

# Immobilization of *Acidithiobacillus ferrooxidans* on monolithic packing for biooxidation of ferrous iron

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## Abstract

The oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) in solution using *Acidithiobacillus ferrooxidans* has industrial applications exclusively in the regeneration of ferric iron ( $\text{Fe}^{3+}$ ) as an oxidizing agent for the removal of hydrogen sulfide from waste gases, desulfurization of coal, leaching of non-ferrous metallic sulfides and treatment of acid mine drainage. The aim of this investigation was to increase the bio-oxidation rate of ferrous sulfate by using immobilized cells. Rate of  $\text{Fe}^{2+}$  oxidation was determined in a packed-bed bioreactor configuration with monolithic particles being used as support material. Biooxidation of ferrous iron by immobilized cells was investigated in repeated batch culture and continuous operation using a laboratory scale packed-bed bioreactor. On this account, effects of process variables such as dilution rate and initial concentrations of  $\text{Fe}^{2+}$  on oxidation of ferrous sulfate were consequently investigated. During repeated batch culture, the immobilized-cells were stable and showed high constant iron-oxidizing activities. A maximum  $\text{Fe}^{2+}$  oxidation rate of 6.7 g/l/h was achieved at the dilution rate of  $2 \text{ h}^{-1}$ , while no obvious precipitate was detected in the bioreactor.

**Keywords:** Ferrous iron oxidation; *Acidithiobacillus ferrooxidans*; Packed-bed bioreactor; Dilution rate.

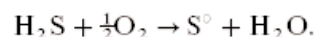
## INTRODUCTION

The biological oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) by *Acidithiobacillus ferrooxidans* is potentially a useful industrial process for the treatment of acid mine

drainage and in the regeneration of ferric iron ( $\text{Fe}^{3+}$ ) as a leaching agent in hydrometallurgical processes (Longa *et al.*, 2004). It also has an important role in the process of desulfurization of sour gases and coal (Daoud and Karamanev, 2006).

Removal of  $\text{H}_2\text{S}$  from gas is based on two steps consisting of absorption involving the chemical reaction of the gas in a  $\text{Fe}^{3+}$  solution, in which the  $\text{Fe}^{3+}$  ion is converted to ferrous sulphate and  $\text{H}_2\text{S}$  is oxidized to its sulphur form. In the second step, the biological oxidation of  $\text{Fe}^{2+}$  in solution leads to the formation of  $\text{Fe}^{3+}$  ions again, thus keeping the cycle going (Malhotra *et al.*, 2002).

The biological oxidation of  $\text{Fe}^{2+}$  ions, produced during the absorption step, to  $\text{Fe}^{3+}$  ions involves the biocatalytic activity of *A. ferrooxidans* and may be regarded as the regeneration of the absorbing solution. The following overall reaction is performed:



Advantages of this process with respect to conventional treatment processes for  $\text{H}_2\text{S}$  (e.g., the Claus process) are mild pressure and temperature conditions representing typical conditions for biotechnological processes; besides, the process is a closed-loop operation without the input of chemicals or output of wastes, thus involving less expenses. In fact, it converts all sulfides to elemental sulfur which can be easily separated from the liquid phase, therefore avoiding possible oxidation to sulfates or thiosulfates. Thus, the economic viability and efficiency of this process depends on the extent of biooxidation efficiency of  $\text{Fe}^{2+}$  ions to  $\text{Fe}^{3+}$  ions which ensures recycling of the oxidant to the first stage of the process (Pagella and Favari, 2000).

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In recent years, most studies have been aimed at improving the rate of bio-oxidation of  $\text{Fe}^{2+}$  by using several types of reactors in which bacteria are attached to supports such as rotating biological contactors (Garcia, 1992), packed-bed and fluidized-bed reactors (Karamanev, 1991). These applications provide a large surface area for bacterial attachment and reduce the loss of biomass (Mazuelos *et al.*, 2001).

The packed-bed reactor is an attractive choice because it is simple and cheap to install and operate. Such reactors with different packings have been used for immobilization of *A. ferrooxidans* by adhesion. Packings include low grade sulfide minerals (Carranza and Garcia, 1990), calcium alginate (Lancy and Tuovinen, 1984), P.V.C. rings (Livesey *et al.*, 1977), polyurethane foam (Armentia and Webb, 1992), glass beads (Ginsburg and Karamanev, 2007; Grishin *et al.*, 1988), activated carbon (Ghauri *et al.*, 2007; Grishin *et al.*, 1988) and siliceous stones (Ginsburg and Karamanev, 2007). Most of these packings combine the advantages of adhesion with those of entrapment, but none of them are practical on an industrial scale (Longa *et al.*, 2004).

At low HRT-values ( $\text{HRT} < 1/\mu_{\text{max}}$ ), where  $\mu_{\text{max}}$  is the maximum specific growth rate ( $\text{h}^{-1}$ ) and HRT is the hydraulic residence time, growth is favored and suspended cells are washed-out from the system, thus biomass is capable of biofilm formation, whereas at high HRT-values ( $\text{HRT} > 1/\mu_{\text{max}}$ ) growth of suspended bacteria is favored instead (Ebrahimi *et al.*, 2005).

There are several factors that play substantial roles in the rate of oxidation of  $\text{Fe}^{2+}$  by *A. ferrooxidans*. These factors include  $\text{Fe}^{2+}/\text{Fe}^{3+}$  iron concentration, cell and oxygen concentrations, pH, temperature and reactor type (Daoud and Karamanev, 2006).

Accordingly, studies were carried out using batch experiments for assessment of optimal environmental conditions, such as initial  $\text{Fe}^{2+}$  concentration and pH, for efficient bio-oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  ions. The effective bio-oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  could be achieved efficiently in the pH range of 1.4-1.8, at 30°C during optimum growth of culture (Malhotra *et al.*, 2002).

The aim of this work is to evaluate monolithic particles as a support for immobilization of *A. ferrooxidans* in a packed bed bioreactor for the purpose of  $\text{Fe}^{2+}$  bio-oxidation. Also, the other major objective of this study was to determine the feasibility of using this carrier for immobilization of *A. ferrooxidans*, and simul-

taneously establish a procedure for *A. ferrooxidans* immobilization on a laboratory scale, that would be simple, fast, and easily reproducible and which can be suitably adapted to the industrial scale.

## MATERIALS AND METHODS

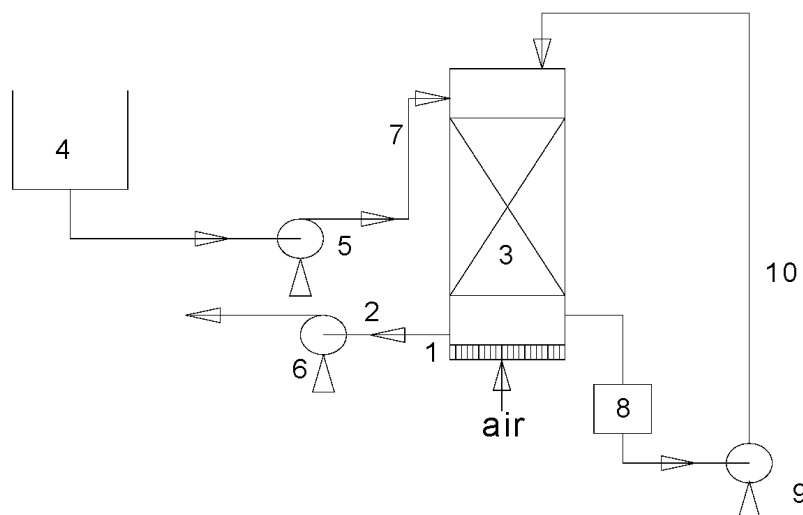
**Microbial strains and growth media:** *Acidithiobacillus ferrooxidans* (DSM 584), obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), was used in this study. *A. ferrooxidans* was grown in modified 9K basal salts (Silverman and Lundgren, 1959) medium which consisted of:  $\text{K}_2\text{HPO}_4$ , 0.4 g/l;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.4 g/l;  $(\text{NH}_4)_2\text{SO}_4$ , 0.4 g/l. The pH of the medium was adjusted to 1.6 with concentrated  $\text{H}_2\text{SO}_4$ . All chemicals used in the growth medium were of laboratory grade, and purchased from Merck, Germany.

**Solid support preparation:** In the present work, utilization of monolithic particles as a support for immobilization of *A. ferrooxidans* was investigated. The monoliths were made by extrusion of cordierite, a ceramic material specially tailored for application in exhaust cleaning (provided by Iran Delco Co). Each wall surface of the monolith acts as a suitable place for biofilm formation because it displays excellent adhesive surface characteristics. The monolith channels were square-shaped with a diameter of approximately 1 mm. Chemical composition of ceramics was  $2\text{MgO} \cdot 2\text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2$ .

Crushed monolithic particles were used as bacterial-support, sieved to provide particles between 3 and 4 mm in size. The sieved solids were then washed with water under pressure to remove slimes. Finally, the particles were washed with 2N  $\text{H}_2\text{SO}_4$  and, later, with water, in order to remove soluble substances.

**Bioreactor for continuous ferrous ions oxidation:** Figure 1 shows a schematic diagram of the bioreactor used to evaluate the biological oxidation of  $\text{Fe}^{2+}$  iron in this study. Air was fed in at the bottom of the column and fresh solution was fed in at top. Solution outlet was placed at 600 mm above the bottom.

Part of the effluent was recirculated to the top of the bioreactor to supply a constant temperature inside the bioreactor and increase the reactant residence time in the biocatalyst bed.



**Figure 1.** Schematic diagram of bioreactor: 1-air distributor; 2- effluent; 3- packed bed; 4-feed tank;5-feed pump; 6- effluent pump; 7- influent; 8- recycle vessel; 9- recycle pump; 10- recycle stream.

The fresh medium compositions were based on different  $\text{Fe}^{2+}$  concentrations and flow rates. The pH was adjusted to 1.6 with  $\text{H}_2\text{SO}_4$ . Air was introduced into the bioreactor at a rate of 500 ml/min in all tests. All experimental equipments were placed at  $30^\circ\text{C}$  in a thermostatically controlled room.

The following procedure was used to fix the bacterial film onto the solid support: Two cultures of *A. ferrooxidans*; one representing a 50% (v/v) inoculum from a spent, iron grown culture and the other representing a 50% (v/v) of fresh culture medium were fed into the bioreactor that was randomly filled with the monolithic particles. The culture medium used was a synthetic medium based on an aqueous ferrous sulphate solution (6.6 g/l of  $\text{Fe}^{2+}$ ) with a pH of 1.6.

When 95% of the  $\text{Fe}^{2+}$  ions were consumed by microorganisms under batch conditions, cultures were discharged and a fresh iron grown culture was added to the reactor. This process was repeated several times (each successive culture was considered as a new step towards biofilm formation) until 95% of the  $\text{Fe}^{2+}$  ions in each batch culture was oxidized after approximately 24 h. A continuous-recycling flow mode of operation was then initiated by passing the culture medium into the bioreactor. The recycle mode was carried out until 95% conversion of  $\text{Fe}^{2+}$  was attained. Because this mode was performed at a high dilution rate, the suspended cells were washed out as a result, thus allowing for biooxidation to be achieved by the immobilized cells. Consequently, immobilized cells were obtained which demonstrated favorable adhesion on

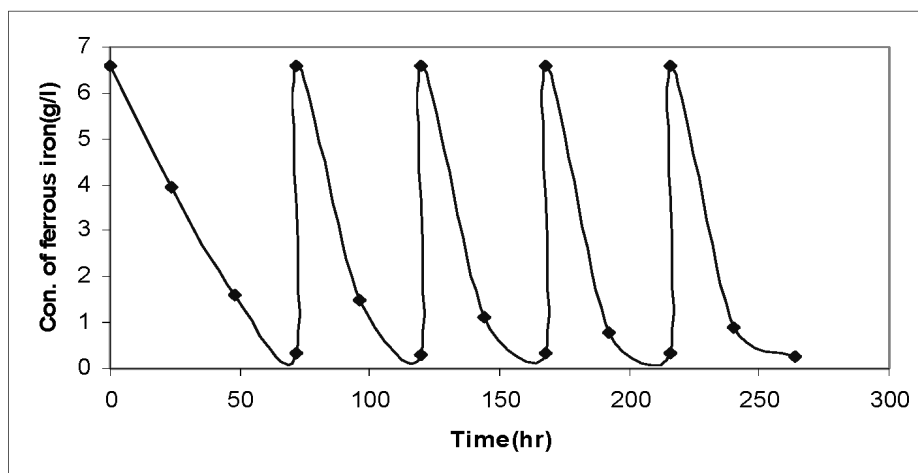
the support particles, hence representing an adhesion biofilm.

The continuous mode of operation started with the same liquid flow rate as in the continuous-recycling operation carried out for the biofilm formation. When the bioreactor reached steady-state conditions, the liquid flow rate was increased; however, the other experimental conditions were kept constant. Conditions were considered as steady-state, when  $\text{Fe}^{2+}$  concentration was less than 10%. Samples were then taken from the effluent stream of the bioreactor and analyzed for  $\text{Fe}^{2+}$  concentration.

**Analytical methods:** The  $\text{Fe}^{3+}$  ion concentration in the solution was determined by the sulfosalicylic acid spectroscopy method (Varian Techtron VIS spectrophotometer, model 635, Techtron, USA) (Karamanev *et al.*, 2002). Differences between concentrations of total iron and  $\text{Fe}^{3+}$  ions were used to obtain the  $\text{Fe}^{2+}$  ion concentration in solution.

## RESULTS

The performance of biological oxidation depends on the support media. The adhesion of iron oxidizing bacteria on the surface of the support media must be due to the forces of adsorption, because these bacteria do not produce any extra-cellular polymeric substances during the oxidation of  $\text{Fe}^{2+}$ . Biooxidation of  $\text{Fe}^{2+}$  was performed in an immobilized reactor which showed



**Figure 2.** Repeated batch oxidation of ferrous iron by immobilized *A. ferrooxidans*, showing ferrous iron concentration in solution as a function of time for repeated batches,  $T=30^{\circ}\text{C}$ ,  $\text{pH}=1.6$ .

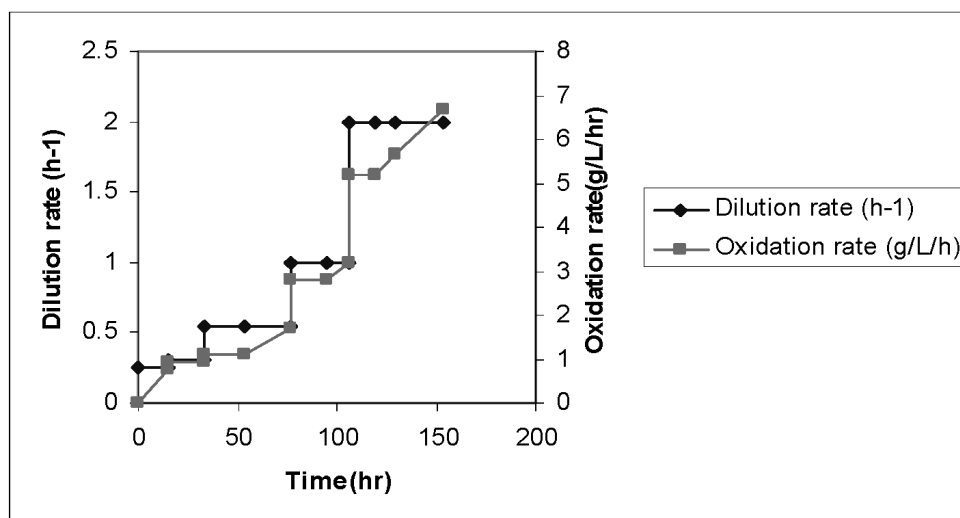
high biooxidation efficiency.

In order to study the stability of catalytic capability of *A. ferrooxidans* absorbed on to the monolithic particle, batch oxidation was performed. When  $\text{Fe}^{2+}$  conversion of over 95% had occurred; fresh medium was fed into the reactor. Figure 2 shows the development course of iron-oxidization.

The continuous operation was carried out at different dilution rates (D) based on the liquid volume of the bioreactor. The effects of initial  $\text{Fe}^{2+}$  concentrations, on the bio-oxidation process at different dilution rates can be seen in Figures 3 to 6.

## DISCUSSION

**Immobilization of *A. ferrooxidans* on monolithic packing:** Figure 2 shows the development course of iron-oxidization. In the first batch culture, a relatively long period, 3 days, is necessary for complete iron oxidation, but the second and the third batch cultures need approximately 48 h. Thereafter,  $\text{Fe}^{2+}$  in each batch culture is oxidized completely within approximately 24 h. The immobilized cells obtained are stable and demonstrate a high constant iron-oxidizing activity.



**Figure 3.** Continuous oxidation of ferrous iron by immobilized *A. ferrooxidans* in the packed-bed bioreactor, initial ferrous concentration 3.5 g/l,  $T=30^{\circ}\text{C}$ ,  $\text{pH}=1.6$ .

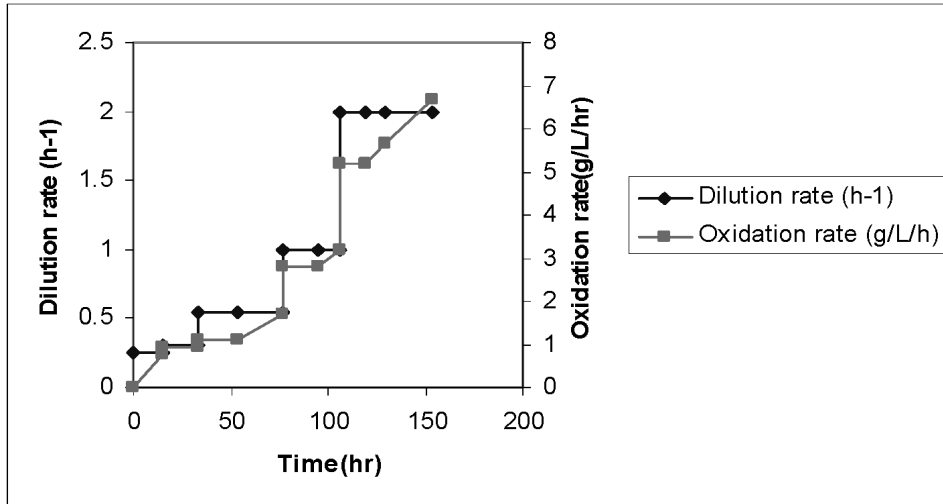


Figure 4. Continuous oxidation of ferrous iron by immobilized *A. ferrooxidans* in the packed-bed bioreactor, initial ferrous concentration 6 g/l, T=30°C, pH=1.6.

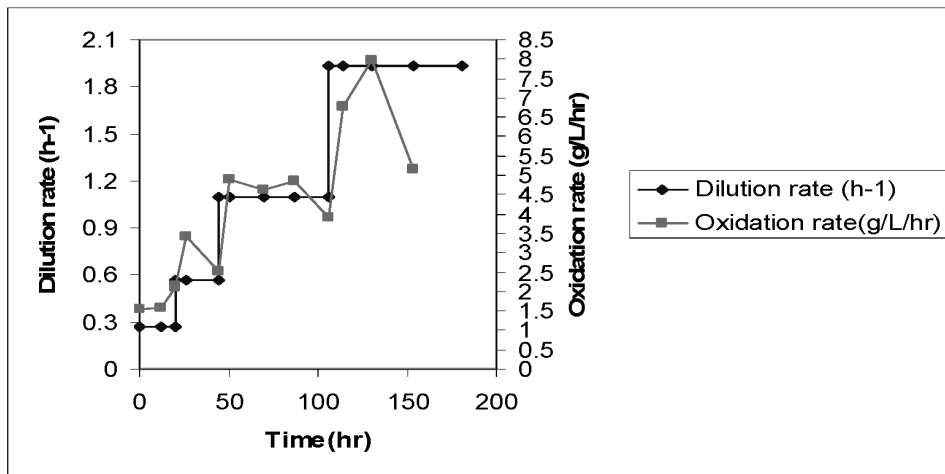


Figure 5. Continuous oxidation of ferrous iron by immobilized *A. ferrooxidans* in the packed-bed bioreactor, initial ferrous concentration 16 g/l, T=30°C, pH=1.6.

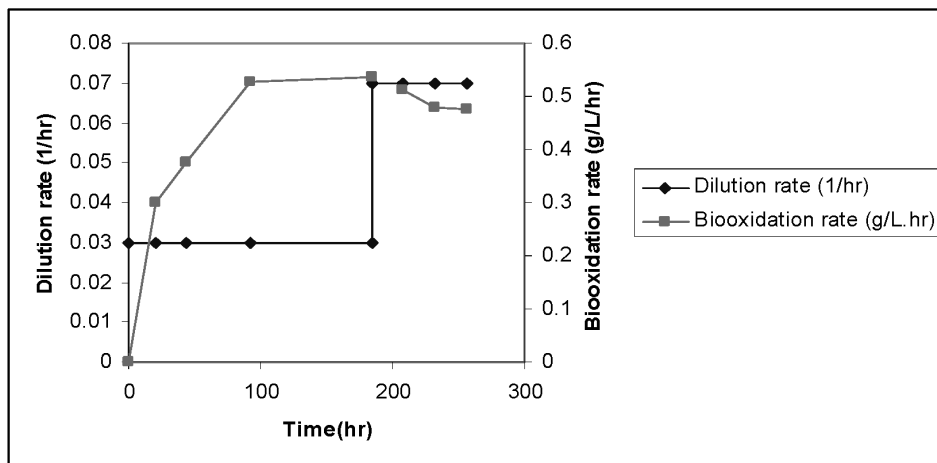
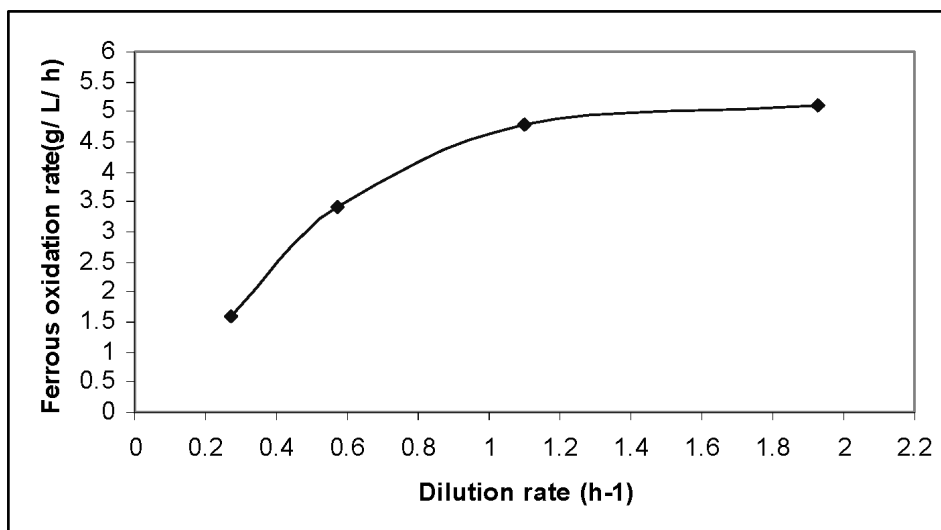


Figure 6. Continuous oxidation of ferrous iron by immobilized *A. ferrooxidans* in the packed-bed bioreactor, initial ferrous concentration 21.3 g/l, T=30°C, pH=1.6.



**Figure 7.** Continuous oxidation of ferrous iron by immobilized *A. ferrooxidans* in the packed-bed bioreactor, showing the effect of dilution rate on the oxidation rate of ferrous iron, T=30°C, pH=1.6 and Fe<sup>2+</sup>= 6 g/l.

#### **Influence of dilution rate on continuous oxidation of ferrous iron in a packed-bed bioreactor:**

The results of these experiments indicate that in the concentration range of 3.5-6 g/l of Fe<sup>2+</sup> ions, the oxidation rates increase in parallel with a rise in the initial substrate concentrations. The reactor shows a good oxidation efficiency of Fe<sup>2+</sup>, at a liquid flow rate of 1.2 l/h; while, by increasing the substrate concentration to 16 and 21.5 g/l, the oxidation efficiency of Fe<sup>2+</sup> decreases significantly. The maximum oxidation rates are 6.7 and 7.9 g/l/h for 3.5 and 6 g/l of initial Fe<sup>2+</sup> ion concentrations, respectively.

The monolith used as a packing only has been reported by Park *et al.* (2005), who have suggested that the monolith does not function as an appropriate support for oxidizing bacteria. However, in this research, for an initial iron concentration below 6 g/l, the monolith has demonstrated a very good efficiency. For the higher initial Fe<sup>2+</sup> ion concentrations, the quantities of jarosite have been detectable and the risk of clogging has been high.

In fact the immobilized cells have an advantage in a continuous operation. In the process investigated in this study, the bacteria on the particles' surface were resistant to washout. In free cell reactors complete washout of bacteria occurs at D = 0.11 h<sup>-1</sup> (Park *et al.*, 2005); whereas, in the present study, using immobilized cells, maximum Fe<sup>2+</sup> biooxidation rate is achieved at D = 2 h<sup>-1</sup>.

Bio-oxidation rates at steady-state for each dilu-

tion rate are plotted in Figure 7. It can be seen clearly that increasing the dilution rate results in a higher Fe<sup>2+</sup> iron oxidation rate. At a dilution rate of 2 h<sup>-1</sup> or lower all Fe<sup>2+</sup> iron is oxidized. When the initial Fe<sup>2+</sup> iron concentration in the solution was 6 g/l, a maximum Fe<sup>2+</sup> biooxidation rate of 6.7 g/l/h occurs.

#### **CONCLUSION**

*A. ferrooxidans*, DSM 584 was grown in 9k medium which was immobilized on monolithic particles, that were 3-4 mm in diameter. The packed-bed column that included the immobilized cells was studied for the purpose Fe<sup>2+</sup> iron conversion. The monolithic particles investigated in this study were found to be suitable material for *A. ferrooxidans* immobilization which is consequently appropriate for Fe<sup>2+</sup> oxidation mainly as a result of its advantages over other commonly used substrates.

The immobilized reactor was studied under batch and continuous culture conditions, in which Fe<sup>2+</sup> starting with an initial concentration of 6.7 g/l was oxidized completely within approximately 24 h under batch conditions.

Continuous oxidation was affected by the dilution rate and initial the Fe<sup>2+</sup> ion concentration. In all cases, an increase in the dilution rates resulted in an increase in the Fe<sup>3+</sup> productivity until a maximum value was achieved, after which a decrease in productivity was

observed. The bio-oxidation rates were 6.7 g/l/h and 7 g/l/h for 3.5 g/l and 6 g/l of initial Fe<sup>2+</sup> ions concentrations, respectively. For higher initial concentrations of 16 g/l and 21.3 g/l, bio-oxidation rates were 0.9 g/l/h and 0.55 g/l/h, respectively.

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