Biological iron oxidation by *Acidithiobacillus ferrooxidans* in a packed-bed bioreactor

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The aim of the work described here was to obtain the preliminary kinetic data required to design an industrial bioreactor and to integrate the system into a process to eliminate the H_2S present in biogas. An analysis of the evolution of bioreactor performance with time (150 days) was performed in order to assess the feasibility of this technique on an industrial scale. A good oxidation rate was obtained despite the transport problems encountered due to occlusion of the porous support.

Keywords:
Acidithiobacillus ferrooxidans; immobilization; polyurethane foam; biogas; hydrogen sulfide

Introduction

Biogas generated during the anaerobic digestion of biodegradable substances is a sub-product with a high energetic value because of its methane content. The use of this gas as a combustible within the same waste treatment plants can reduce the operational costs.

One of the disadvantages inherent in the exploitation of biogas as an energy source is the presence of hydrogen sulfide, usually in proportions that vary between 0.1 and 0.5 %. The removal of H_2S can be achieved by absorbing it in a ferric sulfate solution, a process that produces ferrous sulfate and elemental sulfur.

$$H_2S_{(g)} + Fe_2(SO_4)_{3(aq)} \rightarrow S_{(s)} + 2FeSO_{4(aq)} + H_2SO_{4(aq)}$$

Sulfur can then be separated and ferric sulfate is regenerated by biological oxidation using *Acidithiobacillus ferrooxidans*.

$$2FeSO_{4(aq)} + H_2SO_{4(aq)} + \frac{1}{2}O_2 \xrightarrow{Acidithiobacillus\ ferroxidans} Fe_2(SO_4)_{3(aq)} + H_2O$$

The aim of the work described here was to investigate the continuous oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* immobilized on a polyurethane foam support and to obtain the kinetic data necessary for industrial scale-up of the process. A packed-bed bioreactor configuration, involving simultaneous upward flow of the gas and liquid phase was used.

Biological iron oxidation has been studied using a variety of experimental systems with batch and continuous-flow modes of operation. More recent efforts have used fixed-film approaches, a process that essentially involves various configurations of packed-bed and fluidized-bed reactors with an inert carrier matrix.[1]

The use of polyurethane foam as a support for the passive immobilization of viable *Acidithiobacillus ferrooxidans* cells has been reported to give good results.[2–6] The polyurethane is macroporous and offers lower diffusion resistance to substrate transfer.[7] For this reason, together with its low cost, we found polyurethane foam to be a suitable support for industrial applications.

The chemical reaction involved in this process has been described previously by *Asai et al.*[8] These authors noted that the absorption rates of H_2S increased substantially with the $Fe_2(SO_4)_3$ concentration and pH; the highest rates were obtained between 8 and 17 g l^{-1} Fe^{3+} and with a pH of 2. The bioreactor operating conditions were therefore established to provide the balance between biological oxidation and chemical absorption.

Material and methods

Microorganism and medium

The *Acidithiobacillus ferrooxidans* strains used as inoculum in all the tests were kindly supplied by the Biohydrometallurgy Group of the University of Seville (Spain). The material was originally isolated from mine waters.[9]

The microorganisms were grown in a mineral medium containing the following compounds (per litre of demineralized water): $FeSO_4 \cdot 7H_2O$, 5–15 g;

$(NH_4)_2SO_4$ 3 g; $MgSO_4$ 0.5 g; K_2HPO_4 0.5 g; KCl 0.1 g; $Ca(NO_3)_2$ 0.01 g; $ZnSO_4 \cdot 7H_2O$ 5 mg; $CuSO_4 \cdot 5H_2O$ 0.5 mg; $MnSO_4 \cdot 4H_2O$ 0.5 mg; $CoSO_4 \cdot 7H_2O$ 0.5 mg; $Cr_2(SO_4)_3 \cdot 15H_2O$ 0.25 mg; $Na_2B_4O_7 \cdot 10H_2O$ 0.25 mg; $Na_2MoO_4 \cdot 2H_2O$ 0.25 mg; $NaVO_3$ 0.05 mg. The pH was adjusted to 1.6 with 5 mol L^{-1} H_2SO_4 and the solution was sterilized by filtration.[10]

Laboratory biofilm bioreactors

Figure 1 shows a schematic diagram of the experimental set up. The top part of the bioreactor, where the support particles were placed, consisted of a column with a diameter of 3.5 cm and a length of 27 cm. The volume occupied by support particles was 260 mL. The bottom part of the bioreactor consisted of a column with a length of 14 cm and inlets for the medium and air. The top part of the system incorporated outlets for the effluent and air. The total volume of liquid in the bioreactor was 450 mL.

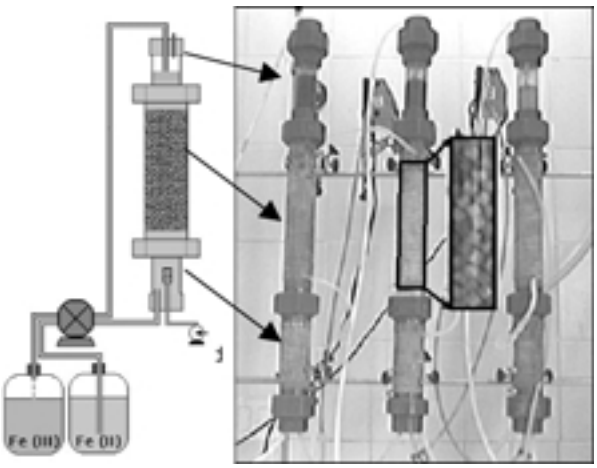

Fig. 1 – *Schematic diagram of the experimental set-up*

In order to control the temperature, the reactor was fitted with a jacket and regulated by a thermostatic water circulator set to 30 °C. Fresh medium was fed by peristaltic pumps and the column was aerated at a flow rate of 180 mL min^{-1}. A dissolved oxygen sensor was used to follow this variable during the fermentative processes.

Immobilization procedure

Acidithiobacillus ferrooxidans cells were immobilized in polyurethane foam cubes of 1 cm length. The cubes had a density of 20 kg m^{-3} and a porosity of around 96 %. Batch culture for the immobilization of cells was performed in 500 ml Erlenmeyer flasks containing 200 ml of mineral medium (5 g L^{-1} Fe^{2+}) and 70 biomass support particles. The medium was inoculated with cell suspension, (φ = 10 %), obtained from a culture in exponential growth and incubated on a rotary shaker for 48 hours at 30 °C. Before complete consumption of the substrate (ferrous iron) had occurred, the spent medium was replaced by fresh medium, followed by five consecutive runs without inoculation. Subsequently, 165 support particles were placed in the bioreactor and one batch culture was carried out with the same medium as used for the kinetic studies. The above procedure is suitable to obtain a support with a uniform colonisation level. In this way, the inoculation of the biological reactor can be carried out to give reproducible conditions.

Kinetic studies. Operating conditions

Each set of experiments started with reactors running according to the immobilization process described. The bioreactor was then switched on to continuous mode and the feed flow rate was adjusted at constant value. The bioreactor was then run until the steady-state was reached. The steady--state conditions were assumed to be established when the ferrous iron concentration in the effluent current altered by less than 5 % during a minimum period of 48 hours. Ferric and ferrous iron concentrations, pH and concentration of bacterial cells in the effluent, were measured during the course of the experiments.

Experiments were performed in media containing 5, 10 and 15 g L^{-1} of Fe^{2+} and dilution rates between 0.1 and 0.5 h^{-1} (calculated according to the total volume of liquid in the bioreactor).

Influence of the process time. Operating conditions

The bioreactor, started-up according to the conditions described above, was maintained in continuous operation for 150 days with dilution rates between 0.1–0.5 h^{-1} and a feed flow rate of 15 g L^{-1} of ferrous iron. During the operational period it was necessary to change the air diffusers due to occlusion produced by ferric precipitates.

Analytical procedures

The concentration of cells in free suspension was determined by direct microscopic counting using a Neubauer chamber (haemacytometer) of 0.1 mm depth and 1/400 mm^2 area and by estimating the total protein concentration.[11]

A modified version of the 1,10-phenanthroline method described by *Vogel*[12] was used to determine the concentration of ferrous iron and total iron. Ferric iron was calculated by subtracting ferrous iron from total iron.

Samples of the matrix materials were removed for study by scanning electron microscopy at the

beginning and the end of column operation. The samples were fixed with 2.5 % glutaraldehyde for 2 h at 4 °C and then rinsed twice with cacodilate buffer (0.1 mol L^{-1}, pH 7), fixed with 1 % osmium tetroxide (pH 7) for 1 h at 23 °C, and then dehydrated by critical-point drying. The dried samples were coated with gold and examined using a Jeol JSM-820 scanning electron microscope.

Results and discussion

Kinetic study of ferrous iron oxidation

A plot of steady-state values for oxidation rates (r_s) versus dilution rate (D) (based on the total volume of the liquid in the bioreactor) showed, that, for a medium containing 5 g L^{-1} Fe^{2+}, the oxidation rate increased proportionally with D (Figure 2). The ferrous iron conversion was in the range 91–97 % (Figure 3).

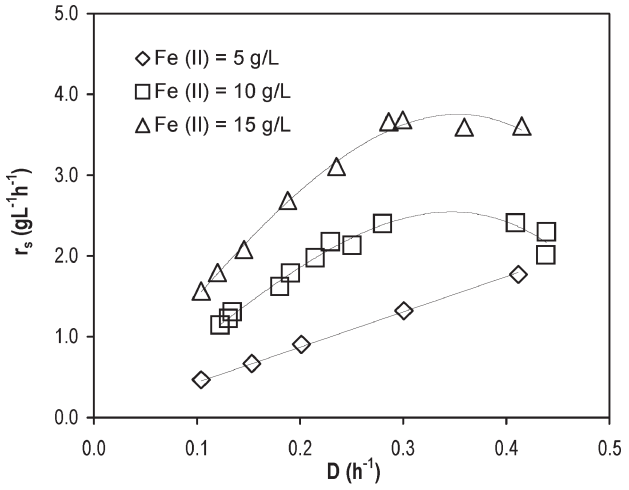

Fig. 2 – *Dependence between oxidation rate and the dilution rate in a biofilm reactor with T. ferrooxidans (steady-state conditions)*

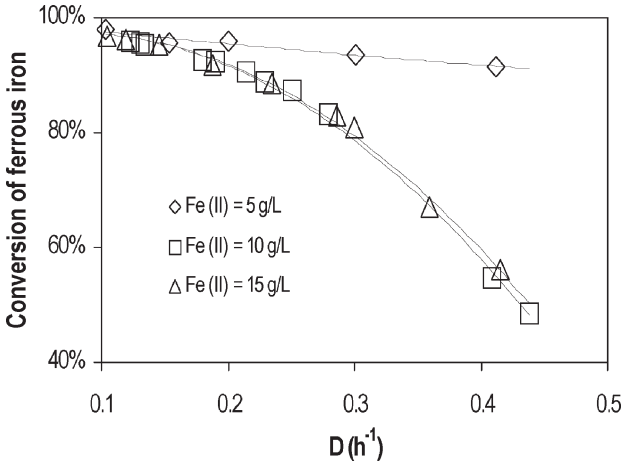

Fig. 3 – *Dependence between conversion of ferrous iron (%) and the dilution rate in a biofilm reactor with T. ferrooxidans (steady-state conditions)*

Two phases could be distinguished for media containing 10 and 15 g L^{-1} Fe^{2+}. At low dilution rates the oxidation rate increased proportionally with D. For dilution rates between 0.3 and 0.4 h^{-1} the ferrous iron oxidation rate reached a plateau and then decreased slightly. However, the ferrous iron conversion decreased continuously. These results show that the microbial activity is affected by iron concentration. Many authors have reported the phenomenon of inhibition by substrate and product.[3,13–18] This phenomenon will be very important when the process is used on an industrial scale, limiting the flow rate of the feed to about 15 g L^{-1} Fe^{2+}.

In Figure 4 the concentration of free cells in suspension is plotted against dilution rate for each set of experiments performed. A similar performance was also observed for the series involving 5, 10 and 15 g L^{-1} Fe^{2+}. An increase in the concentration of biomass in suspension for dilution rates greater than the maximum specific growth rates (0.08 and 0.25 h^{-1}),[19,20] was observed. The presence of suspended cells in the liquid phase at this dilution rate can be considered as being a consequence of the growth of immobilized cells. The possibility of the suspended cells originating by detachment of the biofilm from the support can be discounted as the cells were mainly isolated without forming aggregates. This effect has also been observed during the bacterial culture on biodisk reactors,[21,22] where the mechanical friction of the biofilm is minimal.

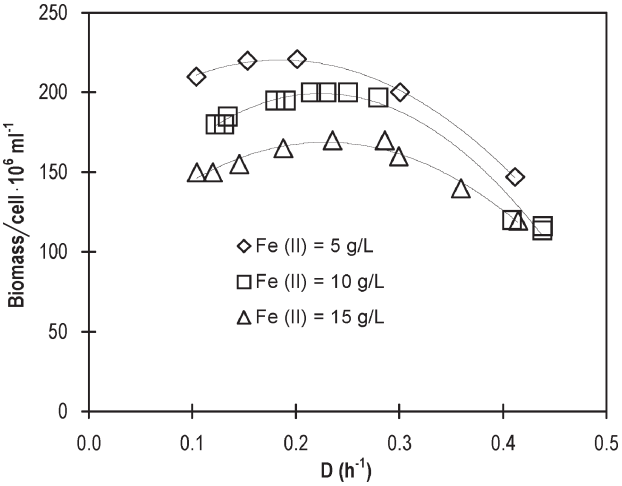

Fig. 4 – *Dependence between concentration of free suspended cells and the dilution rate in a biofilm reactor with T. ferrooxidans (steady-state conditions)*

The cell concentrations in suspension were found to be higher for the experiments involving Fe^{2+} at 5 g L^{-1}, followed by those at 10 and 15 g L^{-1}. This situation is in agreement with the biofilm model suggested by *Karamanev*,[23] where the bacteria adsorbed over the surface of porous jarosite,

formed during the process. Furthermore, the bacteria themselves do not constitute part of the mechanical structure of the biofilm.

Formation of the precipitate depends on the Fe^{3+} concentration in the medium and so it seems reasonable that higher the concentration, the larger the surface area available for colonisation and the lower the concentration of biomass in suspension.

Another major factor for the industrial application of this process is the accumulation of precipitates on the support. In the set of experiments under discussion, the final precipitate was found to accumulate on the support at rates ranging between 0.1 and 0.8 g L^{-1} h^{-1}.

This implies that a one cubic metre reactor working continuously for 100 days would accumulate approximately 2000 kg of precipitate.

Evolution of the performance of the bioreactor with process time

The technological feasibility of this process on an industrial scale depends on the bioreactor performance with respect to process time. In order to obtain information about this aspect, a reactor was operated continually for 150 days with media containing 15 g L^{-1} Fe^{2+} .

The deposits of ferric precipitates on the air diffusers forced us to stop the process periodically in order to perform the necessary cleaning. During these interruptions the reactor was discharged, the diffuser was changed, and the reactor was started-up again with a fresh medium in a discontinuous regime. When all the Fe^{2+} had been consumed (approximately 12 hours) the feed was connected.

The bioreactor maintained a constant oxidation rate of 1.7 g L^{-1} h^{-1} with conversions of 98% during a period of 80 days at a constant dilution rate (0.11 h^{-1}).

After 80 days had elapsed, the dilution rate was successively modified (0,18–0,6 h^{-1}) and the oxidation rate was estimated during the steady-state conditions. This study was performed in order to check if the formation of ferric precipitates in the supports had significantly affected the system performance. The results were compared with the data obtained in the kinetic study (Figure 5). For dilution rates between 0.1 and 0.25 h^{-1} the oxidation rates are similar irrespective of the operation mode. Nevertheless, as the rate of dilution was increased, the oxidation rate decreased in a continuously operating reactor. This change could represent a loss in performance caused by the obstruction of the support pores by the ferric precipitates, a situation that would restrict the transport of the substrates inside. In order to check that the extent of pore occlusion of the porous in the support was due to the formation of ferric precipitates, photomicrographs were obtained once the experiment was over (Figure 6).

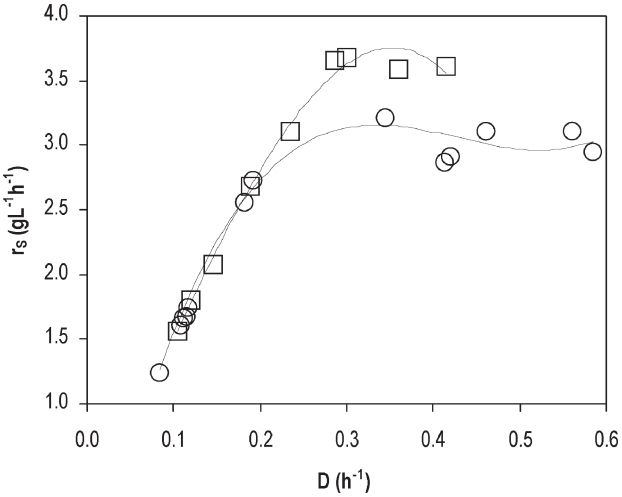

Fig. 5 – *Steady-state values of iron oxidation rate (□ Kinetic studies); (○ Influence of the process time)*

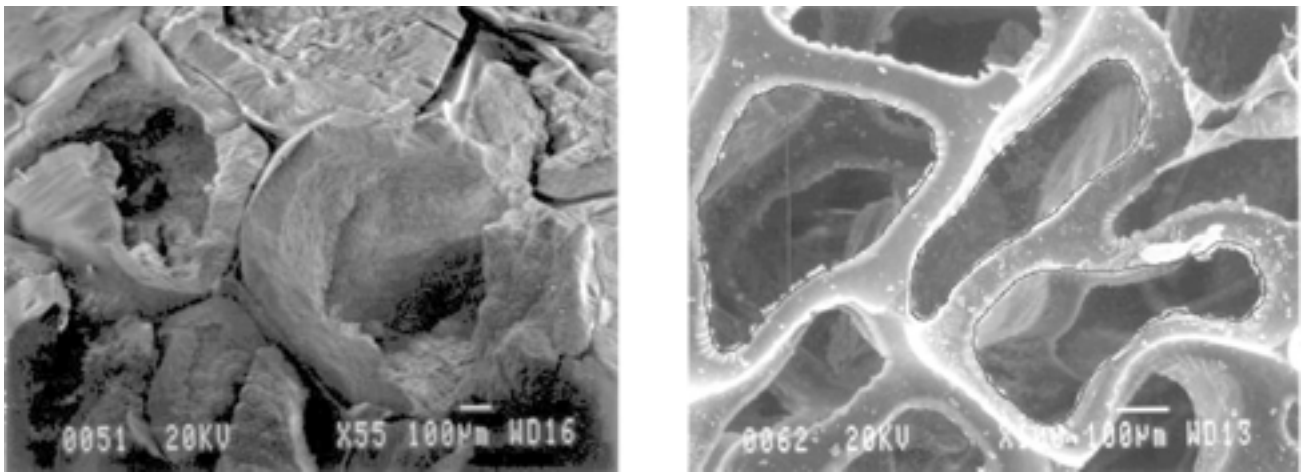

Fig. 6 – *Scanning electron micrograph of support particles. Left: fresh polyurethane foam biomass support particle. Right: after termination of continuous operation mode.*

It can be seen that precipitates had appeared in the pores and this could well be the reason for the decrease in reactor performance.

The results discussed above show that the volume of a biological reactor integrated in a system for the purification of biogas, can be calculated. A sewage treatment plant for a population of 200,000 can produce a volume of biogas of approximately 400 m^3 h^{-1} with an average volume fraction of 7,000 ppm H_2S. Bearing in mind that an oxidation rate of 3.7 g L^{-1} h^{-1} corresponds to a dilution rate of 0.3 h^{-1}, a mass concentration of ferrous iron of approximately 15 g L^{-1} would be necessary for a 2 m^3 bioreactor. In conclusion, it can be stated that the biological regeneration of ferrous sulfate in the process for the removal of H_2S in biogas is not a constraining factor for the industrial application of this process.

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