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# Kinetic study of biological ferrous sulphate oxidation by iron-oxidising bacteria in continuous stirred tank and packed bed bioreactors

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**Abstract**

This paper describes kinetic study of biological ferrous sulphate oxidation by *Thiobacillus ferrooxidans* iron-oxidising bacteria in submerged culture and immobilised in nickel alloy fibre as matrix support. In this way, two types of bioreactors has been used: a continuous stirred tank reactor (CSTR) for free cells and a packed bed bioreactor for immobilised biomass. A mathematical expression has been developed to explain kinetic behaviour of micro-organism in bioreactors as function of the main process parameters. Model predictions of ferrous iron oxidation rate were found closely to experimental data and provide high coefficient. Comparison between oxidation rates for two bioreactors showed that process with a biofilm reactor is more stable than the bioreactor with free immobilised biomass.


*Keywords:* Kinetic study; Ferrous oxidation; *Thiobacillus ferrooxidans*; Stirred tank; Packed bed

## 1. Introduction

*Thiobacillus ferrooxidans* is an acidophilic bacterium that has the ability to oxidise ferrous to ferric iron in the presence of atmospheric oxygen and carbon dioxide. This bacterium is a dominant organism in the process of value metal extraction by microbial leaching of pyritic ores. The product of the oxidation is an acidic solution of Fe(III), a potent chemical oxidant, that has been exploited in the treatment of acidic mine effluents processes for the removal of sulphide hydrogen from sour gases and bioremediation of contaminated soils by heavy metals.

Most of the previous research on this biological oxidation has been concerned with practical aspects of improving the overall leaching rates of metals from sulphide ores by studying the effect of such variables as temperature, pH, nutrient concentration, particle size and mineral type. From an engineering point of view, the principal factor affecting the cost effectiveness of industrial processes is the rate of reaction; so, it is necessary to improve it.

Biological iron oxidation has been studied in several experimental systems with batch and continuous-flow modes of operation [1]. Because of the interest in the kinetic aspects of the oxidation, attempts have been made to improve the ferrous iron oxidation rate by the use of various reactor designs employing biological contacting devices. These have included bacteria in suspended and in fixed-film applications to provide a large surface area for their attachment [2]. Initial work with *T. ferrooxidans* was primarily concerned with the development of rotating biological contactors [3,4]. More recent efforts have addressed other fixed-film approaches, which essentially involve various configurations of packed-bed [5,6] and fluidised-bed reactors with inert carrier matrix materials [7].

In the present work, we have studied the behaviour of biological oxidation of ferrous sulphate in a continuous stirred tank reactor (CSTR) with free suspended cells of

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*T. ferrooxidans* and in a packed-bed reactor with immobilised cells in nickel alloy fibre as matrix support. With experimental data obtained in these studies, we have proposed a kinetic equation in order to calculate theoretical ferrous oxidation rates as function of the input substrate concentration and the substrate conversion. Finally, it is possible to establish a comparison between the reaction rates in packed-bed bioreactor with immobilised cells and that in a bioreactor with free suspended cells.

## 2. Materials and methods

### 2.1. Microorganism and growth conditions

The strain of *T. ferrooxidans* used in this study was isolated from the Rio Tinto mines of Huelva (Spain) and kindly made available by the Biohydrometallurgy Group of the University of Seville (Spain). This strain has the same properties and characteristics of strain used by Nemati and Webb [8] obtained from National Collection of Industrial and Marine Bacteria (NCIMB 9490).

The bacteria were grown in a medium proposed by Silverman and Lundgren [9]: $(NH_4)_2SO_4$ 3.0 g $l^{-1}$; $MgSO_4$ 0.5 g $l^{-1}$; $K_2HPO_4$ 0.5 g $l^{-1}$; KCl 0.1 g $l^{-1}$; $Ca(NO_3)_2$ 0.01 g $l^{-1}$ and a variable concentration of $FeSO_4$, depending on the experiment to be performed.

*Thiobacillus ferrooxidans* was immobilised on nickel alloy fibre according to the procedure described in Gómez et al. [10].

### 2.2. Analytical methods

Ferrous sulphate oxidation was monitored by determining the residual ferrous iron concentration at various intervals. The 1,10 phenanthroline method of Vogel [11] was used. In order to determine the ferrous iron concentration, a 10-μl sample was placed in a tube and diluted with 1.0 ml of distilled water. The pH was adjusted to between 3.0 and 6.0 with 2 mol $l^{-1}$ sodium acetate, 0.8 ml of 1,10-phenanthroline solution was added and, finally, an additional 10 ml distilled water. The absorbance at 515 nm was measured after 5–10 min. In order to determine total iron concentration, 1.0 ml hydroxylamine hydrochloride, as a reducing agent, was added to the sample instead of 1.0 ml distilled water and the same procedure followed. A calibration curve of known $FeSO_4$ concentrations was used to calculate the iron concentrations. The concentration of iron (III) in solution was calculated by subtracting the average iron (II) concentration from the total iron concentration measured at each point in time.

### 2.3. Total cell number

In order to measure the total biomass adhered to the matrix support, a known amount of nickel alloy fibre was placed in a flask with 5 ml of oxalic acid 10% (w/v), at each 'draw and fill' cycle. After 10 min, the support was rinsed with 5 ml of distilled water for 10 min. Then, the rinsings were added to the previous cell suspension obtained.

The biomass concentration was determined by direct counting using a Neubauer chamber counter of 0.02 mm depth and 1/400 $mm^2$ area under an optical microscope.

In some cases, it was necessary to dilute the samples with basal salt solutions because of the high biomass concentrations.

Each measurement was made in duplicate to minimise the experimental errors inherent in working with microbial populations.

### 2.4. Bioreactors

Ferrous sulphate oxidation by free *T. ferrooxidans* cells was studied using an automatic continuous stirred tank reactor with an inlet for medium and air, and outlet for effluent at the top. A working volume of 5 l, aeration rate of 0.5 vvm and agitation rate of 200 rpm was used. The temperature was maintained at 30 °C with an external heat exchanger and flow rates of both inlet and outlet were regulated with peristaltic pumps controlled by automatic control equipment. Sampling of the effluent was performed from the end of the effluent tubing at the reservoir and analysed of ferrous, total iron and biomass.

In the case of immobilised *T. ferrooxidans,* ferrous sulphate oxidation was studied using a glass column ($1 \times 0.05$ m) with an inlet for medium and air at the bottom and outlet for effluent at the top. Nickel alloy fibre with immobilised *T. ferrooxidans* was placed in the column and a layer of sintered glass was placed at the bottom of the matrix layer to keep this inside the column. A working volume of 1350 ml and aeration rate of 0.675 ml $min^{-1}$ were used. The temperature was maintained at 30 °C and flow rates of both inlet and outlet were regulated with automatically controlled peristaltic pumps. Sampling of the effluent was performed from the end of the effluent tubing at the reservoir and analysed for ferrous iron and total iron.

Both bioreactors were operated in batch mode until complete oxidation of iron were achieved, then the reactor was switched to continuous operation. Steady-state operation was considered to be established when the ferrous iron concentration varied by less than 5% during a period of time equal to the theoretical retention time.

Several media with different initial concentration of ferrous iron (from 1000 to 8500 mg $l^{-1}$) were tested. The

initial pH of the media was adjusted to 1.8. For each medium, various dilution rates were applied: 0.01–0.06 $h^{-1}$ for CSTR, and 0.08–0.25 $h^{-1}$ for packed-bed reactor.

## 3. Results and discussion

### 3.1. Operation in continuous stirred tank reactor

Several experimental runs were carried out to determine the influence of dilution rate and ferrous iron concentration in the influent on rate of ferrous iron oxidation. As we mentioned above, bioreactor was operated as an independent unit and was started with a 10% (vol/vol) inoculum of a spent, iron-grown culture of *T. ferrooxidans*. The evolution of ferrous, ferric iron biomass is shown in Fig. 1. Initially, ferrous iron decreased and ferric iron and total biomass increased; until minimum and maximum values, respectively, were achieved. These values are in dependence on the operational conditions in the run. In this moment, steady-state conditions have been achieved, a continuous-flow mode of operation was initiated. The time to achieve steady-state conditions varied depending on the flow rate, but normally this state was reached within 150 h before experimental run was established.

After 300 h, the amount of precipitate deposited in the walls of the bioreactor gradually increased. The precipitate also caused problems in the outlet tubing and in the distribution system of feed air, where it had to be cleaned to prevent blocking. The extensive precipitation in the bioreactor had a detrimental effect on iron (II) oxidation rate and oxygen mass transfer rate to the medium.

The evolution of ferrous and ferric iron concentration in steady-state conditions versus dilution rate and

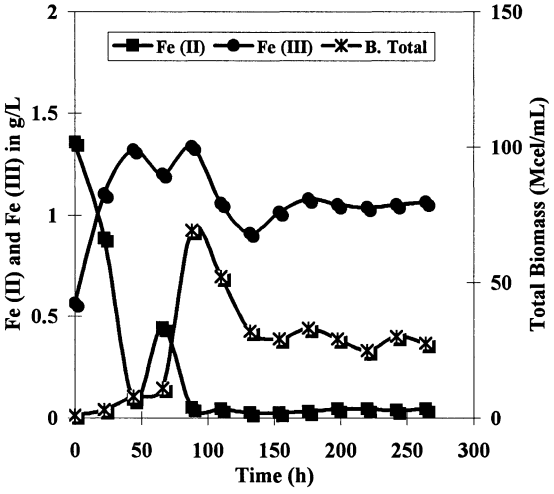


Fig. 1. Evolution of total biomass, ferrous and ferric iron in a continuous stirred tank bioreactor.

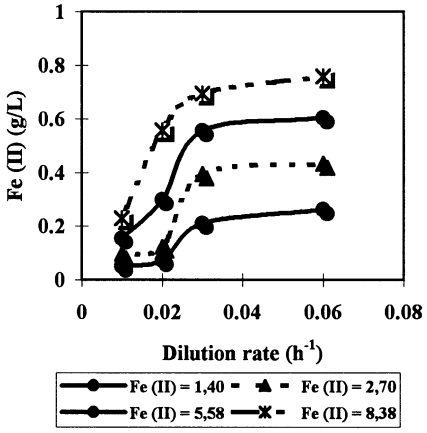


Fig. 2. Steady-state ferrous iron concentration in a continuous stirred tank bioreactor as function of dilution rate.

ferrous iron concentration in the influent are shown in Figs. 2–4, respectively. In general, it can be observed that Fe(II), Fe(III) and total biomass curves have similar progress.

When an influent Fe(II) concentration is below 2.70 g $l^{-1}$, Fe(III) and total biomass were not affected because ferrous iron acts as a limiting substrate for growth. In the same way, when the dilution rate is above 0.03 $h^{-1}$, there is no dependence between these variables and feed iron concentration.

In Fig. 5 it is possible to see that ferrous iron oxidation rate was accelerated by increasing dilution rates from 0.01 to 0.06 $h^{-1}$. These data confirm that operating conditions were below critical with respect to hydraulic loading. Ferrous sulphate oxidation rates are comparable with those published by Karamanev et al. [2], Torma [12] and Kelly et al. [13] for this process when feed ferrous iron is below 6 g $l^{-1}$.

Results obtained can be expressed by a kinetic equation that allows calculating theoretical ferrous oxidation rate as function of influent Fe(II) and Fe(II)

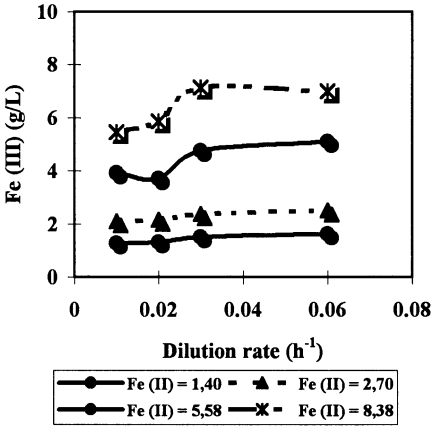


Fig. 3. Steady-state ferric iron concentration in a continuous stirred tank bioreactor as function of dilution rate.

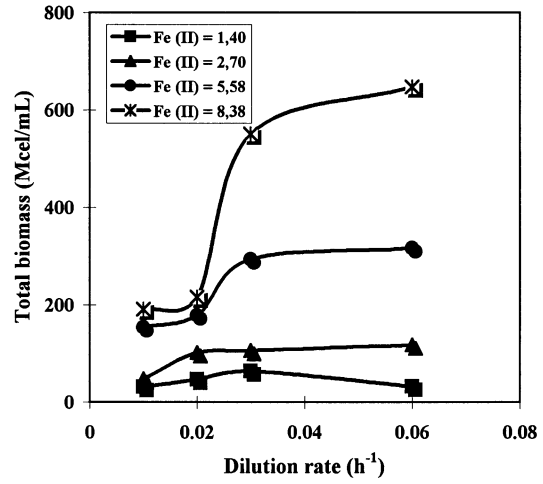


Fig. 4. Steady-state biomass concentration in a continuous stirred tank bioreactor as function of dilution rate.

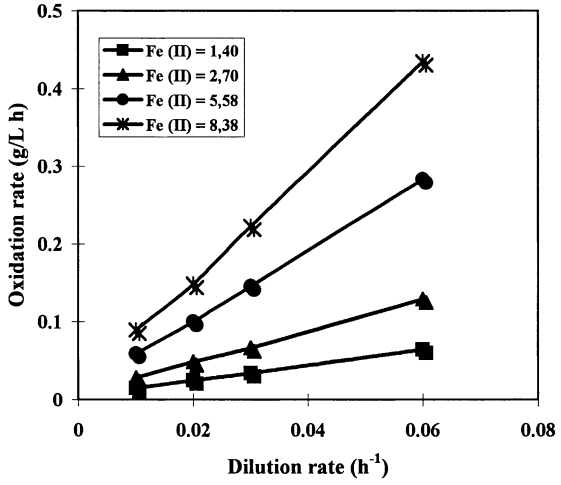


Fig. 5. Experimental data of ferrous iron oxidation rate in a continuous stirred tank bioreactor.

concentration in the reactor under steady-state conditions. As a previous step, it is necessary to develop mass balance for this reactor. The model was develop, using the following main assumptions:

1) The liquid medium in the bioreactor is perfectly mixed.
2) Oxygen transfer rate in the bioreactor is high.
3) Total volume is constant and equal to $V_R$.
4) Ferrous iron acts a limiting substrate.
5) The process is in a continuous regime.
6) Biomass growth rate is the same for free suspended cells obtained previously [14] in batch culture.

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S + K_I \cdot P}$$

where $\mu_{max}$, $K_S$ and $K_I$ are parameters characteristics of the microorganism; $S$ is the substrate concentration and $P$ is the product concentration.

So, mass balance can be expressed in this mathematical form:

$$V_R \frac{dS}{dt} = Q \cdot S_0 - Q \cdot S - V_R \cdot r_S$$

where $V_R$ is the reactor volume; $Q$ is the input and output flow rate; $S_0$ is the input limiting substrate concentration; $S$ is the limiting substrate concentration and $r_S$ is the substrate utilisation rate. In the case of perfectly mixed liquid:

$$r_S = D(S_0 - S)$$

where $D$ is dilution rate. If we consider there is no cellular death, it is possible to assume that dilution rate ($D$) is equal to specific growth rate ($\mu$). So, the model can be rewritten as a function of $r_S = f(S, S_0)$ in this form:

$$r_S = \frac{\mu_{max} \cdot S \cdot (S_0 - S)}{K_S + S + K_I(S_0 - S)}$$

A non-linear regression procedure [15] was used to perform the mathematical fitting of the coefficients. The application of this algorithm to set of experimental data previously referred gives the following values of the parameters: $\mu_{max} = 0.22\ h^{-1}$; $K_S = 0.92\ g\ l^{-1}$ and $K_I = 0.21$. These data are in agreement with literature and high theoretical-experimental determination coefficient ($r^2 = 0.92$) provides good application of this model.

### 3.2. Operation in packed-bed bioreactor with immobilised biomass

Kinetics for continuous oxidation of ferrous iron by immobilised cells of *T. ferrooxidans* were studied in a packed-bed bioreactor. Once inoculated with nickel alloy fibre particles, the bioreactor was started in batch culture until 95% of ferrous iron was oxidised. In this moment, a continuous flow mode of operation was initiated. During the operation of the bioreactor, sampling from different parts of the bed was not possible. Hence, the assessment of biomass hold-up was performed at the end of each experimental run. This was done by taking a know amount of nickel alloy fibre from various parts of the bed. The particles were soaked in a flask with 5 ml of oxalic acid 10% (w/v) for 10 min. After this time, the support was rinsed with 5 ml of distilled water for 10 min. After that, the rinsing was added to previous cell suspension obtained. The biomass concentration was determined by direct counting using a Neubauer chamber counter with optical microscope.

Occasionally, biomass concentration in the effluent was measured and it showed that this concentration is despicable with immobilised biomass.

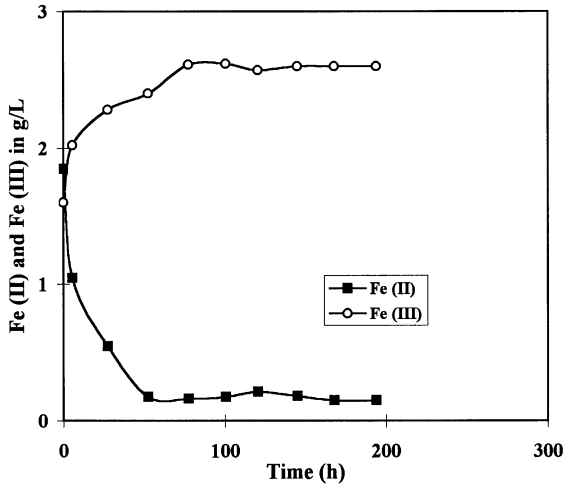


Fig. 6. Evolution of ferrous and ferric iron in a packed-bed bioreactor.

So, ferrous and ferric iron in the effluent were measured daily. A typical evolution of these variables is shown in Fig. 6.

Results obtained from several experimental run at different initial concentrations of ferrous iron and dilution rates allowed to calculate ferrous iron conversion and oxidation rate. At operational conditions studied, the mean conversion is 93% and this result is comparable with those obtained by other authors working in different bioreactors: Nemati et al. [8] working in a packed-bed reactor with *T. ferrooxidans* immobilised in polyurethane foam particles and Halfmeier et al. [16] with *T. ferrooxidans* immobilised in Syran rings.

In general, it can be stated that final conversion has no significant variation when initial concentration of ferrous iron increased, except for 8.38 g $l^{-1}$, where a minimal value was recorded. This fact can be explained because at this ferrous iron concentration can be appear substrate inhibitory effects that it was demonstrate by batch cultures studied previously by ourselves [14] and by Nikolov et al. [17] working on rotating biodisks.

Evolution of ferrous, ferric iron and biomass concentration in steady-state conditions at different feed ferrous iron concentration and dilution rates are shown in Figs. 7–9, respectively.

Two types of behaviour can be observed in Fig. 7: on one hand, when feed ferrous iron concentration is below 5.58 g $l^{-1}$, ferrous iron concentration in effluent has no dependence with the dilution rate and only increased slightly at high values of this variable; on the other hand, when feed ferrous iron is 8.38 g $l^{-1}$, results showed a strong dependence with the dilution rate of the system. The reason for this observation may lie in the inhibitory effect of the substrate concentration at this feed ferrous iron concentration.

Particularly in this case, a decline in ferrous iron concentration was recorded when increased dilution rate

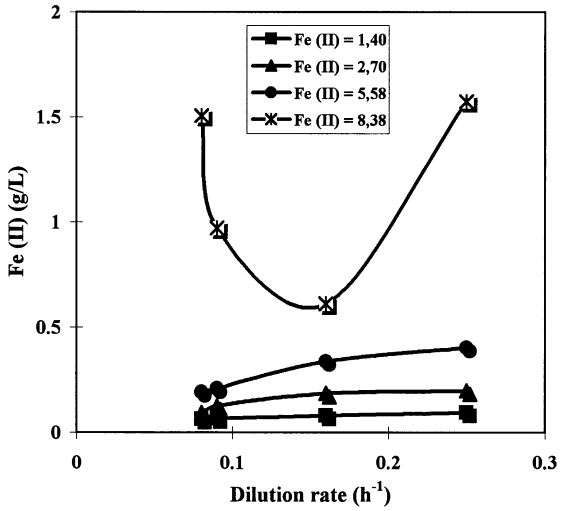


Fig. 7. Steady-state ferrous iron concentration in a packed-bed bioreactor as function of dilution rate.

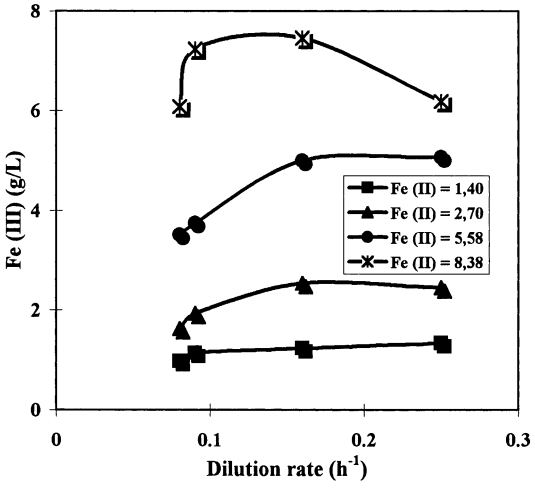


Fig. 8. Steady-state ferric iron concentration in a packed-bed bioreactor as function of dilution rate.

reached a minimum value at 0.16 $h^{-1}$. This response of the system is a consequence of working at operational conditions near those optimal, because the microorganism has a maximum specific growth rate of 0.14 $h^{-1}$. Further increase on dilution rate resulted in higher ferrous iron concentration due to the washout of the bacterial cells is more important than adhesion to the matrix support.

Fig. 9 presents the biomass concentration at the end of the experiment. In this set, the strong influence of dilution rate on this variable can be seen. At an initial concentration of 1.40 g $l^{-1}$ of Fe(II), biomass concentration increased as the dilution rate increased, with a maximum at a dilution rate of 0.16 $h^{-1}$; further increase of dilution rate has no effect on biomass. This fact can be explained because bacterial growth has a limitation with feed substrate concentration. Employing higher

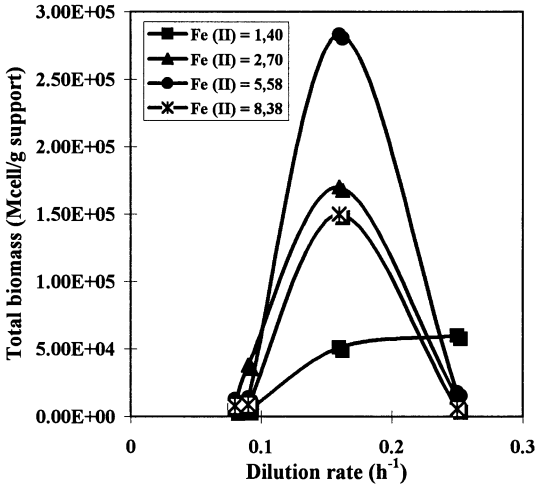


Fig. 9. Steady-state biomass concentration in a packed-bed bioreactor as function of dilution rate.

initial concentrations of ferrous iron, evolution of biomass has a Gauss type evolution with a maximum of 0.16 $h^{-1}$. Moreover, these values are higher with increased feed ferrous iron concentration. For 8.38 g $l^{-1}$ of ferrous iron, an inversion was recorded in this behaviour and a decrease was obtained in this maximum value for those observed with less feed iron concentration. Again, this fact can be explained by substrate inhibitory effects.

Results obtained can be expressed by a kinetic equation to calculate theoretical ferrous oxidation rate as function of influent Fe(II) concentration and Fe(II) concentration in the bioreactor under steady-state conditions. Firstly, it was necessary to develop a mass balance for the reactor. The main assumptions for determination of this dependence were:

1) Perfect mixing of the liquid in the bioreactor. In this bioreactor the mixing is close to perfect because high aeration generates intensive mixing.
2) Micro-organisms are uniformly immobilised on the surface of the bioreactor as a monolayer of constant thickness. The biofilm growth rate is usually much lower than the rate of the biochemical reaction and, therefore, this assumption is valid within a small time period in many of the biofilm reactors.
3) There is no diffusion limitation of the substrate and product. In a thin biofilm, the limiting substrate concentration drop is small and there is negligible effect of the diffusion.
4) There is no external mass transfer limitation, such as bioreactors with intensively hydrodynamics like biodisk reactors.
5) The free suspended micro-organisms do not affect the bioreaction rate in the biofilm reactor, because the biofilm reactors work at high dilution rates when the free suspended micro-organism are washed out.
6) No kinetic changes of the bioprocess after fixation of the micro-organisms.
7) The process is in a continuous steady-state regime.
8) Death cellular rate is negligible to growth rate.

So mass balance can be expressed in this mathematical form:

$$V_R \frac{dS}{dt} = Q \cdot S_0 - Q \cdot S - V_R \cdot r_S$$

where $V_R$ is the reactor volume; $Q$ is the input and output flow rate; $S_0$ is the input limiting substrate concentration; $S$ is the limiting substrate concentration and $r_S$ is the substrate utilisation rate. If we consider that there is no variation of substrate concentration and the reactor volume is constant, the model can be rewritten in this form:

$$r_S = \frac{\mu_{max} \cdot X}{Y_{XS}} \cdot \frac{S}{K_S + S + K_I \cdot (S_0 - S)} = D(S_0 - S)$$

In this equation $X$ is the concentration of the biomass in the biofilm and $Y_{XS}$ is the biomass-substrate yield coefficient. This parameter has been evaluated as $2.07 \times 10^7$ cells $g^{-1}$ of Fe(II) oxidised.

In addition, we have assumed that product formation can be estimated by the difference between input substrate ($S_0$) and substrate concentration in the bioreactor ($S$). So, this assumption implies that $Y_{PS}$ is closed to 1 in the range of concentrations studied.

The application of non-linear algorithm to set of experimental data previously referred gives the following values of the parameters: $\mu_{max} = 0.12\ h^{-1}$, $K_S = 0.98$ g $l^{-1}$ and $K_I = 2.30$. So, it is important to notice that kinetics of immobilised biomass can be represented for the equation proposed in batch culture, but the coefficients change its values. These values are in the range proposed in the literature for systems with immobilised biomass.

The comparison of the experimental with theoretical rates provides a high coefficient ($r^2 = 0.94$) and so, the good application of the model proposed can be concluded.

### 3.3. Comparison between oxidation rates for continuous stirred tank reactor and packed-bed bioreactor

Once kinetic models have been proposed, it is necessary to check out the sensitivity of these models for two types of bioreactor versus changes in the parameters; basically the influence of some changes in value parameters over kinetic coefficients has been studied: $\mu_{max}$, $K_S$ and $K_I$. These data are represented in Figs. 10–12. It is possible to see the change in

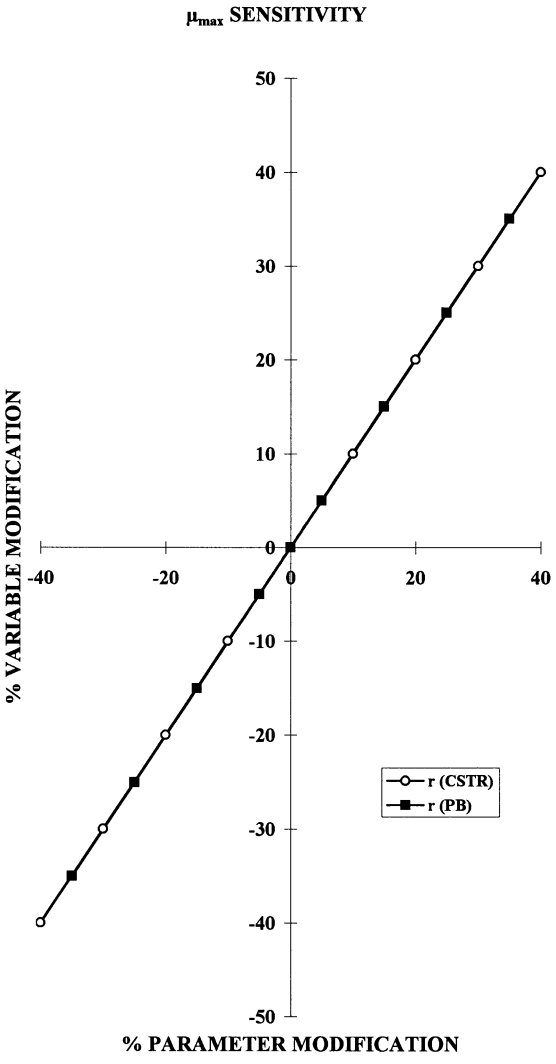


Fig. 10. Analysis of $\mu_{max}$ sensitivity.

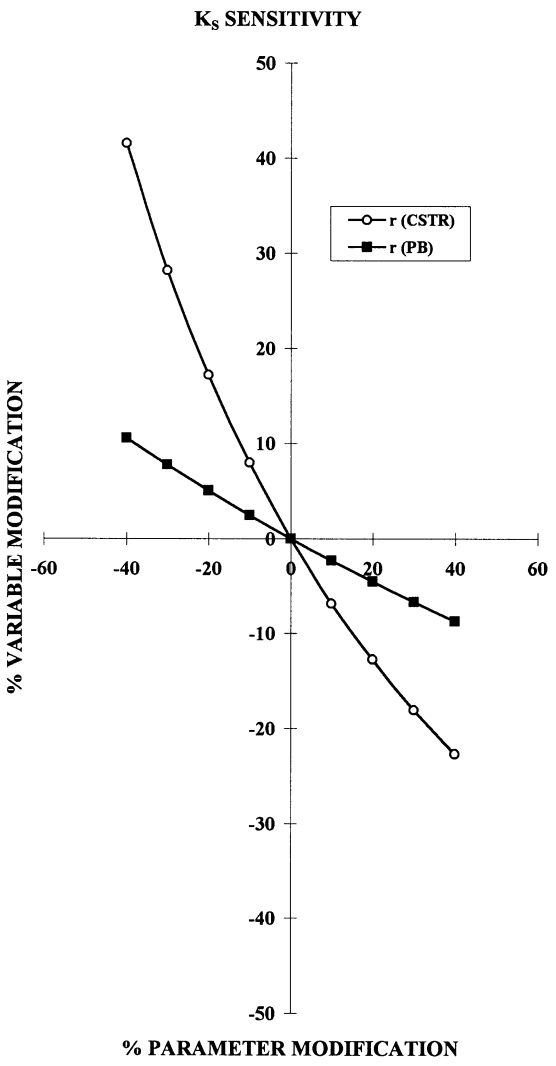


Fig. 11. Analysis of $K_S$ sensitivity.

oxidation rate value by a modification of kinetic parameters included in the equation. It is necessary to explain that these data are theoretical in order to check model application. This type of analysis is very useful for simulation of the model at different operating conditions not studied in this work.

Results obtained for model sensitivity on maximum growth rate are shown in Fig. 10. In this case, changes in $\mu_{max}$ affects the ferrous iron oxidation in equal percentage to the variable modification in two cases studied.

In Figs. 11 and 12 results are shown for $K_S$ and $K_I$, respectively. In general, it can be observed that these parameters have an important influence over ferrous iron oxidation rate in the bioreactors studied, but the type of influence is not equal in each model. In this way, CSTR model is more sensible to changes in $K_S$ and ferrous iron oxidation calculated for packed-bed bioreactor has high sensitivity for changes in inhibition by product coefficient.

So, it is possible to establish an impact order: $\mu_{max} > K_S > K_I$ for CSTR and $\mu_{max} > K_I > K_S$ for packed-bed bioreactor.

After that, the main aim of this study is to determine the ratio between the reaction rates in a continuous stirred tank reactor ($r_{S(CSTR)}$) and that in a packed-bed bioreactor with immobilised biomass ($r_{S(PB)}$) of *T. ferrooxidans* as a function of the main process parameters: the input substrate concentration $S_0$ and the substrate conversion.

As we mentioned before, ferrous iron oxidation rate is equal to:

$$r_S = \frac{\mu_{max} \cdot S \cdot (S_0 - S)}{K_S + S + K_I(S_0 - S)} \quad (1)$$

and taking into account that the conversion is:

$$x = 1 - S/S_0,$$

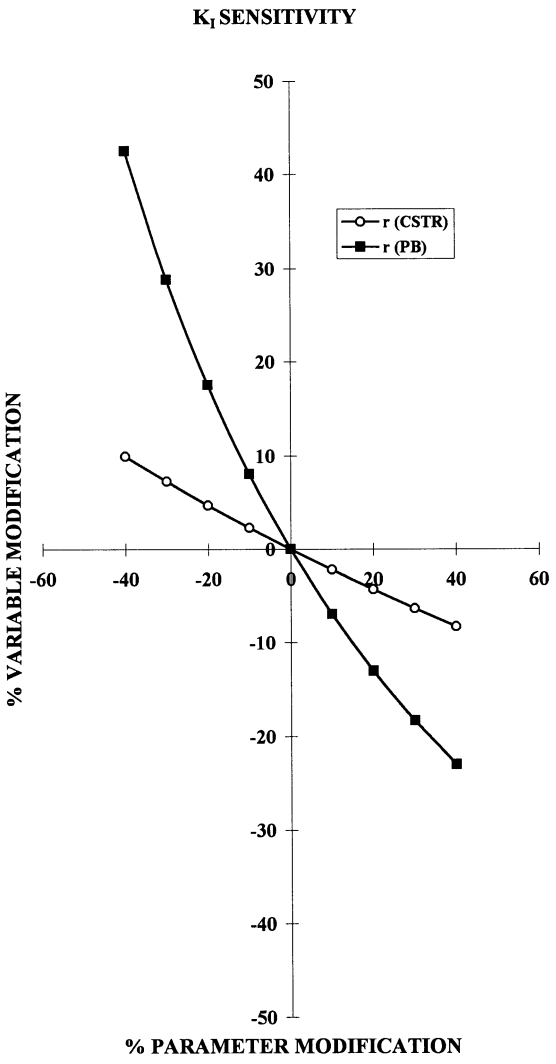


Fig. 12. Analysis of $K_I$ sensitivity.

Eq. (1) written in terms of substrate conversion, becomes:

$$r_{S(CSTR)} = \frac{\mu_{max} \cdot x \cdot S_0^2 \cdot (1 - x)}{K_S + S_0 + S_0 \cdot x \cdot (K_I - 1)} \quad (2)$$

The dependence between the oxidation rate and the conversion in a bioreactor with free suspended cells is given in Fig. 13.

When the biomass is fixed on solid support, the material balance of the limiting substrate is given by the following equation:

$$r_{S(PB)} = \frac{\mu_{max} \cdot X}{Y_{XS}} \cdot \frac{S}{K_S + S + K_I \cdot (S_0 - S)} \quad (3)$$

Eq. (3), written in terms of the substrate conversion becomes:

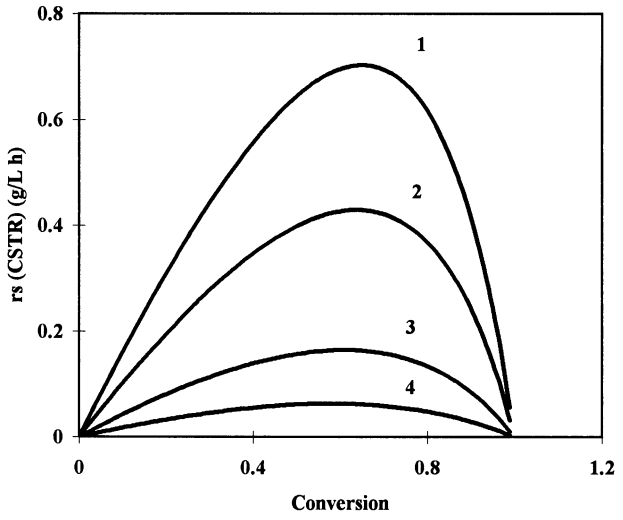


Fig. 13. Effect of input substrate concentration on oxidation rate in a continuous stirred tank bioreactor.

$$r_{S(PB)} = \frac{X}{Y_{XS}} \cdot \frac{\mu_{max} \cdot S_0 \cdot (1 - x)}{K_S + S_0 + S_0 \cdot x \cdot (K_I - 1)} \quad (4)$$

If one analyzes the theoretical curves given in Figs. 13 and 14, it can be seen that change of the input substrate concentration in a bioreactor with free suspended cells influences the process rate much stronger than in a biofilm reactor. Therefore, stability of the process in a biofilm reactor is greater than that of a free suspended cultivation. This again shows that the reactors with immobilised biomass are more stable than the bioreactor with free suspended cells.

The ratio between two types of bioreactor could be determined by dividing Eq. (4) to Eq. (2). The result is given below:

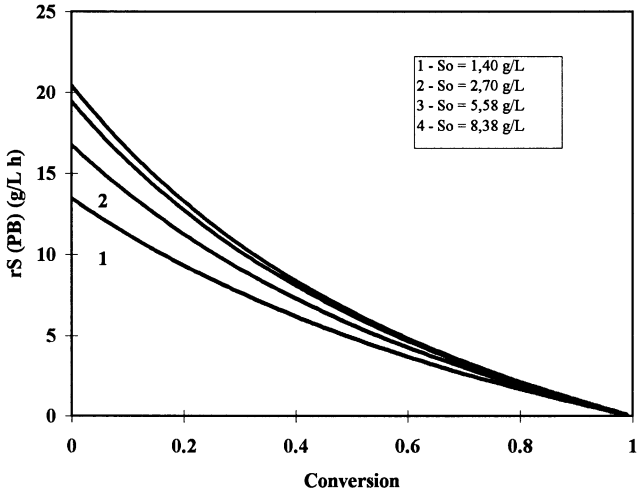


Fig. 14. Effect of input substrate concentration on oxidation rate in a packed-bed bioreactor.

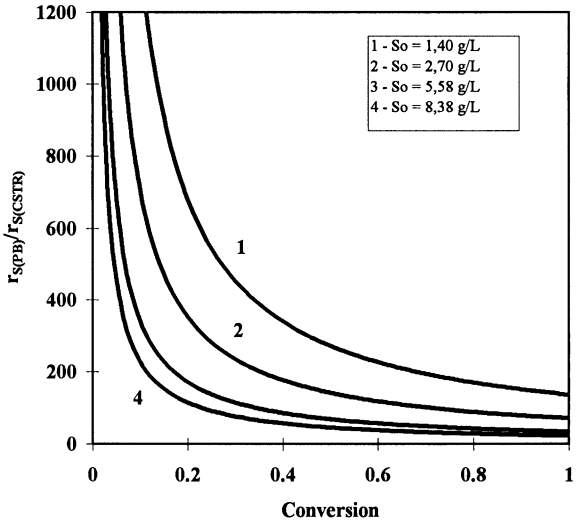


Fig. 15. Comparison of oxidation rate of bioreactors studied as function of input substrate concentration and conversion.

$$r_{S(PB)}/r_{S(CSTR)} = \frac{X}{Y_{XS}} \cdot \frac{1}{x \cdot S_0} \quad (5)$$

Therefore, the volumetric reaction rate in a biofilm reactor is higher than that in a bioreactor with free suspended cells as the fixed micro-organisms concentration is higher than the yield coefficient, the input substrate and the conversion is smaller. A simple functional analysis shows that when the substrate is totally consumed ($x \to 1$), this ratio becomes equal to the complex $X/YS_0$. At $x$ approaching to zero, the ratio between the rates becomes infinity, i.e. the biofilm reactor is incomparably better than a bioreactor with suspended biomass. Another important conclusion is that the biofilm reactor is preferable to the bioreactors with suspended cells when the input substrate concentration is lower. The dependence between the ratio of bioreaction rates and conversion is plotted in Fig. 15.

This ratio is valid not only for the kinetic model proposed, but also for any type of the function $\mu(S)$ when this function is the same in the biofilm and in free suspended bioprocess, and when $Y$ is constant. In fact, the right side of Eq. (5) represents the ratio of biomass concentration, because $YS_0x = X_{\text{suspended cells}}$. In fact:

$$r_{S(PB)}/r_{S(CSTR)} = \frac{X_{\text{immobilised}}}{X_{\text{suspended}}}$$

This equation contains the value of the immobilised biomass concentration as function of ferrous iron oxidation rate in two types of bioreactors.

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