INFLUENCE OF ZINC ON FERROUS IRON BIOOXIDATION: BIOLOGICAL OR PHYSICAL NATURE?

Alfonso Mazuelos*, Nieves Iglesias, Rafael Romero, Miguel Ángel Mejías, Francisco Carranza

Department of Chemical Engineering of the University of Seville (Spain)

Address:

Departamento de Ingeniería Química de la Universidad de Sevilla

c/ Profesor García González, Edificio Química

41012 Sevilla

Spain

* Corresponding author: A. Mazuelos

e-mail: mazuelos@us.es

fax: +34 954 55 64 47

Abstract

Ferrous iron bio-oxidation is negatively affected by the presence of heavy metals. Although the available information relates this phenomenon to purely biological aspects, it is contradictory with respect to tolerance levels, mechanisms and kinetics. This dispersion of results may be due to the empirical nature of the approaches which are based on batch cultures and fail to consider the conditions of aeration of the biomass.

In the present work, the influence of Zn^{2+} in the range of 0 to 40 g/L is tested in continuous packed-bed bioreactors, by studying oxygen partial pressure and aeration flow rate as variables. Results show that when oxygen is the limiting reagent under identical aeration conditions, the bio-oxidation rate decreases by 0.8% per gram per litre of Zn^{2+}. The cause of this result is purely thermodynamic; the solubility of oxygen in the medium decreases in equivalent proportions of the bio-oxidation rate owing to the salting-out effect.

This finding leads to redesign of reactors for continuous ferrous iron bio-oxidation with the presence of Zn^{2+}, whereby special attention is paid to the aeration system and its control during the operation.

Keywords: bio-oxidation, ferrous iron, bioreactor system, oxygen solubility, zinc tolerance, immobilized cells, mass transfer, biofilm.

1. INTRODUCTION.

The bio-oxidation of ferrous to ferric iron, catalysed by microorganisms is based on the reaction:

$$Fe^{2+}_{(aq)} + \frac{1}{4}O_{2\,(g)} + H^{+}_{(aq)} \xrightarrow{microorganisms_{(S)}} Fe^{3+}_{(aq)} + \frac{1}{2}H_2O_{(l)}$$

It is a heterogeneous process in which both the catalyst and the reagents are in different phases, and, therefore not only is it influenced by biochemical factors by also the physical phenomena of mass transfer.

There are many publications on the kinetics of microbial ferrous-iron oxidation, mainly related to the use of *Acidithiobacillus ferrooxidans* and, to a lesser extent, *Leptospirillum ferroxidans* and *Leptospirillum ferriphilum* [1, 2]. Literature relating to the effect of dissolved metals is less abundant and, in some cases, contradictory.

Dissolved metals slow down the bio-oxidation kinetics. This effect has been studied in solutions containing varying concentrations of metallic ions such as Cr^{3+}, Cu^{2+}, Cd^{2+}, Co^{2+}, As^{3+}, Ag^{+}, Zn^{2+}, Ni^{2+}, Al^{3+}, Mg^{2+}, Na^{+}, K^{+}, Pb^{2+}, Sn^{2+}, Mn^{2+}, $Hg^{2+,}$ UO_2^{2+} and Th^{2+}. According to their impact on the process these metals could be classified into two groups:

- Toxic metals, those that strongly inhibit cell growth and could be lethal to microorganisms in concentrations in the order of mg/L, such is the case of Ag, Hg, U and Th. These metals are toxic because they inhibit the cell metabolic activity [3-5].
- Tolerated metals, which are those that are tolerated by cells but impose an inhibitory effect on the cell.

Regarding the latter group, the information in the literature is sparse. The extent and mechanism of inhibition (competitive, non-competitive and uncompetitive inhibition) remain unclear, as does the tolerance of microorganisms to these metals. In addition to

the variability of strains used as inoculum and of the type of bioreactor employed to carry out exploratory cultures, this inconsistency in the information may have originated from the following reasons:

First, most studies related to the influence of heavy metals on biooxidation rate are based on batch cultures [3-14]. Boom et al. [15, 16] analyzed the advisability of examining the kinetic of ferrous iron biooxidation in batch and continuous cultures and concluded that batch culture experiments were inappropriate for the attainment of testable results, due to the impossibility of achieving steady states which allow the effect of any variable of composition on the cells to be observed in a reasonable time. It is inherent to batch culture systems that cells from inoculation are always subject to changing conditions.

In addition, if any variable related to the composition is changed, a shorter or longer lag phase shows that the cells are not balanced to stimuli in their environment and need to reorganize their metabolic functions. In studies cited above the lag phases in many cases are much longer than the periods of growth. These disadvantages are overcome with continuous systems, in which the attainment of steady states is possible.

Second, the aforementioned authors based their studies on tests that fail to take into account the oxygenation conditions of culture (partial pressure of oxygen in the gas, gas-liquid transfer area or flow aeration), whereby these conditions are diverse. In their studies, only as sensitive variables Fe^{2+} and inhibitor metal concentrations are taken. The limitations of oxygenation in bio-oxidation processes is shown to be a proven fact by many authors, these limitations markedly influence when the cells are immobilized on a solid support [17-23]. This is essentially a physical limitation of a fluid-dynamic and thermodynamic nature. The fluid-dynamic aspects are related to the contribution of the convective and molecular mechanisms and to the gas-liquid interfacial area

generation. From the standpoint of thermodynamics, it should be noted that the solubility of oxygen in aqueous saline solutions, such as nutrient media, in contact with air at atmospheric pressure and temperature around 30ºC, is less than 8 ppm, and is further reduced as the concentration of ionic species in the medium is increased [24, 25]. These oxygen concentrations are much lower than the concentrations to which ferrous iron is usually fed in bio-oxidation tests, in the order of g/L. Hence, confusion can be expected in the identification of the limiting reagent.

It has been demonstrated that the ionic strength is a significant variable to be considered when bio-oxidation is studied quantitatively. In this context, the effect of Al^{3+}, K^{+}, SO_4^{2-}, NO_3^{-}, Na^{+} and Cl^{-} on the medium has been reported [23-29]. It has been postulated that the increase of ionic strength can result in a decrease in the concentration of the dissolved gases O_2 and CO_2, and in an increase of energy required to cope with the high ionic strength, for example, in order to counteract the osmotic pressure exerted on the cell by the surrounding solution [27, 28].

In the present work the influence of Zn^{2+} in the range of 0 to 40 g/L is tested in continuous packed-bed bioreactors, by studying oxygen partial pressure and aeration flow rate as variables. For fixed aeration conditions, the effect of this metal on the bio-oxidation is correlated with the decrease of oxygen solubility owing to the salting-out effect.

Literature points out, amongst others, the potential application of ferrous iron bio-oxidation in the regeneration of ferric iron in hydrometallurgical processes based on the indirect bioleaching of sulphide metallic ores [30-32]. A commercial bioleaching plant for the treatment of Zn concentrates will generate liquors with concentrations of Zn^{2+} in the range of 20 to 40 g/L, by taking into account the Zn grade in concentrates and a pulp density between 10-15% w/w in leaching reactors [33].

Highest yields for bio-oxidation have been achieved in continuous flooded packed-bed bioreactors (3,500 g/h·m^2 for a 1-metre-hight bed) [22]. In these reactors, the microorganisms are attached to the bed by forming a biofilm, which allows the accumulation of high cell concentrations.

The aim of the present paper is to study the influence of zinc in ferrous iron bio-oxidation in order to understand its nature with the intention of improving the design of the bioreactor for hydrometallurgy applications.

2. MATERIALS AND METHODS

2.1. Inoculum

A mixed culture obtained from Riotinto Mine acid drainage waters was used as inoculum. It mainly consists of autotrophic bacteria such as *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*, and some heterotrophs (*Ferrimicrobium spp.* and A*cidiphilium spp*). Identification of bacterial microorganisms was done by a molecular culture-independent method [34]. Briefly, DNA was purified from the sample and used in PCR with 16S rRNA primers. Amplicon was cloned and sequenced, and the obtained nucleotide sequence compared against databases.

The culture is routinely maintained on a modified Silverman and Lungren 9K nutrient medium at pH 1.25 (adjusted with concentrated H_2SO_4).

In discontinuous tests, with neither forced aeration nor agitation, the specific generation of ferric iron rate is 0.055h^{-1}.

2.2. Batch biooxidation preliminary assays

In order to study the tolerance and the adaptation of the inoculum to Zn^{2+}, three series of consecutive static cultures were carried out (Zn^{2+} concentration 10, 20 and 30 g/L). These cultures were conducted in 250 mL Erlenmeyer flasks containing 80 mL of 9K

nutrient medium at pH 1.25. $ZnSO_4$ was added in the necessary amount to reach Zn^{2+} concentrations of 10, 20 and 30 g/L, depending on the case. 20 mL of the last culture grown in the same series were added as inoculum. The temperature was set at 31ºC. Ferrous iron concentration was measured during the culture growth as function of time. Specific biooxidation rate was calculated as the maximum slope in the plot $Ln[Fe^{3+}]$ against time.

2.3. Continuous biooxidation assays

Continuous biooxidation assays were carried out in two flooded packed bed bioreactors, named bioreactor A and bioreactor B. This kind of bioreactor consists of a column packed with inert solid particles with inlets for liquid and gas at the bottom [22]. The liquid stream, carrying ferrous iron, and gas, bearing oxygen, are continuously fed, rising through the bed and filling all the hollow space (flooding the bed).

Both bioreactors, A and B, had a diameter of 4.2 cm and different heights: bioreactor A 10 cm and bioreactor B 30 cm. In both cases the bed consisted of siliceous stone particles, between 6 and 8 mm in size and randomly packed. The porosity of the bed was 0.42. These particles are the biomass support that forms a biofilm. The biofilm formation process was performed as described in Mazuelos et al [35].

Air was supplied under a pressure of 0.5 bar and through a 1 mm nozzle. Air flow rate was controlled using a standard glass tube flow-meter P/N 10420 Cole Palmer calibrated to air (accuracy ± 7 cm^3min^{-1}).

The liquid stream consisting of an aqueous solution of $FeSO_4$ and $ZnSO_4$, adjusted to pH 1.5 using concentrated H_2SO_4, was fed to the reactor by a peristaltic pump through an 8 mm hole. The liquid flushed out by overflow. The liquid flow-rate was measured in the outlet stream with a 250-mL probe during two hours (standard deviation: 2.2 mL/h).

The bioreactors were placed in a thermostatically controlled room, maintained at 31ºC.

Both bioreactor operations can reach steady states. The operation was considered in steady state if the ferrous iron concentration 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.

Zn^{2+} influence

The study of Zn^{2+} influence was performed running bioreactor A. The gaseous stream feed was always air, with selected flow rates of 250 or 500 mL/min. Liquid was fed at 88 mL/h, with a Fe^{2+} concentration of 7 g/L.

These assays were carried out in two consecutive stages:

Stage 1: with 250 mL/min air flow rate, Zn^{2+} concentration was gradually increased from 0 to 40 g/L in liquid feed.

Stage 2: with 500 mL/min air flow rate, Zn^{2+} concentration was gradually decreased from 40 to 0 g/L in liquid feed.

During the test, changes in Zn^{2+} concentration and air flow rate were performed once the steady state was reached.

Oxygen partial pressure influence

To study the influence of the partial pressure of oxygen in the fed gas, bioreactor B was used. The gas fed to the reactor during the test was sometimes air and other times pure oxygen. When feeding pure oxygen, CO_2 was bubbled into the liquid before putting it into the bioreactor, since this gas is necessary for autotrophic bacterial growth. The gas flow rate (air or pure oxygen) was 600 mL/min.

In order to prevent ferrous iron from being the limiting reactant in the biooxidation process, its concentration in the outlet solution was higher than 12 g/L in each steady state. This requires the liquid flow rate to be modified in the range 89-419 mL/h and the

ferrous iron concentration to be kept about 22 g/L. These tests were performed without Zn^{2+} and with a Zn^{2+} concentration of 23 g/L.

Transient state by change in oxygen partial pressure in the fed gas

In bioreactor B, the transient state caused by replacing the air stream fed with pure oxygen keeping the other variables constant was studied. The evolution of ferrous iron concentration in the outflow regarding time was followed.

This reactor was initially operating under steady state. In this steady state Fe(II) and Zn(II) concentration in feed were of 21.1 g/L and 23.0 g/L, respectively, and flow rates were 89 mL/h for liquid and 600 mL/min for air. In these conditions, the liquid volume measured in bioreactor B was 0.26 L.

2.4. Fe^{2+} and Zn^{2+} analysis

Fe^{2+} concentration was determined by standard potassium dichromate solution in an automatic titrator with 0.05N $K_2Cr_2O_7$ (accuracy ± 3mg/L). Zn^{2+} concentration was determined by AAS (accuracy ± 0.01 mg/L).

2.5. Models for estimating oxygen solubility in electrolyte solutions.

Narita et al. [24] and Tromans [25] present models for estimating oxygen solubility in electrolyte solutions.

The Narita et al equation is the following:

$$\log\left(\frac{S}{S_0}\right) = -\sum K_i \cdot C_i \qquad (1)$$

where:

S: the solubility of O_2 in the electrolyte solution.

S_0 : the solubility of O_2 in pure water.

K_i : the "solubility constant" for an electrolyte *i* (empiric value).

C_i : molarity of species *i*.

K_i is 0.108 for Zn^{2+}, 0.121 for SO_4^{2-} and 0.069 for HSO_4^-.

At 25ºC the thermodynamic equilibrium relationship between SO_4^{2-} and HSO_4^- species concentrations is given by the following equation:

$$\log\left(\frac{[SO_4^{2-}]}{[HSO_4^-]}\right) = pH - 1.91 \qquad (2)$$

Tromans' equation is:

$$s_i = \phi \cdot s_{aq} \qquad (3)$$

$$\phi = \left[1 + \kappa \cdot c_i^{\,y}\right]^{-h} \qquad (4)$$

where:

s_i : the molal solubility of O_2 in the presence of ionic species *i*.

s_{aq} : the molal solubility of O_2 in pure water.

c_i : the molal concentration of the ionic species *i*.

κ, *y* and *h* are empiric parameters for each electrolyte.

For $ZnSO_4$, κ=0.233, *y* =1.01 and *h*=2.656.

When there are more ionic species in water,

$$\frac{s_{aq}}{P_{O_2}} = k \cdot \phi_1 \left(\prod_2^z \phi_i\right)^n \qquad (5)$$

where,

P_{O_2} : the oxygen partial pressure (atm).

k : the solubility constant for oxygen in pure water ($mol \cdot kg^{-1} \cdot atm^{-1}$).

ϕ_i : the value of ϕ for the ionic species *i*.

ϕ_1 : the lowest value of ϕ_i.

n : an empirical exponent, 1>n>0, that has been shown to have a likely value near 0.8.

3. RESULTS AND DISCUSSION

3.1. Batch biooxidation tests

The percentage of oxidized ferrous iron versus time in batch biooxidation tests for the first and the last culture of each series is plotted on figure 1. For each Zn^{2+} concentration similar results were observed regardless the serial number of re-inoculation. The average values of specific biooxidation rate (standard deviation in brackets) were 0.042 (0.002), 0.033 (0.001) and 0.019 (0.002) h^{-1} at 10, 20 and 30 g/L of Zn^{2+} concentration respectively.

Therefore it can be said that the inoculum tolerates and has been adapted to each tested Zn^{2+} concentration and that there is an inverse relationship between biooxidation kinetic and Zn^{2+} concentration.

3.2. Influence of Zn^{2+} on continuous biooxidation

Figure 2 shows continuous ferrous iron biooxidation rates (Q_{biox}) that were obtained when Zn^{2+} concentration in feed was varied between 0 and 40 g/L, for the two flow rates tested (250 or 500 mL/min).

Q_{biox} is calculated from the following equation:

$$Q_{biox} = flow\,rate \cdot \left([Fe^{2+}]_i - [Fe^{2+}]_e \right) \qquad (6)$$

Where,

$[Fe^{2+}]_i$: Fe^{2+} concentration in feed (g/L).

$[Fe^{2+}]_e$: Fe^{2+} concentration leaving bioreactor (g/L).

In figure 2 two almost parallel straight lines with negative slopes are observed, being the highest y-intercept the one belonging to the highest air flow rate.

This means that:

- Zn^{2+} negatively affects the biooxidation process, its influence being proportionally higher the higher its concentration.
- For each Zn^{2+} concentration, increases in air flow rate results in a quantitative increase in the ferrous-iron biooxidation rate.

For each air flow rate tested, a function that relates biooxidation rate with Zn^{2+} concentration can be fitted:

- For air flow rate 500 mL/min: $Q_{biox} = -0.0044 \cdot [Zn^{2+}] + 0.6082$ (7)
- For air flow rate 250 mL/min: $Q_{biox} = -0.0038 \cdot [Zn^{2+}] + 0.4967$ (8)

The effect of air flow rate on biooxidation shows that, in the tested conditions, the oxygen transfer from the air bubbles to the biomass has an important influence on the biooxidation process, in spite of the fact that the amount of oxygen fed is very much higher than the stoichiometric one (5100% for air flow rate 250 mL/min).

Figure 2 shows the biooxidation rates obtained in continuous tests and the predicted ones by Narita et al and Tromans (equations (1) and (3), respectively) basing the calculations on the biooxidation rates obtained without Zn^{2+} and supposing that the slowing down in the biooxidation kinetic in presence of Zn^{2+} is due only to the decrease in oxygen solubility.

It can be observed that the biooxidation rates predicted from the calculation of relative solubilities following Narita and Tromans models fit experimental values of biooxidation rate. This fact led us to postulate that oxygen is the limiting reactant, that it is practically consumed by the biomass and that the possible biological effects associated with Zn^{2+} ion are masked by diffusional processes of oxygen transfer from bubbles to the vicinity of the biomass.

Figure 3 shows the percentage drop in continuous biooxidation rate for the two aeration flow rates assayed when bioreactor A is operated and the percentage decrease in the

specific rate obtained in batch tests against Zn^{2+} concentration. On equal Zn^{2+} concentrations, the effect of Zn^{2+} is stronger if the liquid is not stirred. Considering that the effect of Zn^{2+} on biooxidation is basically related to mass transfer processes, this result indicates a logical fact: in a static system, diffusional barriers for oxygen transfer are considerably higher than in a system where some degree of mixing exists.

3.3. Study of the influence of oxygen partial pressure on continuous Fe^{2+} biooxidation

Table 1 shows results obtained in continuous biooxidation tests operating bioreactor B. In these assays the air stream was replaced by pure oxygen in the absence of Zn^{2+} and with 23 g/L of Zn^{2+} in the liquid feed.

It can be observed that the biooxidation rate is modified in a similar factor in absence and in presence of Zn^{2+} when the oxygen partial pressure in the gas feed is modified. Also, it can be said that Narita and Tromans' models allow the estimation the biooxidation rate when Zn^{2+} is fed. This result reinforces the hypotheses that the biooxidation process is limited by oxygen transfer phenomena, and that the main effect of Zn^{2+} fed is the change to oxygen solubility in liquid medium.

It must be remembered that Narita et al. and Tromans experimentally prove that the relative oxygen solubility due to the introduction of a salt in liquid medium is not affected by a change in oxygen partial pressure.

3.4. Transient state by change in oxygen partial pressure in fed gas

The results shown in section 3.3 come from steady states. The relative difference between them may be a consequence both of the change in oxygenation conditions in cells initially placed in the bioreactor and the cell population evolution response to that change. If we expect to discriminate the first said effect and, therefore, if oxygen transfer really limits the biooxidation process and the amount of biomass placed in the

bioreactor does not, it is necessary to study the transient state evolution with insufficient time for there to be important changes in cell population.

This was done replacing the air stream in feed with an oxygen stream, keeping the other variables constant. The system response was analysed following the evolution of Fe^{2+} concentration in the outlet stream (figure 4).

In the un-steady state the instantaneous biooxidation rate can be calculated from the mass balance equation, where it is necessary to include the accumulation term.

$$\frac{dm}{dt} = M_i - M_e - R_b \quad (9)$$

where,

m is mass of Fe^{2+} (g).

$\frac{dm}{dt}$ is the accumulation term (g/h), and represents the rate of change of mass of $Fe^{2+}$with time measured at a particular instant in the bioreactor. This accumulation term is calculated from:

- The polynomial that fits the Fe^{2+} concentration leaving bioreactor against time, given by the following equation:

$$\left[Fe^{2+}\right]_e = -0.002903t^4 - 0.007630t^3 + 0.699366t^2 - 5.119208t + 12.793921 \quad (10)$$

- The liquid volume in biorreactor B; $(V_L)_B$ = 0.26 L

- Considering continuous stirred tank reactor as flow model [22, 37].

$$\frac{dm}{dt} = \frac{d([Fe^{2+}]_e \cdot (V_L)_B)}{dt} = (V_L)_B \cdot \frac{d[Fe^{2+}]_e}{dt} \quad (11)$$

M_i is mass flow rate of Fe^{2+} entering the bioreactor at any time and is calculated as

$$M_i = [Fe^{2+}]_i * liquid\ \ flow\ rate \quad (12)$$

In this test, ferrous iron concentration in feed solution, $[Fe^{2+}]_i$, was 21.1 g/L and the liquid flow rate was 89 mL/h.

M_e is mass flow rate of Fe^{2+} leaving the bioreactor at any time (g/h).

$$M_e = [Fe^{2+}]_e * liquid\ \ flow\, rate \qquad (13)$$

R_b is the instantaneous biooxidation rate (g/h).

Figure 5 shows instantaneous biooxidation rate Rb against time: the experimental Rb calculated from $[Fe^{2+}]$ experimental data and the theoretical Rb from $[Fe^{2+}]$ calculated by eq. 10.

An immediate response can be observed when air is replaced with oxygen: instantaneous biooxidation rate rises from 0.74 g/h to 2.1 g/h. This confirms the fact that when the bioreactor was fed with air, oxygen was really the limiting reactant, there being enough biomass in the bioreactor to almost triple the biooxidation rate. However, according to Henry's law, the change in air stream fed implies an increase in the oxygen solubility close to 5 times. The fact that an equivalent increase in instantaneous biooxidation rate is not achieved suggests that if pure oxygen is fed there is not enough biomass in the bioreactor to completely metabolise all the dissolved oxygen, because in liquid, at least during the two hours after changing, there is excess of Fe(II) (figures 4 and 5). Therefore, few seconds after changing, the process would go from being controlled by oxygen to be controlled by the amount of biomass. After 2 or 3 hours a decrease in instantaneous biooxidation rate is observed and it is attributed to the Fe^{2+}control (figure 5).

In summary, the results show that in presence of Zn^{2+}, when oxygen is the limiting reactant, diffusional processes of mass transfer are the key-factor, masking the biological effects.

This way of interpretation opens up new possibilities for improvement in the design of reactors for ferrous iron biooxidation, which have already been tested successfully in the design and operation of an indirect bioleaching pilot plant treating a Zn-Pb rougher

concentrate that will be published in detail shortly [33]. The bed volume of the bioreactor in this pilot plant was of 163 L. Zn and Fe concentrations in feed were of 20 g/L. In this case, and taking into account mainly economic aspects, mass transfer was improved by increasing the turbulent flow.

4. CONCLUSIONS.

The results of this work conclude that:

- When air is used, the oxygen fed to the bioreactor is in excess over the stoichiometric. Despite this fact, oxygen in liquid is the limiting reagent.
- In such conditions, the study of biooxidation rate is seriously compromised by the aeration conditions (gas flow rate and oxygen partial pressure).
- Zn^{2+} negatively affects the biooxidation process. The loss of efficiency is proportional to Zn^{2+} concentration in the medium.
- The drop in the efficiency of biooxidation processes in presence of Zn^{2+} is due to the slowdown of oxygen transfer from gas to cells as a consequence of its solubility decrease.
- The biological effects associated to Zn^{2+} are masked by physical phenomena of mass transfer.
- It is possible to eliminate the negative effects of Zn^{2+} on biooxidation kinetics by improving the conditions for mass transfer. In this paper, this has been demonstrated at lab scale, increasing the oxygen partial pressure in the gas phase.

5. ACKNOWLEDGEMENTS.

This work was carried out in the frame of BioMinE (European project contract NMP1-CT-500329-1). The authors acknowledge the financial support given to this project by

the European Commission under the Sixth Framework Programme for Research and Development.

References:

[1] Nemati, M., Harrison, S.T.L., Hansford, G.S., Webb, C. Biological oxidation of ferrous iron by *Thiobacillus ferrooxidans*: a review on the kinetic aspects. Biochemical Engineering Journal 1998, 1, 171-190

[2] Ojumu, T.V., Petersen, J., Searby, G.E., Hansford, G.S. A review of rate proposed for microbial ferrous-iron oxidation with a view to application to heap bioleaching. Hydrometallurgy 2006, 83, 21-28.

[3] García O., Da Silva L.L.. Differences in growth and iron oxidation among *Thiobacillus ferrooxidans* cultures in the presence of some toxic metals. Biotechnology Letters 1991, 3 (8), 567-570.

[4] Leduc, L.G., Ferroni, G.D., Trevors, J.T., Resistance to heavy metals in different strains of *Thiobacillus ferrooxidans*. World Journal of Microbiology and Biotechnology 1997, 13, 453-455.

[5] De, G.C., Oliver, D.J., Pesic, B.M. Effect of heavy metals on the ferrous iron oxidizing ability of *Thiobacillus ferrooxidans*. Hydrometallurgy 1997, 44, 53-63.

[6] Tuovinen, O.H., Niemelä, S.I., Gyllenberg, H.G. Tolerance of *Thiobacillus ferrooxidans* to some metals. Antonie van Leeuwenhoek 1971; 37: 489-496.

[7] Das, A., Modak, J.M., Natarajan, K.A., Technical note: Studies on multi-metal ion tolerance of *Thiobacillus ferrooxidans*. Minerals Engineering 1997; 10, 7: 743-749.

[8] Kupka, D., Kupsakova, I. Iron (II) oxidation kinetics in Thiobacillus ferrooxidans in the precense of heavy metals. Biohydrometallurgy and the Environment toward. The Mining of the 21st century, part A, 1999, 387-396.

[9] Cabrera, G., Gómez, J.M., Cantero, D., Influence of heavy metals on growth and ferrous sulphate oxidation by *Acidithiobacillus ferrooxidans* in pure and mixed cultures. Process Biochemistry 2005, 40, 2683-2687.

[10] Cabrera, G., Gómez, J.M., Cantero, D., Kinetic study of ferrous sulphate oxidation of *Acidithiobacillus ferrooxidans* in the presence of heavy metals ions. Enzyme and Microbial Technology, 2005; 36: 301-306.

[11] Li, H.M., Ke, J-J, Influence of Cu(II) and Mg(II) on the growth and activity of Ni(II) adapted *Thiobacillus ferrooxidans*. Minerals Engineering, 2001, 14, 113-116.

[12] Li, H.M., Ke, J-J, Influence of Ni(II) and Mg(II) on the growth and activity of Cu(II) adapted *Thiobacillus ferrooxidans*. Hydrometallurgy, 2001, 61, 151-156.

[13] Shiers, D.W., Blight, K.R., Ralph, D.E., Sodium sulphate and sodium chloride effects on batch culture of iron oxidising bacteria. Hydrometallurgy, 2005, 80, 75-82.

[14] Nurmi, P., Özkaya, B., Kaksonen, A.H., Tuovinen, O.H., Puhakka, J.A. Inhibition kinetics of iron oxidation by Leptospirillum ferriphilum in the presence of ferric, nickel and zinc ions. Hydrometallurgy 2009, 97, 137-145.

[15] Boon, M., Ras, C., Heijnen, J.J., The ferrous iron oxidation kinetics of *Thiobacillus ferrooxidans* in batch cultures. Applied Microbiology Biotechnology, 1999, 51, 813-819.

[16] Boon, M., Meeder, T.A., Thöne, C., Ras, C., Heijnen, J.J., The ferrous iron oxidation kinetics of *Thiobacillus ferrooxidans* in continuous cultures. Applied Microbiology Biotechnology, 1999, 51, 820-826.

[17] Boon, M., Heijnen, J.J. Gas-liquid mass transfer phenomena in biooxidation experiments of sulphide minerals: A critic review of literature data. Hydrometallurgy, 1998, 48, 187-204.

[18] Savic, D.S., Veljkovic, V.B., Lazic, M.L., Vrvic, M.M., Vuvecit, J.L. Effects of the oxygen transfer rate on ferrous iron oxidation by *Thiobacillus ferrooxidans*. Enzyme and Microbial Technology, 1998, 23, 427-431.

[19] Nikolov, L., Valkova-Valchanova, M., Mehochev, D. Oxidation of high ferrous iron concentration by chemolithotrophic Thiobacillus ferrooxidans in packed-bed bioreactors. J. Biotechnology, 1988, 7, 87-94.

[20] Diz, H.R., Novak, J.T., Modeling biooxidation of iron in packed-bed reactor. Journal of Environmental Engineering, 1999, February, 109-116.

[21] Nemati, N., Webb, C., Combined biological and chemical oxidation of ferrous sulphate using immobilised *Thiobacillus ferrooxidans*, 1999, Journal of Chemical Technology and Biotechnology, 74, 562-570.

[22] Mazuelos, A., Carranza, F., Palencia, I., Romero, R., High efficiency reactor for the biooxidation of ferrous iron. Hydrometallurgy 2000; 58: 269-275.

[23] Mazuelos, A., Romero, R., Palencia, I., Carranza, F., Borjas, F.J., Oxygen transfer in ferric iron biological production in a packed-bed bioreactor. Hydrometallurgy, 2002, 65, 2002, 15-22.

[24] Narita, E., Lawson, F., Han, K.N. Solubility of oxygen in aqueous electrolyte solutions. Hydrometallurgy 1983; 10: 21-37.

[25] Tromans, D. Modeling Oxygen Solubility in water and electrolyte solutions. Ind. Eng. Chem. Res. 2000; 39: 805-812.

[26] Blight, K.R., Ralph, D.E. Effect of ionic strength on iron oxidation with batch cultures of chemolithotrophic bacteria. Hydrometallurgy 2004; 73:325-334.

[27] Blight, K.R., Ralph, D.E. Aluminium sulphate and potassium nitrate effects on batch culture of iron oxidising bacteria. Hydrometallurgy 2008, 92: 130-134.

[28] Ojumu, T.V., Petersen, J., Hansforf, G., The effect of dissolved cations on microbial ferrous iron oxidation by *Leptospirillum ferriphilum* in continuous culture. Hydrometallurgy 2008; 94: 69-76.

[29] Candy, R.M., Blight, K.R., Ralph, D.E., Specific iron oxidation and cell growth rates bacteria in batch culture, Hydrometallurgy, 2009, 98, 148-155.

[30] Rohwerder, T., Gehrke, T., Kinzler, K., sand, W., Bioleaching review part A: Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl. Microbiol. Biotechnol. 2003; 63: 239-248.

[31] Carranza, F., Palencia, I., Romero, R., Silver catalyzed IBES process: application to a spanish copper-zinc sulphide concentrate. Hydrometallurgy 1996; 44: 29-42.

[32] Carranza, F., Iglesias, N., Romero, R., Palencia, I. Kinetics improvement of high-grade sulfides bioleaching by effects separation. FEMS Microbiology Reviews 1993; 11: 129-138.

[33] Frías, C., Carranza, F., Sánchez, F., Mazuelos, A., Frades, M., Romero, R., Díaz, G., Iglesias, N., New developments in indirect bioleaching of zinc and lead sulphide concentrates, proceedings of the 6th International Symposium Hydrometallurgy 2008, p. 497-505.

[34] Steffan, R.J. and Atlas, R.M. Polymerase Chain Reaction: Applications in Environmental Microbiology. Ann. Rev. Microbiol. 1991, 45, 137-161.

[35] Mazuelos, A., Palencia, I., Romero, R., Rodriguez, G., Carranza, F., Ferric iron production in packed bed bioreactors: influence of pH, temperature, particle size, bacterial support material, and type of air distributor. Minerals engineering, 2001; 14, 5: 507-514.

[36] Bailey, J.E., Ollis, D.F., "Biochemical Engineering Fundamentals", 2nd ed., New York: McGraw-Hill; 1986, p. 457-512.

Tables:

Table 1**.** Study of the influence of oxygen partial pressure on continuous Fe^{2+} biooxidation; results obtained operating bioreactor B. Air stream is replaced by pure oxygen in the absence of Zn^{2+} and with 23 g/L of Zn^{2+} in the liquid feed.

Table 1

Steady state	$[Fe^{2+}]_i$ (g/L)	$[Fe^{2+}]_o$ (g/L)	Liquid flow rate (mL/h)	P_{O2} (atm)	$[Zn^{2+}]$ (g/L)	Q_{bioox} (g/h)	Narita [24] Q_{bioox} (g/h)	Tromans [25] Q_{bioox} (g/h)
1	22.50	14.50	115	0.21	0.00	0.92±0.01	-	-
2	21.10	12.78	89	0.21	23.00	0.74±0.01	0.75	0.74
3	20.97	13.00	419	1	0.00	3.34±0.04	-	-
4	22.36	12.80	293	1	23.00	2.80±0.03	2.74	2.70

Figures:

Figure 1: Percentage of oxidized ferrous iron versus time in batch biooxidation tests for the first and the last culture of each series. N is the serial number of re-inoculation.

Figure 2: Continuous biooxidation test performed running bioreactor A, together with the predicted ones by Tromans and Narita et al models: ferrous iron biooxidation rate (Q_{biox}) versus Zn^{2+} concentration in feed. Gaseous stream: air; flow rate: 250 and 500 mL/min. Liquid stream: Fe^{2+} concentration: 7 g/L; flow rate; 88 mL/h. Error bars represent the standard deviation.

Figure 3: Percentage drop in continuous biooxidation rate for the two aeration flow rates assayed when bioreactor A is operated and the percentage decrease in the specific rate obtained in batch tests against Zn^{2+} concentration.

Figure 4: Continuous biooxidation test running bioreactor B: evolution of Fe^{2+} concentration in the outlet stream as a result of replacing the air stream fed with pure oxygen. Feed composition: 21.1 g/L of Fe(II) and 23.0 g/L of Zn(II); liquid flow rate: 89 mL/h; gas flow rate: 600 mL/min.

Figure 5: Continuous biooxidation test running bioreactor B: evolution of instantaneous biooxidation rate as a result of replacing the air stream fed with pure oxygen. Feed composition: 21.1 g/L of Fe(II) and 23.0 g/L of Zn(II); liquid flow rate: 89 mL/h; gas flow rate: 600 mL/min.

Figure 1

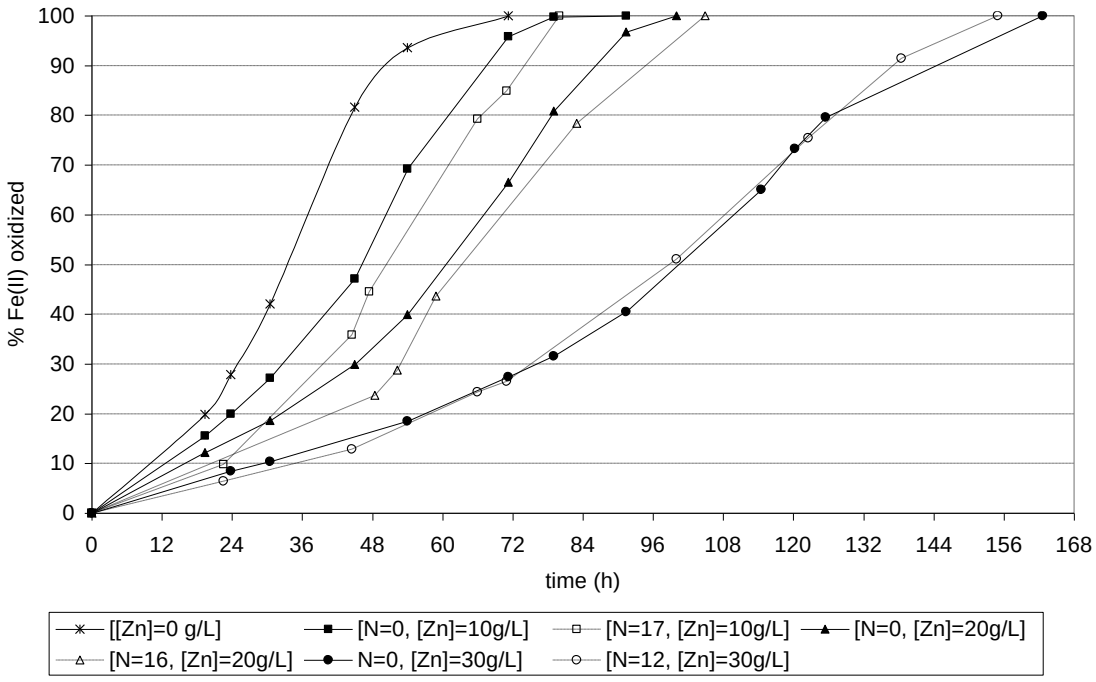

% Fe(II) oxidized
time (h)
0
10
20
30
40
50
60
70
80
90
100
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
[[Zn]=0 g/L]
[N=0, [Zn]=10g/L]
[N=17, [Zn]=10g/L]
[N=0, [Zn]=20g/L]
[N=16, [Zn]=20g/L]
N=0, [Zn]=30g/L]
[N=12, [Zn]=30g/L]

Figure 2

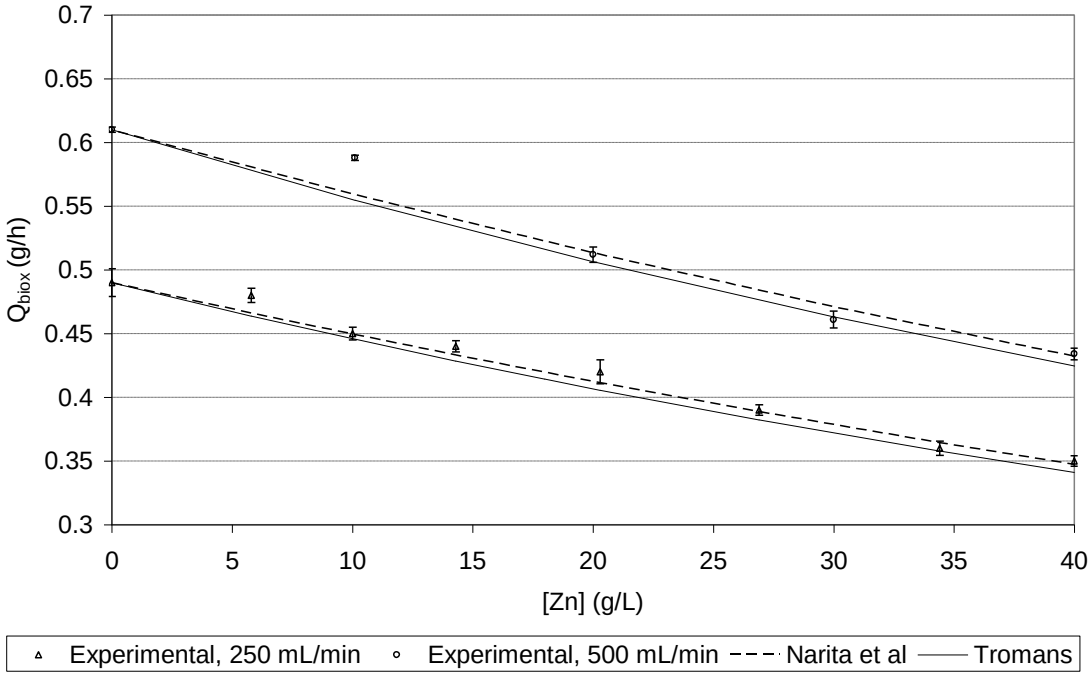

0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
Q_{biox} (g/h)
0
5
10
15
20
25
30
35
40
[Zn] (g/L)
Experimental, 250 mL/min
Experimental, 500 mL/min
Narita et al
Tromans

Figure 3

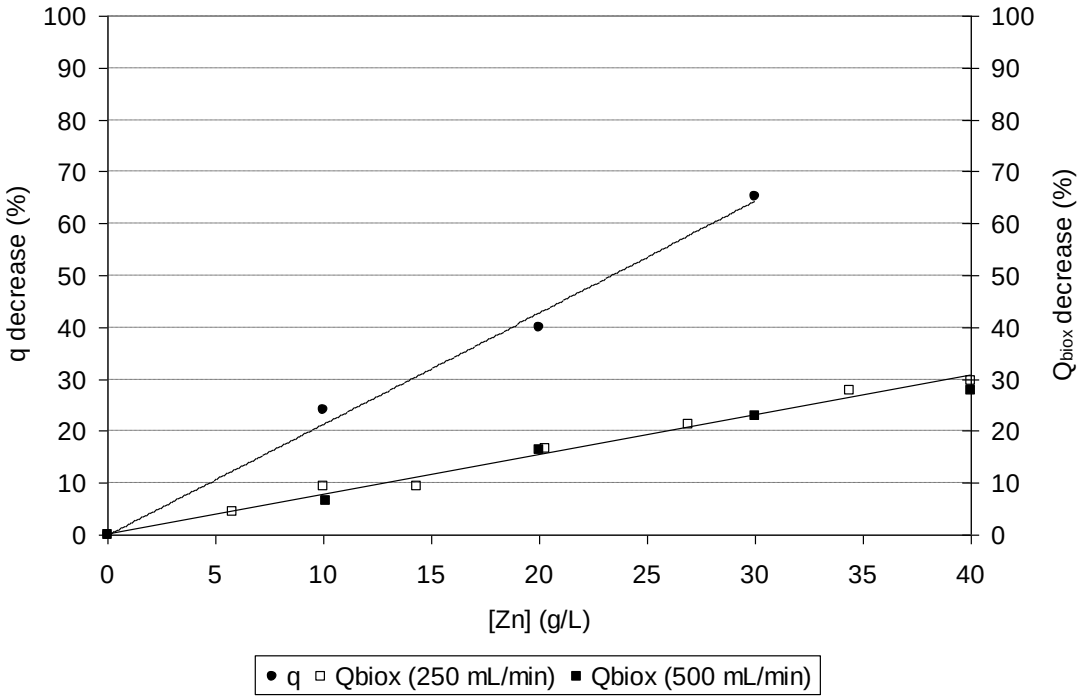

100
90
80
70
60
50
40
30
20
10
0
q decrease (%)
Qbiox decrease (%)
0
5
10
15
20
25
30
35
40
[Zn] (g/L)
q
Qbiox (250 mL/min)
Qbiox (500 mL/min)

Figure 4

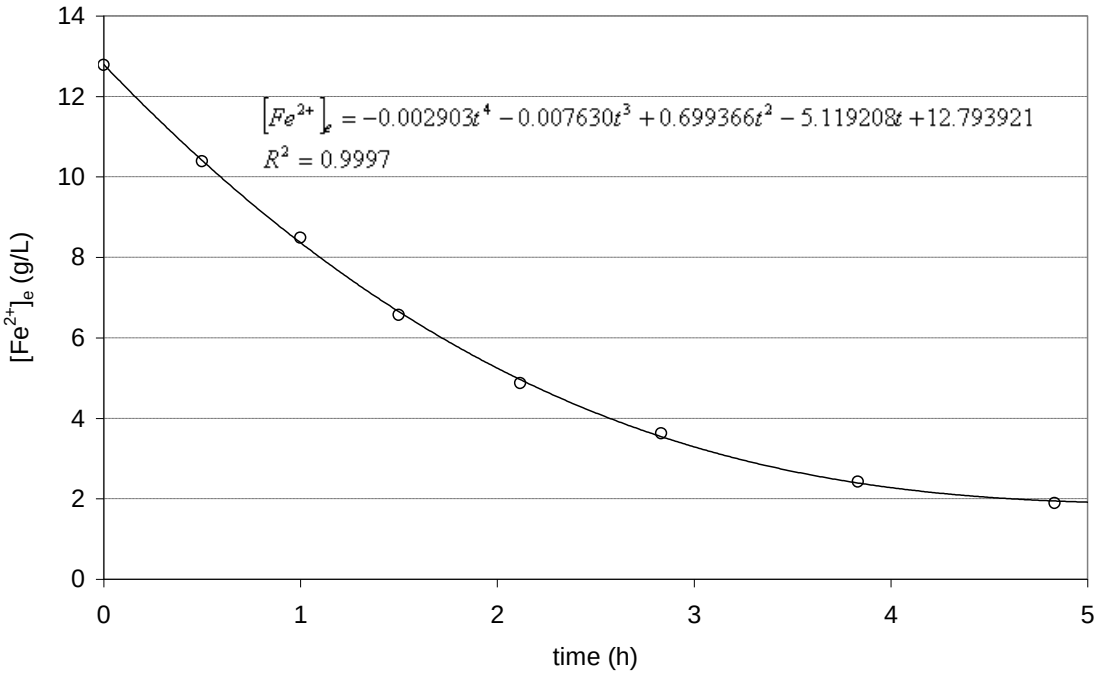
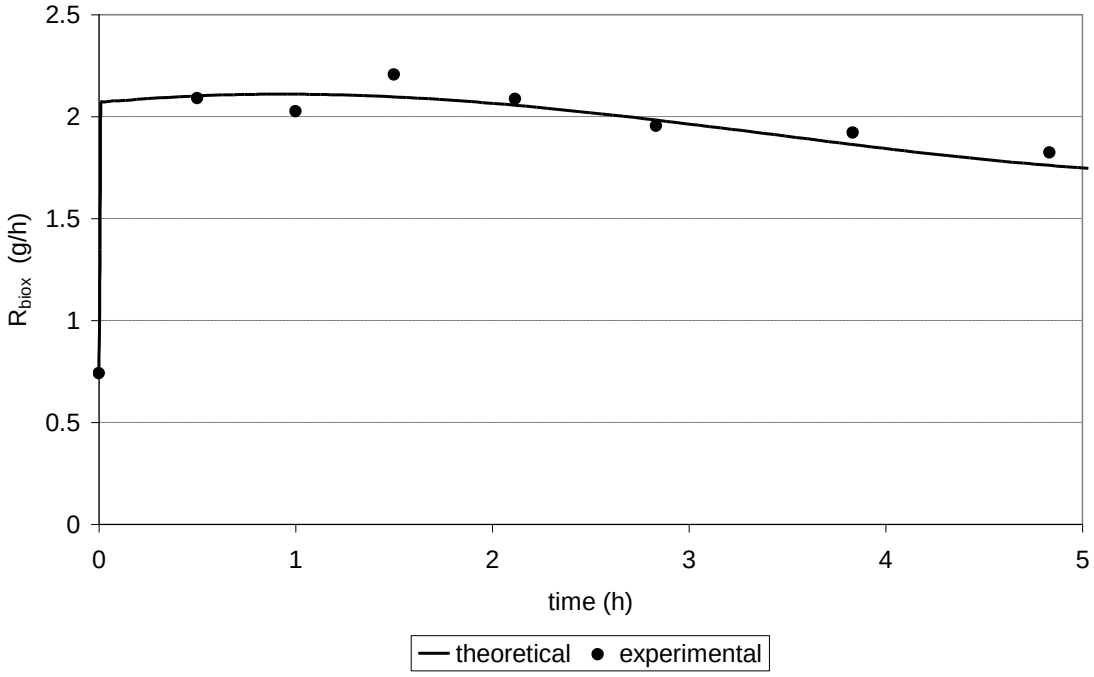

$[Fe^{2+}]_e = -0.002903t^4 - 0.007630t^3 + 0.699366t^2 - 5.119208t + 12.793921$
$R^2 = 0.9997$
$[Fe^{2+}]_e$ (g/L)
time (h)
0
2
4
6
8
10
12
14
0
1
2
3
4
5

Figure 5


2.5
2
1.5
1
0.5
0
R_{biox} (g/h)
0
1
2
3
4
5
time (h)
theoretical
experimental