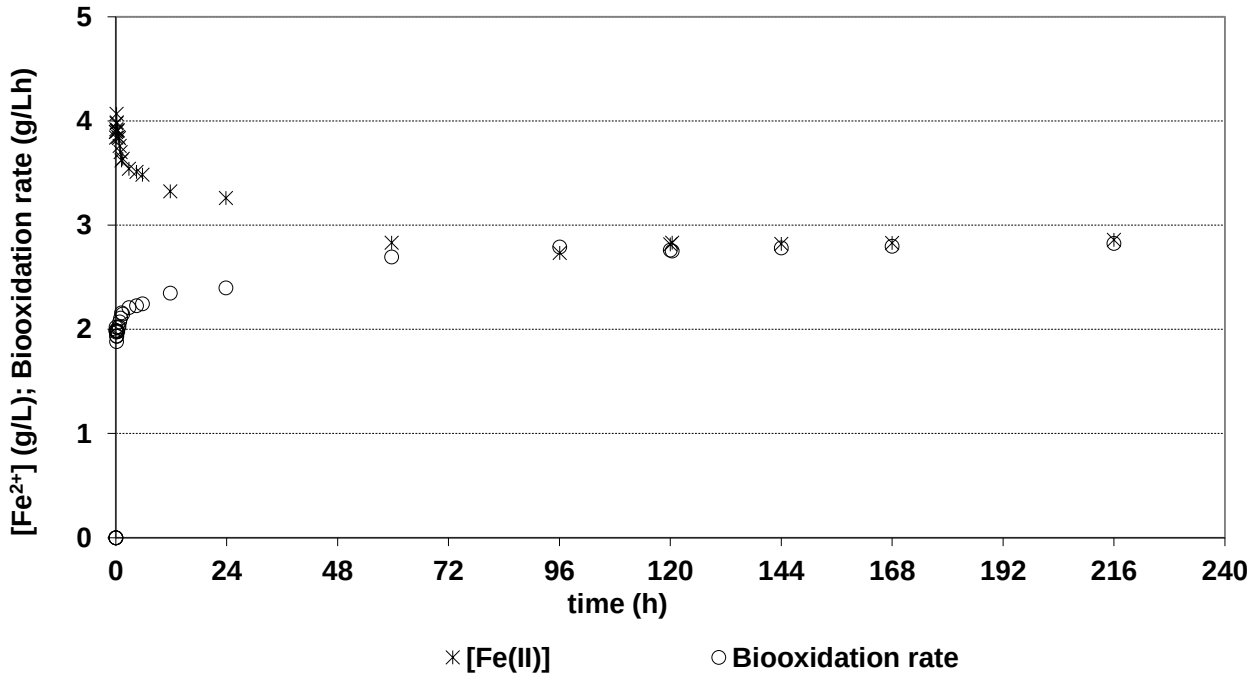

Figure 9: Results for [Fe^{2+}] and bio-oxidation rate shown against time after removing 20 g/L of Cu^{2+} from the feed.