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Kinetic equation for growth of *Thiobacillus ferrooxidans* in submerged culture over aqueous ferrous sulphate solutions

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Abstract

This paper proposes a useful equation for ferrous sulphate oxidation processes by *Thiobacillus ferrooxidans* in batch operations. It is based on the equation proposed by Liu and Branion and predicts the specific growth rate as a function of the concentration of substrate (Fe^{+2}) and product (Fe^{+3}). This expression is based on the thesis that growth of *Thiobacillus ferrooxidans* is competitively inhibited by product. The fit of this equation to the experimental data gives a high theoretical-experimental determination coefficient (r^2 = 95%).

Keywords: *Thiobacillus ferrooxidans*; Ferrous sulphate; Kinetic equation

1. Introduction

Thiobacillus ferrooxidans is a strictly aerobic, gram-negative and chemoautotrophic bacterium which derives the energy for its metabolism from the oxidation of inorganic iron and reduced sulphur compounds (Ingledew, 1982). It is involved in the problem of acid mine drainage of mines and, since it was first isolated by Colmer et al. (1950) during the 1940s, there have been numerous papers referring to the metabolic properties of this micro-organism and its ability to achieve optimum growth under strongly acid conditions (pH < 2.5) (Drobner et al., 1990). Recently, as a result of increasing interest in environmental problems and awareness of the heavy pollution produced from burning coal of high sulphur content, many industries have shown interest in the use of *Thiobacillus ferrooxidans* as a biological solution to desulphurisation of coal (Maloney and Moses, 1991).

In addition to this first application, others have arisen in which this micro-organism is considered

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as an alternative to traditional chemical methods, such as bioleaching of metals, treatment of acid mine drainage, elimination of sulphur from sour gases and removal of hydrogen sulphide from industrial and municipal effluent gases (Jensen and Webb, 1995a). However, in most of these application areas, the results obtained have not been sufficiently satisfactory to bring about the necessary adaptation of the potential of this micro-organism for industrial use (Jensen and Webb, 1995b).

The first step towards an effective industrial usage of *Thiobacillus ferrooxidans* in the above-mentioned processes is the study of the kinetics of oxidation maintained by the micro-organism. There have been several works published on this aspect, focusing on the kinetics of aqueous ferrous sulphate oxidation solutions. From the research undertaken by Lacey and Lawson (1970), Liu et al. (1988), Suzuki et al. (1989), Lizama and Suzuki (1989) and Kumar and Gandhi (1990), various expressions can be selected for the rate of ferrous sulphate oxidation of this micro-organism, but the majority of them are difficult to handle in the design of a bioreactor since they involve many parameters; and fitting a large number of parameters from experimental data is a difficult process.

Therefore, this paper proposes a useful kinetic equation for the growth of *Thiobacillus ferrooxidans* in submerged culture over aqueous ferrous sulphate solutions, in which the number of parameters to be fitted has been considerably reduced, thus facilitating the design of bioreactors.

2. Materials and methods

2.1. Micro-organism

The strain of *Thiobacillus ferrooxidans* used in this study was isolated in the Riotinto mines of Huelva (Spain) and kindly made available by the Biohydrometallurgy Group of the University of Seville (Spain).

2.2. Medium and cultivation conditions

The medium used to grow and maintain the micro-organism was that proposed by Silverman and Lundgren (1959) in g l^{-1}: $(NH_4)_2SO_4$ 3.0; $MgSO_4$ 0.5; K_2HPO_4 0.5; KCl 0.1; $Ca(NO_3)_2$ 0.01 and a variable concentration of $FeSO_4$, depending on the experiment to be performed, in the range between 1000 and 8000 mg l^{-1} of Fe^{+2}.

Erlenmeyer flasks of 500 ml were used, containing 200 ml of medium and 10% of inoculum. The initial pH was adjusted to 2.0, to avoid excessive precipitation of iron III products. Flasks were cultivated at 30°C and 200 rev./min in a rotary shaker; each experiment performed was duplicated.

Experiments were finished when ferrous iron concentration was around 500 mg l^{-1}. The pH varied less than four-tenth in all cases during the experimental processes.

2.3. Analytical methods

The oxidation of ferrous sulphate was monitored by determining its residual concentration in the medium following the 1,10-phenantroline method (Vogel, 1989). In order to measure the concentration of total iron in solution the ferric iron was reduced to ferrous, after filtration of the medium, using hydroxylamine as reducing agent and determining this concentration by the previously mentioned method. Subsequently, the concentration of ferric iron in solution was determined by difference between the ferrous and total iron concentration.

The concentration of total biomass was determined by counting in a Neubauer chamber with optical microscope following the classical method.

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

3. Results

Shown in Fig. 1 are data obtained experimentally for the concentrations of ferrous iron, ferric iron and total biomass. It can be observed that evolution of the process hardly depends on the initial ferrous sulphate concentration.

From experimental data of total biomass (X_i) at each moment (t_i), it is possible to calculate the value of the specific growth rate at that moment (μ_{ci}). The procedure to follow may be based on either numerical differentiation or graphical methods, according to the type and number of experimental data accumulated. In this case, a numerical differentiation procedure has been followed in accordance with the following calculation algorithm:

$$\mu_{c_i} = \left(\frac{dX}{dt}\right)_i \frac{1}{X_i};$$

$$\left(\frac{dX}{dt}\right)_i = \left(\frac{\dfrac{X_i - X_{i-1}}{t_i - t_{i-1}} + \dfrac{X_{i+1} - X_i}{t_{i+1} - t_i}}{2}\right)$$

The results obtained represent the set of values of the specific growth rate for the different concentrations of ferrous iron and ferric iron measured at each moment t_i.

On the basis of the procedure mentioned above, we can look after any correlation between growth and product or substrate concentration and it is possible to propose a useful equation for the specific growth rate of *Thiobacillus ferrooxidans* in aqueous solutions of ferrous sulphate. The equa-

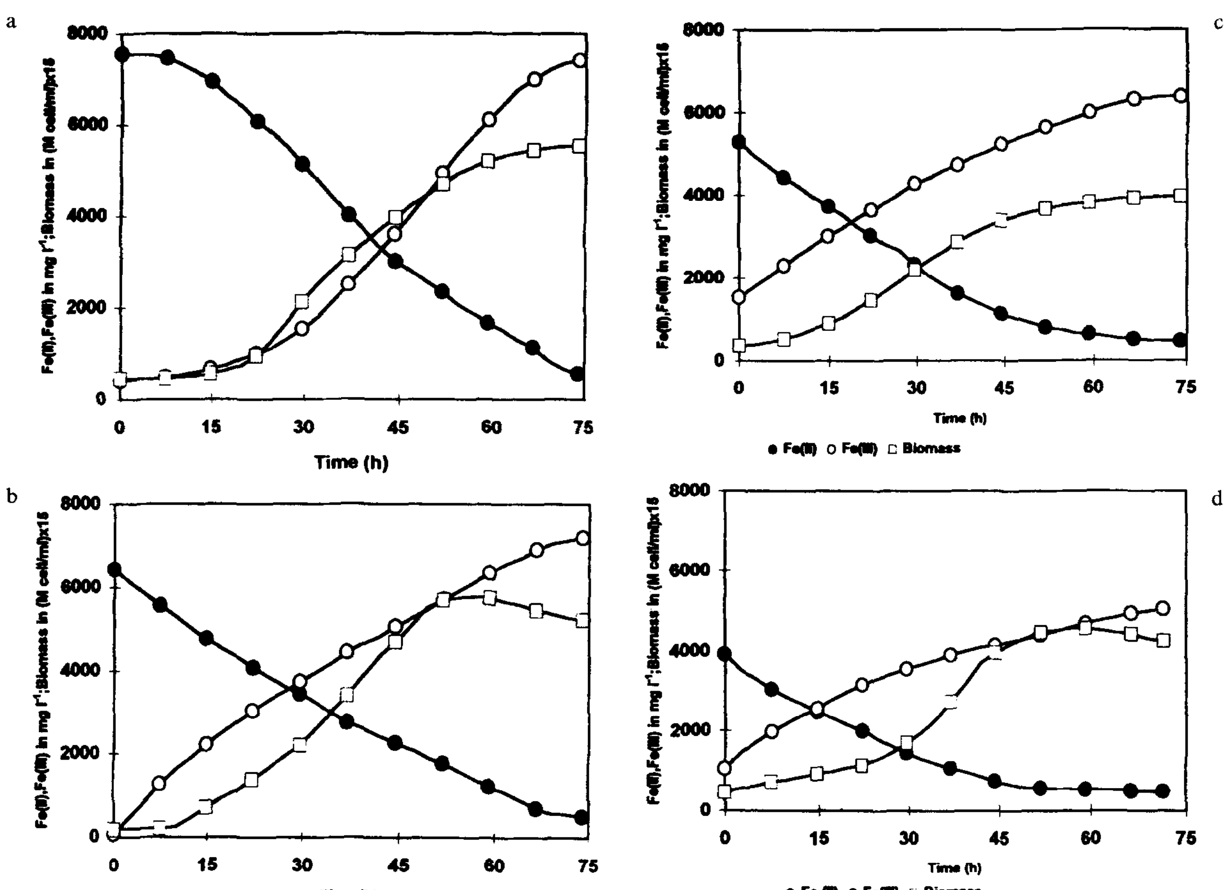

Fig. 1. Evolution of the experimental data for concentrations of substrate, product and total biomass, for different initial concentrations of substrate: (a) 7500 mg l^{-1}; (b) 6500 mg l^{-1}; (c) 5200 mg l^{-1}; (d) 4000 mg l^{-1}; (e) 2700 mg l^{-1}; (f) 1400 mg l^{-1}.

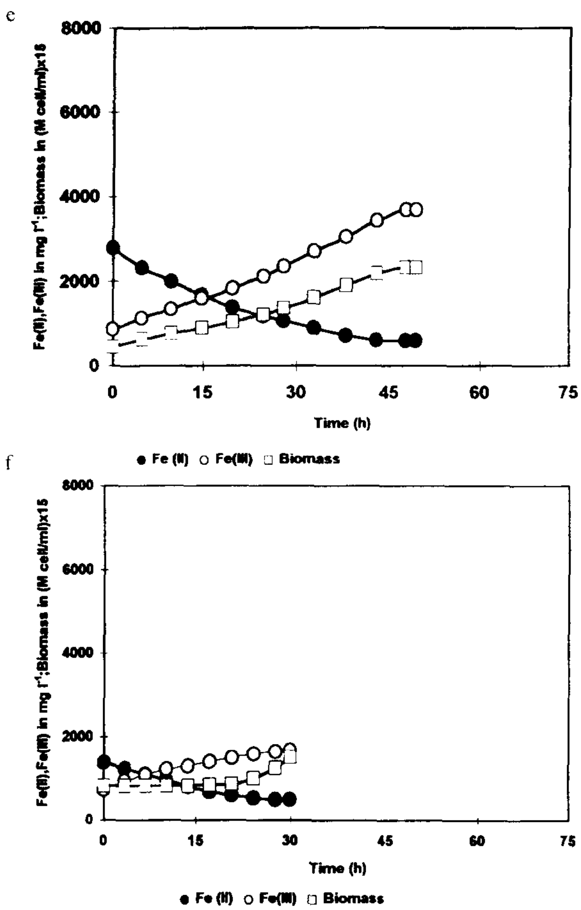

Fig. 1E and 1F.

tion is based on that published by Liu et al. (1988), and predicts the specific growth rate as a function of the concentrations of substrate (Fe^{+2}) and product (Fe^{+3}). The basis of this equation is a kinetic of the competitive inhibition by product type, mathematically represented as follows:

$$\mu_c = \frac{\mu_{max} Fe^{+2}}{K_s + Fe^{+2} + K_i Fe^{+3}}$$

where μ_c is the specific growth rate of the micro-organism, μ_{max} is the maximum specific growth rate, K_s is a parameter representing the degree of affinity of the micro-organism for the substrate and K_i is the constant of inhibition by the product. These parameters, μ_{max}, K_s and K_i are characteristics of the micro-organism and may be influenced to a greater or lesser extent by the level of solvation of the substrate, temperature and pH.

The proposed equation can be theoretically justified by the following considerations. Firstly, the growth of the biomass is a process energetically dependent on the substrate metabolism; thus, the greater the rate at which the cells metabolise the substrate, the greater will be the specific growth rate. Secondly, this metabolism consists of a chain of reactions regulated by enzymes and coenzymes (Ingledew, 1982), in such a way that the rate of the overall process is limited by the rate of the slowest stage. Finally, there exists a typical case of enzymatic kinetic which follows a model inhibition by product.

A non-linear regression procedure based on Marquardt's algorithm was used to perform the mathematical fitting of the coefficients (Marquardt, 1963). The application of this procedure to the set of experimental data of exponential phases of the experiments previously referred to gives the following values for the parameters: $\mu_{max} = 0.14$ h^{-1}, $K_s = 0.94$ g l^{-1} and $K_i = 2.98$ ($r^2 = 0.95$). The regression of the proposed equation is shown in Fig. 2.

The following considerations can be established with respect to this set of fitted values. Firstly, the value of K_s indicates that *Thiobacillus ferrooxidans* has a strong affinity for the ferrous ion during its metabolic activity, in comparison with $K_s = 112$ g l^{-1} of *Saccharomyces cerevisiae* for glucose (Caro et al., 1991) or $K_s = 40.88$ g l^{-1} of *Acetobacter aceti* for ethanol (Romero et al., 1994). Also, the value proposed is very similar to that published by other authors for the same process of oxidation of ferrous sulphate, Lacey and Lawson (1970), $K_s = 1.01$ g l^{-1} (31°C, pH 1.8); Vian et al. (1986), $K_s = 1.12$ g l^{-1} (30°C, pH 2.1) and MacDonald and Clark (1970), $K_s = 0.40$ g l^{-1} (32°C, pH 1.9).

With regard to the coefficient of inhibition by product, its value indicates the existence of a significant inhibitory effect of the ferric ion over the growth of *Thiobacillus ferrooxidans*. However, it is difficult to make comparisons since, although other authors have noted the existence of in-

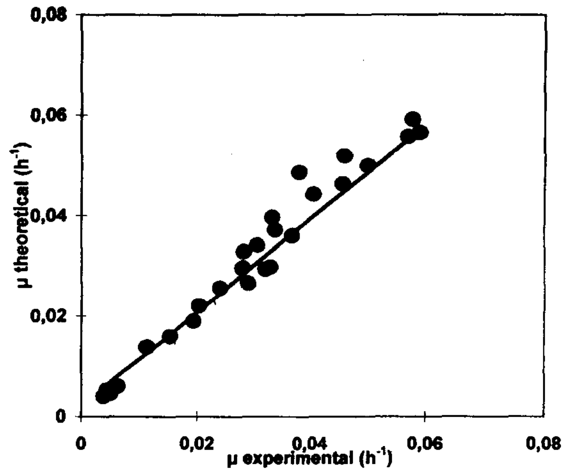

Fig. 2. Representation of the fit between the theoretical and experimental specific growth rates of *Thiobacillus ferrooxidans*.

hibitory effects exerted by the product, their importance has not been quantified from the kinetic point of view (Landesman et al., 1966). In comparison with other micro-organisms, it is possible to notice that the effect of ethanol on *Saccharomyces cerevisiae* growth has an inhibition coefficient by product of 40 g l^{-1}.

Finally, the value obtained for μ_{max} in this study has also proved to be similar to that found by other authors under different experimental conditions. Thus, MacDonald and Clark (1970) established that μ_{max} had a value of 0.145 h^{-1}, working at 32°C and pH 1.9; Kelly and Jones (1978), μ_{max} = 0.143 h^{-1} (30°C, pH 1.6); Vian et al. (1986), μ_{max} = 0.14 h^{-1} (30°C, pH 2.1) and Liu et al. (1988), μ_{max} = 0.11 h^{-1} (35°C, pH 1.8).

A three-dimensional representation of the proposed equation can be seen in Fig. 3.

4. Conclusions

Firstly, it is confirmed that the growth of *Thiobacillus ferrooxidans* in aqueous solutions of

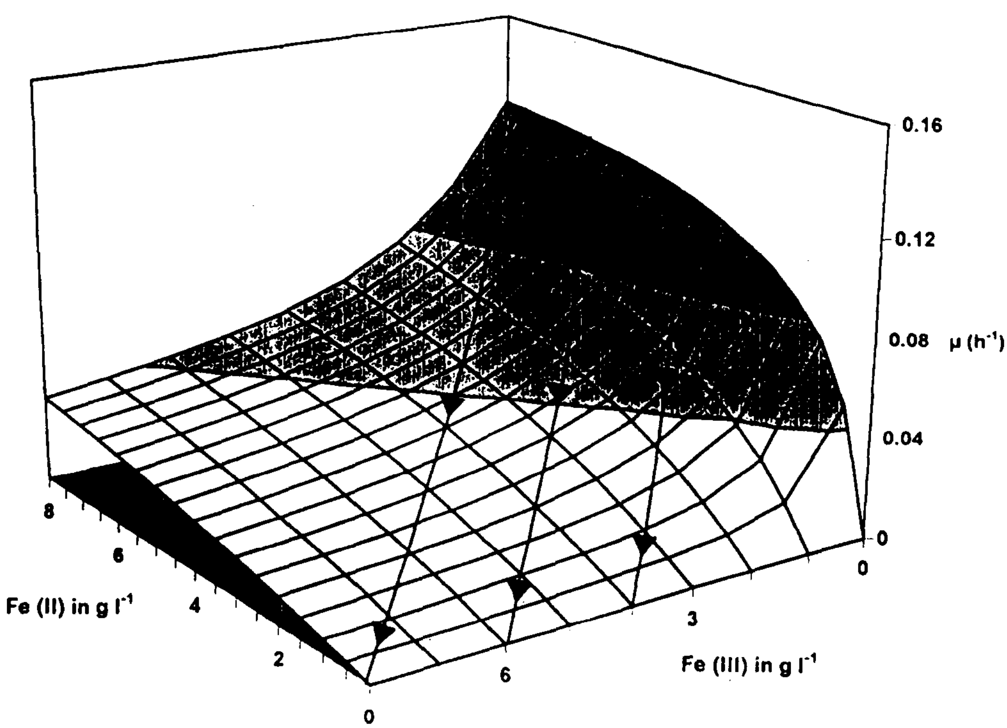

Fig. 3. Three-dimensional representation of the proposed equation for the specific growth rate of *Thiobacillus ferrooxidans*. Line with arrowheads indicates the fermentation course.

ferrous sulphate is affected by a competitive-type inhibition exerted by the product. The proposed equation recognises this effect being constructed on the theoretical foundation of enzymatic action mechanisms.

Furthermore, apart from this theoretical basis, this equation promises to be extremely useful in industrial design because it is expressed in simple mathematical form and it is easy to handle, with easily fitted parameters, under different working conditions; these factors are of basic importance in the design of bioleaching reactors.

Finally, the parameters introduced into the equation have a simple physical meaning and the calculated values show good fitting, so that the calculations provide a high theoretical-experimental coefficient of determination ($r^2 = 0.95$)

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References

Caro, I., Cantero, D. and Pérez, L. (1991) Development of a kinetic model for alcoholic fermentation of must. Biotechnol. Bioeng. 38, 742–748.

Colmer, A.R., Temple, K.L. and Hinkle, M.E. (1950) An iron-oxidising bacterium from the acid mine drainage of some bituminous coal mines. J. Bacteriol. 59, 317–328.

Drobner, E., Huber, H. and Stetter, K.O. (1990) *Thiobacillus ferrooxidans*, a facultative hydrogen oxidiser. Appl. Environ. Microbiol. 56, 2922–2923.

Ingledew, W.J. (1982) *Thiobacillus ferrooxidans*: the bioenergetics of an acidophilic chemolithotroph. Biochim. Biophys. Acta 683, 89–117.

Jensen, A.B. and Webb, C. (1995a) Ferrous sulphate oxidation using *Thiobacillus ferrooxidans*: a review. Process Biochem. 30, 225–236.

Jensen, A.B. and Webb, C. (1995b) Treatment of H_2S-containing gases: a review of microbiological alternatives. Enzyme Microbial Technol. 17, 2–10.

Kelly, D.P. and Jones, C.A. (1978) Factors affecting metabolism and ferrous iron oxidation in suspensions and batch culture of *Thiobacillus ferrooxidans*. In: Murr, L.E., Torma, A.E. and Brierley, J.A. (Eds.), Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena. Academic Press, New York, pp. 20–43.

Kumar, R.S. and Gandhi, K.S. (1990) Modelling of Fe^{+2} oxidation by *Thiobacillus ferrooxidans*. Appl. Microbiol. Biotechnol. 33, 524–528.

Lacey, D.T. and Lawson, F. (1970) Kinetics of the liquid phase oxidation of acid ferrous sulphate by the bacterium *Thiobacillus ferrooxidans*. Biotechnol. Bioeng. 12, 29–50.

Landesman, J., Duncan, D.W. and Walden, C.C. (1966) Iron oxidation by washed cell suspensions of the chemoautotroph *Thiobacillus ferrooxidans*. Can. J. Microbiol. 12, 25–33.

Liu, M.S., Branion, R.M.R. and Duncan, D.W. (1988) The effects of ferrous iron, dissolved oxygen, and inert solids concentrations on the growth of *Thiobacillus ferrooxidans*. Can. J. Chem. Eng. 66, 445–451.

Lizama, H.M. and Suzuki, Y. (1989) Synergistic competitive inhibition of ferrous iron oxidation by *Thiobacillus ferrooxidans* by increasing concentration of ferric iron and cells. Appl. Environ. Microbiol. 55, 2588–2591.

MacDonald, D.G. and Clark, R.H. (1970) The oxidation of ferrous iron sulphate by *Thiobacillus ferrooxidans*. Can. J. Chem. Eng. 48, 669–676.

Maloney, S. and Moses, V. (1991) Microbial desulphurisation of coal. In: Moses, V. and Cape, R.E. (Eds.), Biotechnology, the Science and the Business. Harwood Academic Publishers, London, pp. 581–590.

Marquardt, D.W. (1963) An algorithm for least-squares estimation of non-linear parameters. J. Soc. Ind. Appl. Math. 11, 431–441.

Romero, L.E., Gómez, J.M., Caro, I. and Cantero, D. (1994) A kinetic model for growth of *Acetobacter aceti* in submerged culture. Chem. Eng. J. Biochem. Eng. J. 54, 15–24.

Silverman, M.P. and Lundgren, D.G. (1959) Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. An improved medium and a harvesting procedure for securing high cell yields. J. Bacteriol. 77, 642–647.

Suzuki, Y., Lizama, H.M. and Tackaberry, P.D. (1989) Competitive inhibition of ferrous iron oxidation by *Thiobacillus ferrooxidans* by increasing concentrations of cells. Appl. Environ. Microbiol. 55, 1117–1121.

Vian, M., Creo, C., Dalmastri, C., Gionni, A., Palazzolo, P. and Levi, G. (1986) *Thiobacillus ferrooxidans* selection in continuous culture. In: Lawrence, R.W., Branion, R.M.R. and Ebner, H.G. (Eds.), Fundamental and Applied Biohydrometallurgy. Elsevier, Amsterdam, pp. 395–401.

Vogel, A.I. (1989) Vogel's Textbook of Quantitative Chemical Analysis, 5th edn. Longman Group Ltd., London.