J Chem Technol Biotechnol 79:163–170 (2004)
DOI: 10.1002/jctb.956

Biological oxidation of ferrous iron: study of bioreactor efficiency

MM Mesa,* JA Andrades, M Macías and D Cantero

Departamento de Ingeniería Química, Tecnología de Alimentos y Tecnologías del Medio Ambiente, Facultad de Ciencias, Universidad de Cádiz, Campus Río San Pedro, Spain

Abstract: The bio-oxidation of ferrous iron is a potential industrial process for the regeneration of ferric iron in the removal of H_2S. In the first stage, H_2S is selectively oxidized to elemental sulfur using ferric sulfate. The ferrous sulfate produced is oxidized to ferric sulfate using *Thiobacillus ferrooxidans* for recycle and reuse in the process. The aim of the work described here was to investigate continuous oxidation of ferrous iron by immobilized *T ferrooxidans* and the factors which can directly affect the oxidation rate in order to assess the feasibility of this technique on an industrial scale. An analysis of the evolution of bioreactor performance with time (125 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. On the other hand, the toxic effects due to absorption in the ferric solution of one or more compounds from the gas digester were studied using a ferric iron solution from the absorption process. The results indicate the feasibility of the biological system for the regeneration of the ferric-absorbing solution. Finally, a previous study for the design of an industrial bioreactor to regenerate ferric sulfate solutions, used to remove H_2S from biogas in a wastewater-treatment plant (Jerez de la Frontera, Spain), is introduced. Good biological oxidation performances have been obtained using a pilot plant bioreactor of 500 dm^3.

Keywords: biogas; H_2S; ferrous iron; ferric iron; iron-oxidizing bacteria

INTRODUCTION

Biogas, which is generated from the anaerobic digestion of biodegradable matter, leads to a subproduct that has a high energy value due to its methane content. The use of this gas as a fuel can decrease both energy costs and operational costs in waste treatment plants where this fuel is generated.

The presence of hydrogen sulfide, in the range 0.1–0.5%, is the main drawback in the use of biogas as an energy source. A number of physico-chemical processes such as the dry gas redox process, liquid redox processes and liquid adsorption processes are usually employed for desulfurization of gases containing hydrogen sulfide, however, they have high capital costs, demand large energy inputs and result in the generation of hazardous wastes.[1,2] Apart from this a variety of biochemical processes using various bacterial species have been reported in the literature with the capacity of removing sulfur in various forms from gaseous emissions. These processes are characterized by small capital costs and low energy requirements for H_2S removal.[3] In these processes the major use has been made of bacteria of *Thiobacillus* sp for an effective desulfurization process.[4] Certain photosynthetic bacteria belonging to families Chromatiaceae and Chlorobiaceae are also being used to metabolize H_2S, however, the advantages associated with biochemical processes utilizing these bacterial species suffer from the serious drawback of a slow rate of reaction that makes the processes uneconomic.

A combined chemical–biological method to remove H_2S from gases is based on two steps corresponding to absorption involving chemical reaction of the gas in a solution of ferric sulfate (where the ferric ion is converted to ferrous), and biological oxidation of ferrous ions in the solution to produce ferric ions again. The biological oxidation involves the biocatalytic activity of the bacterium *Thiobacillus ferrooxidans*[5–8] (reclassified as *Acidithiobacillus ferrooxidans*).

The process can be represented as:

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

The elemental sulfur is removed by means of sediment formation and filtration. The resultant

* Correspondence to: MM Mesa, Departamento de Ingeniería Química, Tecnología de Alimentos y Tecnologías del Medio Ambiente, Facultad de Ciencias, Universidad de Cádiz, Campus Río San Pedro, Spain
E-mail: mariadelmar.mesa@uca.es
Contract/grant sponsor: Comisión Interministerial de Ciencia y Tecnología (Spain); contract/grant numbers: AMB93-0353; AMB99-0657-C02-01
(*Received 4 February 2002; revised version received 1 September 2002; accepted 12 September 2003*)
Published online 5 January 2004

solution enters the bioreactor where the bacteria perform the oxidation process according to the following equation:

$$2FeSO_{4(aq)} + H_2SO_{4(aq)} + \tfrac{1}{2}O_2 \xrightarrow{\textit{Thiobacillus ferrooxidans}} Fe_2(SO_4)_{3(aq)} + H_2O$$

The oxidized solution that results from this biological step can be used again in the absorption step, leading to minimal consumption of the oxidant.

One of the advantages of this kind of process is that the reaction between the hydrogen sulfide and the ferric sulfate is very rapid and goes to completion. Furthermore, the reaction does not produce toxic waste or generate other waste products apart from sulfur, process characteristics that minimize associated treatment costs.

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.[9]

Details concerning the chemical reaction have been published by Asai *et al*. These authors noted that the absorption rates increased substantially with both the $Fe_2(SO_4)_3$ concentration and the pH; the highest rates were obtained at concentrations between 8 and 17 g dm^{-3} Fe^{3+} and at pH values between 1.5 and 2.[10] Thus, for large-scale applications it will be necessary that this process operates at a rate which is acceptable.

A major factor for the industrial application of this process is the accumulation of precipitates on the support. During the biological iron oxidation abundant amounts of jarosite (basic iron(III) sulfate) are precipitated. Some authors have observed that these deposits could participate directly in the process of biofilm formation.[11–13] However, when the bioreactor is operated continuously over a long period of time, the formation of ferric precipitates in the supports could represent a loss in performance caused by the obstruction of the support pores, restricting the transport of substrates inside.

During the process of chemical oxidation, when ferric sulfate reacts with H_2S, elemental sulfur is produced. Sometimes the separation of elemental sulfur is not properly achieved. It has been shown that the presence of elemental sulfur in the ferrous sulfate fed to the bioreactor affects the efficiency of the bio-oxidation process. The serial subculture of *T ferrooxidans* in medium containing sulfur has been reported.[19] Another important factor is the presence of ferric ions, which remain unreactive with H_2S. A trend of competitive inhibition by ferric ions in ferrous sulfate solutions has been cited in the literature.[19,20] In previous work,[18] we found an inhibitory effect of ferric iron from 5 g dm^{-3}. Therefore, to integrate the biological oxidation with chemical absorption, the operating conditions of the bioreactor must be established with these considerations in mind.

On the other hand, toxic effects due to absorption in the ferric solution of one or more compounds from the gas digester could be produced. Pagello *et al*[7] have reported that several anions can inhibit the growth of *T ferrooxidans*. In an acid environment, these ions will be complexed with protons. If in this form they have low polar momentum, they can permeate the cell membrane to subsequently dissociate in citosol (which has a near neutral pH). They can, consequently, accumulate inside the cell. This is true, in particular, for the protonated forms of some halogen salts, which are found as gases in normal conditions. For this reason this study is essential for the long-term operation of the system as a closed cycle. Accordingly, studies were carried out using two packed-bed bioreactor configurations with the microorganism immobilized on a polyurethane foam support. The use of polyurethane foam as a support for the passive immobilization of viable *T ferrooxidans* cells has been reported to give good results.[14,15,16,17,18] The polyurethane is macroporous and offers lower diffusion resistance to substrate transfer. For this reason, together with its low cost, we found polyurethane foam to be a suitable support for industrial applications.

In order to perform a comparative study of the results, experiments were also undertaken in which the efficiency of the reactors was not influenced by the factors mentioned above.

Finally, in this paper a pre-design study is presented of an industrial bioreactor project for implantation in a wastewater treatment plant (Jerez de la Frontera, Spain) for recovery of ferric sulfate used for the removal on H_2S in a biogas stream.

MATERIALS AND METHODS

The strain of *Thiobacillus ferrooxidans* (UCA 2) was kindly supplied by the Biohydrometallurgy Group of the University of Seville (Spain). It was originally isolated from mine waters.

Thiobacillus ferrooxidans was grown in a mineral medium containing, per dm^{-3} demineralized water: $FeSO_4.7H_2O$ 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; $ZnSO_4.7H_2O$ 5 mg; $CuSO_4.5H_2O$ 0.5 mg; $MnSO_4.4H_2O$ 0.5 mg; $CoSO_4.7H_2O$ 0.5 mg; $Cr_2(SO_4)_3.15H_2O$ 0.25 mg; $Na_2B_4O_7.10H_2O$ 0.25 mg; $NaMoO_4.2H_2O$ 0.25 mg; $NaVO_3$ 0.05 mg. The medium was adjusted to pH 1.6 with 5 M H_2SO_4 and sterilized by filtration.[21]

The effluent obtained from an absorber (packed column) was used to feed the reactors installed at the waste water treatment plant (WWTP). H_2S was removed from the biogas flowing from the anaerobic sludge digestion within the WWTP at Jerez de la Frontera (Spain).

Analytical methods

A modified version of the 1,10-phenanthroline method described by Vogel[22] was used to determine the concentration of ferrous iron and total iron. Ferric iron was calculated by subtracting ferrous iron from total iron. For the purposes of comparison, ferrous and total iron were determined by titration against 0.017 M potassium dichromate in the presence of *N*-phenylanthranilic acid as indicator.[22]

The Tutweiler method was used to analyse the H_2S contained in the gas effluent in the absorber. The average composition of biogas from the anaerobic digester was 0.5% H_2S and 65% CH_4.

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 M, 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.

Bench-scale bioreactors

The biological oxidation of ferrous sulfate was carried out in packed-bed reactors (Fig 1). The reactors were made of PVC and the bed was made up from polyurethane foam particles that were 1 cm^3 in size. The thermostat consisted of an external jacket that maintained the temperature at 30 °C. Fresh medium was fed by peristaltic pump. Two reactors of different size were used:

Bioreactor (I): Column with a diameter of 3.5 cm and length of biocatalyst bed of 27 cm. Volume occupied by support particles was 0.260 dm^3.

Bioreactor (II): Column with a diameter of 5.7 cm and length of biocatalyst bed of 79 cm. Volume occupied by support particles was 2 dm^3. This reactor was used to regenerate the solution of ferric sulfate required to assess the performance of the absorber.

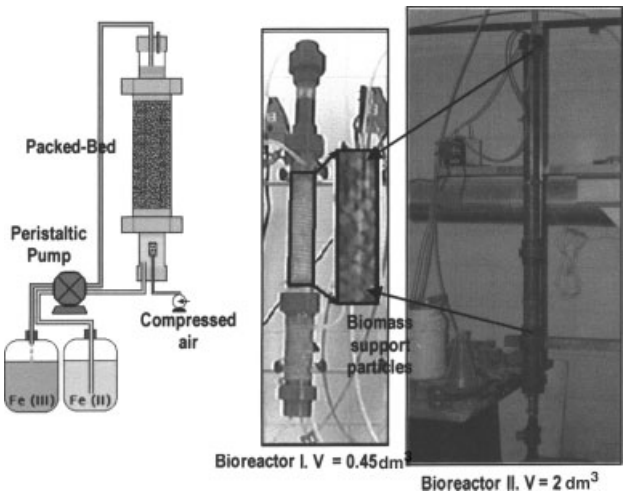

Figure 1. Schematic diagram and photographs of the bench-scale bioreactors.

The total iron content in the culture medium feeding the reactors was maintained at the same level in all experiments with a value of 15 g dm^{-3} and a pH of 1.6. The use of this concentration gave rise to ferric iron concentrations of between 10 and 15 g dm^{-3} in the bioreactor effluent and this was then used for the removal of H_2S. These were found to be the most appropriate conditions for the best absorber performance.[10]

Thiobacillus 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 cm^3 Erlenmeyer flasks containing 200 cm^3 of mineral medium (5 g dm^{-3} Fe^{2+}) and 70 biomass support particles for bioreactor (I) and 400 cm^3 of mineral medium and 140 biomass support particles for bioreactor (II)

The medium was inoculated with cell suspension, 10% (v/v), obtained from a culture in exponential growth and incubated on a rotary shaker for 48 h 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 bioreactor (I) and 1000 in bioreactor (II) and one batch culture was carried out with the same medium as used for the kinetic studies.

The purpose of this repeated batch operation was to sufficiently increase the immobilized cell concentration before continuous operation. The above procedure is suitable to obtain a support with a uniform colonization level. In this way, the inoculation of the biological reactor can be carried out to give reproducible conditions.

At the end of the last batch run the operation was converted into continuous mode. Steady-state operation was considered to be established when the ferrous iron concentration in the effluent varied by less than 5% during a minimum period of 48 h. Ferric and ferrous iron concentrations and pH were measured.

Experiments were performed in media containing 15 g dm^{-3} and dilution rates between 0.5 and 7 g dm^{-3} h^{-1} (based on the volume occupied by the biocatalyst bed).

Absorber

A packed column was used to absorb H_2S into the Fe^{3+} solution and this column contained a random packing of Rasching rings. Figure 2 shows the installation used in this process. The packing column consisted of a PVC tube of 130 cm length and 3.35 cm diameter. The packing material was Rasching rings of 9 mm external diameter and these were supported by a perforated silicone plate. The features of the rings and the bed are outlined in Table 1.

The absorbent was kept in a stainless steel tank of 25 dm^3 capacity. The liquid was fed into the column from the higher level through a spreader

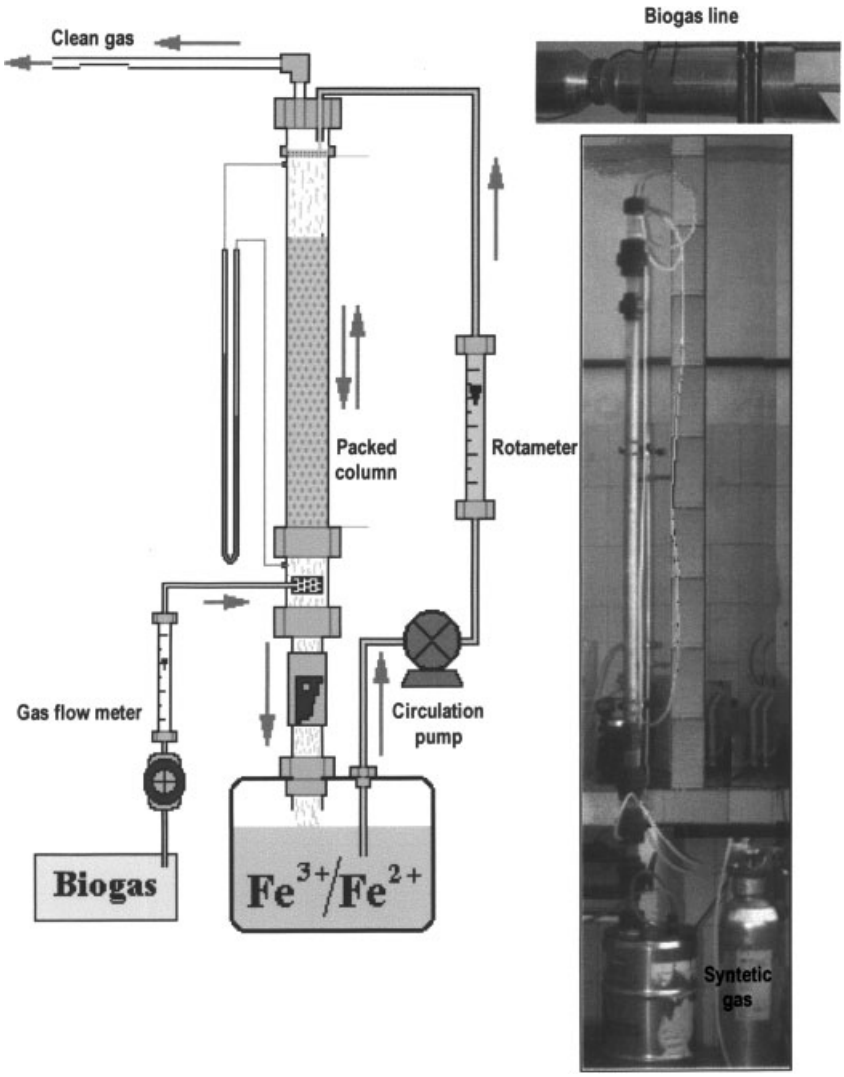

Figure 2. Schematic diagram and photographs of the absorber.

Table 1. Features of the absorption column

Features of the bed			Features of the packing		
Packing weight	M	80.8 g	External-diameter	ϕ_{ext}	0.9 cm
Hole volume	V_h	124 cm^3	Ring thickness	e_p	0.09 cm
Total volume	V_t	163 cm^3	Internal-diameter	ϕ_{int}	0.72 cm
Real density	ρ_r	2.07 g cm^{-3}	Ring height	h_p	1.06 cm
Apparent density	o_{ap}	0.50 g cm^{-3}	Volume	V_p	0.240 cm^3
Column diameter	ϕ_c	3.35 cm	Area	S_p	5.86 cm^2
Porosity	ε	0.76	Specific area	σ_p	24.1 cm^{-1}
Number of particles	n/V	0.98 n.cm^{-3}	Equivalent diameter	ϕ_{eq}	0.7 mm
Specific area	σ_L	5.77 cm^{-1}	Sphericity	φ	0.32
Bed, real length	L	111.7 cm			

plate in order to ensure a uniform distribution over the packing. After passing through the column, the liquid was returned to the storage vessel through a PVC pipe, which also incorporated a sampling point. The gas was introduced into the column base through a side access point located below the bed level.

The pressure drop through the bed was estimated by using a manometer tube at the column head and base. The values were found to be between 2 and 5 cm of water per metre of bed depending on the liquid and gas flows used.

The absorbing solution had an average composition of Fe^{3+} 13.5 g dm^{-3} and Fe^{2+} 0.7 g dm^{-3} and the pH was 2.1. The absorption efficiency of H_2S from the biogas was always maintained above 90% by working with liquid flows between 48 and 58 $dm^3\,h^{-1}$ and gas flows between 60 and 70 $dm^3\,h^{-1}$.

In order to check if the gas resulting from the digesting process contained some product which was

toxic to the microorganism, we used a synthetic gas, with an average composition of H_2S 0.47%, methane 70% and CO_2 29.5%, and biogas from the anaerobic digester. The absorption column was installed in the WWTP at Jerez de la Frontera (Spain), with the gas line directly connected to the biogas outlet of the anaerobic digester.

Plant pilot bioreactor

The bioreactor consisted of a stainless steel tank in which was introduced a stainless steel structure (Fig 3). This structure supported the air diffusers, a coil for temperature control by water circulation, and packed beds.

The packed beds were made of plastic screens with diameter of 10 cm and 100 cm high. The packed beds were divided in five parts to avoid compression of the support particles. Then, 115 support particles having a size of 8 cm^3 were introduced in each packed bed. The total number of packed beds was 28 and the total number of biomass support particles was 16 100.

For efficient oxygen mass transfer, five membrane diffusers, each with a surface of 0.025 m^2, were, used at the bottom of the reactor. The operating air flow rate was 0.5 $m^3\,h^{-1}$.

The working volume of the bioreactor was 500 dm^3. Before the operation was started, the tank was filled with 200 dm^3 of inoculum (incubated in laboratory reactors) and 300 dm^3 medium. The bioreactor operated in batch mode. 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. Once the last step was finished, a continuous flow mode of operation was initiated.

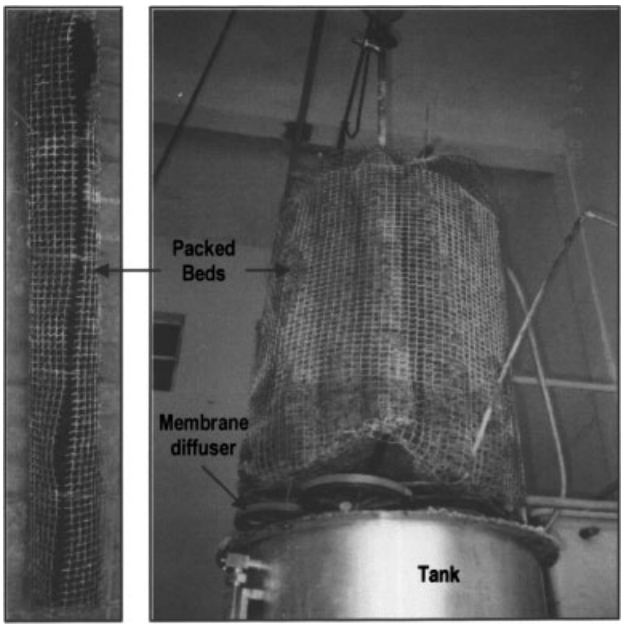

Figure 3. Photographs of internal configuration and packed beds in pilot plant bioreactor.

RESULTS AND DISCUSSION

Evolution of the performance of the bioreactor with process time

The technological feasibility of this process on an industrial scale depends on the bioreactor's 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 dm^{-3} Fe^{2+}. This study permitted us to check if the formation of ferric precipitates in the supports had significantly affected the oxidation rate.

During a period of 80 days, the bioreactor was fed continuously with an Fe^{2+} solution (15 g dm^{-3}), which was prepared in the laboratory, at a flow rate of about 2 g Fe^{2+} $dm^{-3}\,h^{-1}$. The bioreactor maintained a constant oxidation rate of 1.7 g $dm^{-3}\,h^{-1}$ with conversions of 98%.

After 80 days had elapsed, the flow rate was successively modified (2–7 g Fe^{2+} $dm^{-3}\,h^{-1}$) and the oxidation rate was estimated during the steady-state conditions.

Figure 4 represents the oxidation rates obtained versus the different flow rates of feed assayed from day 80 after the reactor start-up.

These results were compared with the data obtained in experiments where the bioreactor efficiency during the first steady state after start-up was studied.[18] In this study each set of experiments started from a non-colonized support and the bioreactor was then switched on to continuous mode until the steady state was reached (independent experimental runs).

For the independent experimental runs, it can be seen that the oxidation rates have a linear relationship with the flow rate of feed until values higher than 4.5 g Fe^{2+} $dm^{-3}\,h^{-1}$ are reached. Above this value, and up to at least 6.5 g $dm^{-3}\,h^{-1}$, the oxidation rate can be considered constant. It might seem that, under these operating conditions, the reactor studied is able to oxidize a maximum of 3.8 g Fe^{2+} $dm^{-3}\,h^{-1}$.

It can be seen from Fig 4 that the oxidation rates are similar in both experimental runs until

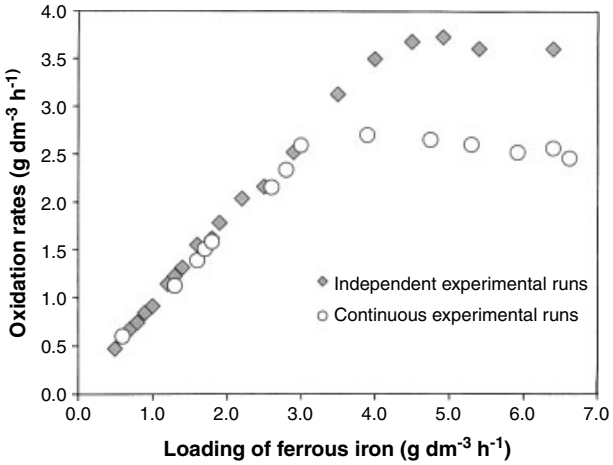

Figure 4. Effect of loading of ferrous iron on oxidation rate for different operating modes.

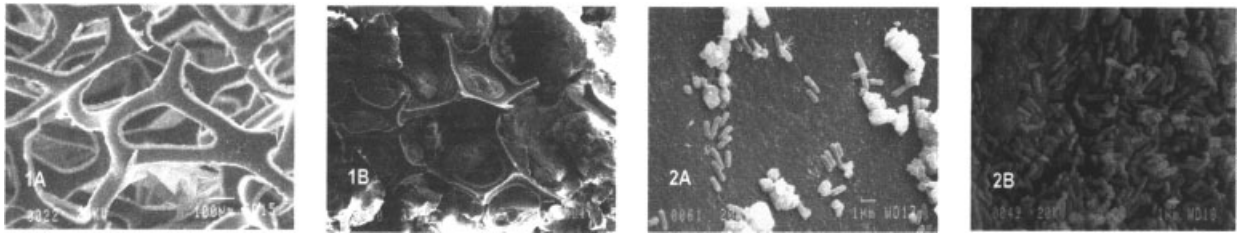

Figure 5. Scanning electron micrographs 1A, 1B, Polyurethane foam support particles. A, at the beginning of continuous operation mode; B, after 80 days of operation. 2A, 2B, Bacterial colonization in surface of polyurethane foam particle in the packed-bed bioreactor: A, at the beginning of continuous operation mode; B, after 80 days of operation.

a flow rate of feed of 3.2 g Fe^{2+} dm^{-3} h^{-1} is used. Nevertheless, as the flow rate increased the oxidation rate decreased in the continuous experimental runs. 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 (Fig 5). It can be seen that precipitates had appeared in the pores and this could well be the reason for the decrease in reactor performance.

Nevertheless, during this period of time, a constant value of oxidation rate was reached. As most bacteria were immobilized on the ferric iron precipitates, in turn, the number of attached bacteria, the Fe^{2+} oxidation rate and Fe^{3+} productivity may be expected to increase constantly. However, new iron deposits covered bacteria previously immobilized, thus preventing these from accessing nutrients. Beside, a gradual increase of bacterial seriously affected nutrient availability, especially oxygen and carbon dioxide, thus limiting growth.

On the other hand, 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 dm^{-3} h^{-1}.

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

The collected data confirmed that kinetics limitation of the biological step in the process could be caused by physical phenomena and mass transfer limitations connected to the formation of the ferric precipitate. The presence of a great amount of solids affected the process performance negatively and finally led to subsequent technological problems (reactor clogging). To summarize, controlling the stability and accumulation of the ferric solution seems to be a basic consideration for practical application of the process on an industrial scale and close-cycle operation.

The following points can be inferred from the data obtained: an industrial reactor that works with a flow rate of feed lower than 3.2 g Fe^{2+} dm^{-3} h^{-1} would not be affected by pore occlusion in the support, due to the precipitation of solids in the reactor, for at least 125 days. If higher feed rates are used, the reactor efficiency gradually decreases to 70% of the maximum value achieved.

Thus, the replacement of the support in an industrial reactor could be undertaken at periods of around 4 months after initial operation without loss of reactor efficiency.

Effects on the bioreactor efficiency of the Fe^{2+} feed obtained by chemical absorption reaction

The experiments designed for evaluation of optimal operating conditions in the absorber are discussed hereunder. In the experimental test performed in the absorber the following parameters were measured: ferric iron concentration, and hydrogen sulfide concentrations in inlet (gas from digester) and outlet gas.

First, we investigated the influence of gas flow and the liquid recirculation rate. A set of nine experiments was arranged with synthetic gas and medium containing 13 g dm^{-3} Fe^{3+} and 0.7 g dm^{-3} Fe^{2+} (from biological reactor). The gas flow rate used in the different tests varied approximately between 60 and 100 dm^3 h^{-1} and the liquid recirculation rate between 40 and 100 dm^3 h^{-1}. The highest conversions (around 90%) were achieved at liquid recirculation rates of 40–60 dm^3 h^{-1} and gas flows of 60–70 dm^3 h^{-1}.

In order to evaluate the effect of ferric iron concentration on absorption rate, tests were carried out in the absorber under the following operating conditions: 13 g dm^{-3} Fe^{3+} and 0.7 g dm^{-3} Fe^{2+}; pH = 1.95; 67 dm^3 h^{-1} gas flow; liquid recirculation rate 47 dm^3 h^{-1}.

Results from this experiment indicated that the ferric iron concentration decreased quickly from the initial concentration to around 9 dm^3 h^{-1}, in approximately 4 h. After this time, this tendency started to become less marked.

The results of kinetic evaluation of H_2S oxidation are shown in Fig 6, where the absorption rates and % elimination are plotted versus the ferric iron concentration.

It may be noted that the absorption rate is maximum in the region between 10 and 13 g dm^{-3}, while it decreases remarkably in the region of lower concentrations.

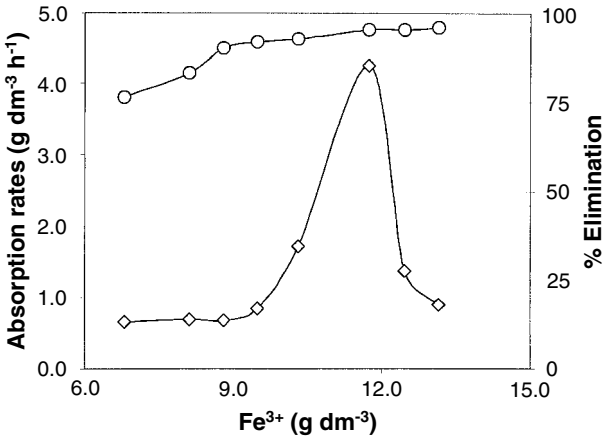

Figure 6. Effect of ferric ions on absorption process.

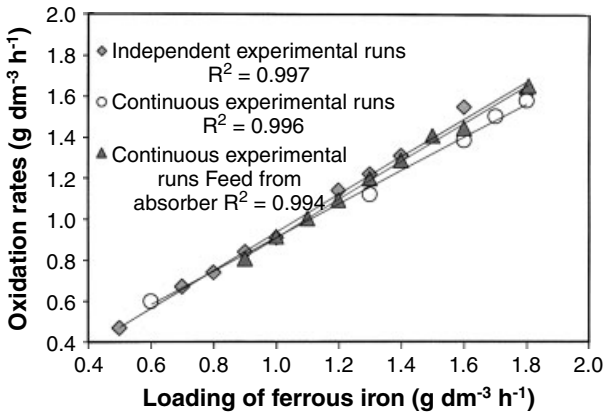

Figure 7. Comparison of the oxidation rate obtained in the different experimental sets.

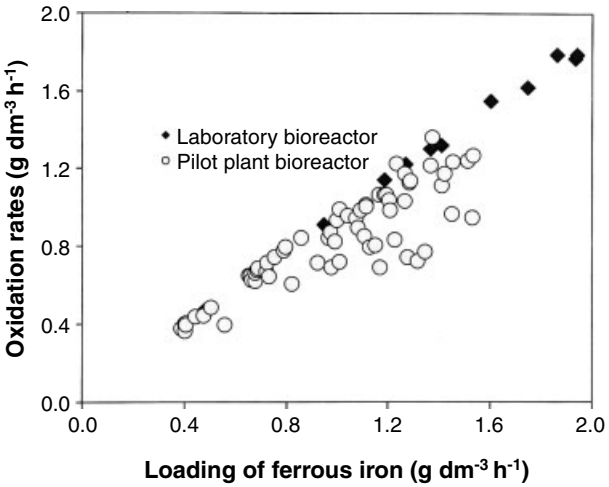

Figure 8. Results of continuous oxidation in pilot plant bioreactor.

Nevertheless, in order to avoid inhibition effects of ferric ions, the absorber was operating until the conversion in the solution exceeded 90% of the initial Fe^{3+}. Then, the settling and filtration of the solution was carried out to separate the elemental sulfur. The resultant solution was used for the bioreactor feed.

Bioreactor (II), which was described previously, was used to investigate the possible presence of any compound in the biogas that could accumulate in the absorbing solution and inhibit the microorganisms' growth in the bioreactor.

Once the reactor had been prepared in the way described above, the effluent—with a higher ferric iron concentration—was fed into the absorption plant.

The absorbing solution was kept in the absorber until the Fe^{2+} concentration had reached a value greater than $13\,g\,dm^{-3}$. At this point the solution was removed from the storage tank and filtered to completely remove the elemental sulfur formed in the absorber. The pH of the resulting solution was adjusted to the appropriate value where necessary and this liquid was used to feed the bioreactor. The reactor continued working with this feed for more than 250 days. The feed rate was always kept between 0.8 and $1.6\,g\,dm^{-3}\,h^{-1}$ to avoid the possible influence of ferric iron precipitation on the support. This condition was monitored in order to ensure that any possible loss in the reactor efficiency was solely caused by the presence of products that had been introduced into the reactor from the anaerobic digester.

For the purpose of comparison, results of oxidation of ferrous iron by immobilized cells of *Acidithiobacillus ferrooxidans* for the three experimental sets are presented in Fig 7.

The rates obtained demonstrate that, in the range of feed rates investigated, there is no question of inhibition or poisoning of the microorganism due to the fact that the feed comes from the absorber.

Pilot plant bioreactor

For 80 days, the bioreactor was continuously fed with fresh medium, containing $15\,g\,dm^{-3}$ ferrous iron, and flow rates of the feed in the range $0.5–1.5\,g\,dm^{-3}\,h^{-1}$.

The results of continuous oxidation of ferrous iron are presented in Fig 8. The results were compared with the data obtained in the laboratory bioreactor. For the flow rate of feed assayed, there is a good correspondence between oxidation rate values.

This study demonstrated that this bioreactor configuration could be adequate for long-term continuous operation of ferrous iron oxidation.

In conclusion, it can be stated that the reactor studied can be scaled-up to an industrial level as there is no evidence for loss of efficiency during a period of operation of at least 4 months. This period is sufficient to make the costs involved in the replacement of the support negligible.

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 concentration of 7000 ppmv H_2S. Bearing in mind that an oxidation rate of $3.7\,g\,dm^{-3}\,h^{-1}$ corresponds to a dilution rate of $0.3\,h^{-1}$, a concentration of ferrous iron of approximately $15\,g\,dm^{-3}$ 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.

ACKNOWLEDGEMENTS

The authors wish to express sincere gratitude to the 'Comisión Interministerial de Ciencia y Tecnología (Spain)' for funding this research through Projects AMB93-0353 and AMB99-0657-C02-01.

REFERENCES

1 Kohl A and Nielsen R, *Gas Purification*, Gulf Publishing Company, Houston, Texas, USA (1997).
2 Malhotra S and Pandey RA, Desulphurization of gaseous fuels with recovery of elemental sulphur: an overview. *Crit Rev Environ Sci Technol* **29**:229–268 (1999).
3 Jensen AB and Webb C, Treatment of H_2S containing gases: a review of microbiological alternatives. *Enzyme Microb Technol* **17**:2–10 (1995).
4 Lens PNL, Visser A, Janseen AHJ, Pol LW and Lettinga J, Biotechnological treatment of sulphate-rich waste waters. *Crit Rev Environ Sci Technol* **28**:41–88 (1998).
5 Halfmeier H, Schäfer-Treffenfeldt W and Reuss M, Potential of *Thiobacillus ferrooxidans* for waste gas purification. Part 1. Kinetics of continuous ferrous iron oxidation. *Appl Microbiol Biotechnol* **40**:416–420 (1993).
6 Halfmeier H, Schäfer-Treffenfeldt W and Reuss M, Potential of *Thiobacillus ferrooxidans* for waste gas purification. Part 2. Increase in continuous ferrous iron oxidation kinetics using immobilized cells. *Appl Microbiol Biotechnol* **40**:582–587 (1993).
7 Pagella C, Perego P and Zilli M, Biotechnological H_2S gas treatment with *Thiobacillus ferrooxidans*. *Chem Eng Technol* **19**:78–88 (1996).
8 Comas J, Balaguer MD and Rigola M, Biological treatment of gases and odours: study of the application of a pilot plant (absorption column and biological reactor) for the treatment of H_2S from a waste gas, in *Chemical Industry and Environment II*, Vol 1, ed by Piccininj N and Delcrenzo R, Politecnico di Torino, Italia, pp 49–58 (1996).
9 Jensen AB and Webb C, Ferrous sulfate oxidation using *Thiobacillus ferrooxidans*: a review. *Process Biochem* **30**:225–236 (1995).
10 Asai S, Konishi Y and Yabu T, Kinetics of absortion of hydrogen sulfide into aqueous ferric sulfate solutions. *AIChE Journal* **36**:1331–1338 (1990).
11 Karamanev DG, Model of the biofilm structure of *Thiobacillus ferrooxidans*. *J Biotech* **20**:51–64 (1991).
12 Pogliani C and Donati E, Immobilisation of *Thiobacillus ferrooxidans*: importance of jarosite precipitation. *Process Biochem* **35**:997–1004 (2000).
13 Mesa MM, Macías M and Cantero D, Mathematical model of the oxidation of ferrous iron by a biofim of *Thiobacillus ferrooxidans*. *Biotechnol Prog* **18**:679–685 (2002).
14 Armentia H and Webb C, Ferrous sulfate oxidation using *Thiobacillus ferrooxidans* cells immobilised in polyurethane foam support particles. *Appl Microbiol Biotech* **36**:697–700 (1992).
15 Nemati M and Webb C, Effect of ferrous iron concentration on catalytic activity of immobilized *Thiobacillus ferrooxidans*. *Appl Microbiol Biotechnol* **46**:250–255 (1996).
16 Nemati M and Webb C, A kinetic model for biological oxidation of ferrous iron by *Thiobacillus ferrooxidans*. *Biotechnol Bioeng* **53**:39–43 (1997).
17 Nemati M and Webb C, Combined biological and chemical oxidation of ferrous sulfate using immobilized *Thiobacillus ferrooxidans*. *J Chem Technol Biotechnol* **74**:562–570 (1999).
18 Mesa MM, Macías M and Cantero D, Biological iron oxidation by *Acidithiobacillus ferrooxidans* in a packed bed bioreactor. *Chem Biochem Eng Q* **16**:69–73 (2002).
19 Kelly DP and Jones CA, Factors affecting metabolism and ferrous iron oxidation in suspension and batch culture of *Thiobacillus ferrooxidans* relevance to ferric leach solution regeneration, in *Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena*, ed by Murr LE and Brierley JA. Academic Press, New York, pp 19–43 (1978).
20 Gomez JM and Cantero D, Kinetic study of biological ferrous sulphate oxidation by iron-oxidising bacteria in continuous stirred tank and packed bed bioreactors. *Process Biochem* **38**:867–875 (2003).
21 Johnson DB, Ghauri MA and Said MF, Isolation and characterization of an acidophilic, heterotrophic bacterium capable of oxidizing ferrous iron. *Appl Environ Microbiol* **58**:1423–1428 (1992).
22 Vogel AI, *Vogel's Textbook of Quantitative Chemical Analysis*. 5th edn. Longman, London, pp 287–310 (1989).