Depósito de investigación de la Universidad de Sevilla

https://idus.us.es/

"This is an Accepted Manuscript of an article published by Elsevier in Minerals Engineering on May 2001, available at: https://doi.org/10.1016/S0892-6875(01)00038-3 ."

FERRIC IRON PRODUCTION IN PACKED BED BIOREACTORS: INFLUENCE OF pH, TEMPERATURE, PARTICLE SIZE, BACTERIAL SUPPORT MATERIAL AND TYPE OF AIR DISTRIBUTOR

Mazuelos, A., Palencia, I., Romero, R., Rodríguez, G., Carranza, F.

Dept. Ingeniería Química. Facultad de Química. Universidad de Sevilla. 41071 Sevilla. Spain

E-mail: mazuelos@cica.es

ABSTRACT

The biooxidatíon of ferrous iron in solution has industrial applications in the regeneration of ferric iron as a leachíng agent of non-ferrous metallic sulphides and in the treatment of acid mine drainage. The airn of this work was the study of several variables (pH, temperature, particle size, bacterial support material and type of air distributor) for the design of a packed bed bioreactor for the ferrous iron biooxidation. Basic criteria of design have been the following:

- Maximum residence time of the liquid for a minimuin-sized reactor. Flooded packed bed reactors have been used in order to meet this requirement.
- The solid material that acts as bacterial support must allow a rapid and permanent biofilm formation and show a good chemical resistance to ferric sulphate and sulphuric acid.
- Constant and homogeneous air supply in the whole bed.

Bioreactor consisted of a column randomly packed with solid particles, and it was fed with an acidic solution of ferrous sulphate. Air and fresh solution were fed in at the boffom of the column from where they flooded the reactor. The inoculum consisted of a mixed culture of bacteria isolated from Riotinto mines drainage waters, and adapted to pH 1.25 in the laboratory, composed mainly of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans.*

A methodology for biofilm formation was established. Maximum ferric iron productivity was 11.1 g l^{-1} h^{-1}. The type of air diffusor has been an important parameter to be taken into account in the design, as the oxygen dissolved in the liquid medium limits the ferrooxidant activity of bacteria.

INTRODUCTION

Biooxidation of ferrous iron is an important reaction in hydrometallurgical leachíng operations, as well as in evaluation of naturally occurring metal sulphide oxidation processes such as the formation of mine acid drainage (Nyavor et al., 1996). The ferric iron produced as a result of the growth of bacteria serves as a lixiviant for the oxidation of the sulphides, and therefore it can be regenerated in a cyclic bioleaching process following the indirect mechanism (Carranza et al., 1993, Palencia et al., 1998). There has been extensive research on ferrous iron biooxidation with application to the treatment of acid mine drainage and the regeneration of leaching solutions. It has been studied in several experimental systems with batch and continuous-flow modes of operation. Attemps 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 fixed-film application to provide a large surface area for their allachment and to reduce the loss of biomass. Recent efforts have addressed various configurations of packed-bed and fluidized-bed reactors with inert biomass - support materials (Nikolov et al., 1986, Nikolov and Karamanev, 1987, García et al., 1989, Carranza et al., 1990, Grishin et al., 1988, Armentia et al., 1992). The packed-bed reactor is an attractive choice because it is simple and cheap to install and operate. The medium can be

fed in either at the top or at the boffom of the bed. If the ferrous sulphate solution is sprayed on to the top of the packing, there are some disadvantages related to the presence of ‘dead zones’. These may be regions that receive too little liquid flow, causing nutrient limitation or the accumulation of metabolic products (H^+, Fe^{3+}) to inhibitory levels or, there may be regions that receive too much liquid and become waterlogged, preventing the penetration of the 0_2 and $C0_2$ essential for bacteria metabolism. This feature together with the low mean residence time inherent in these systems result in low ferric iron productivities and may be partially overcome when flooded packed-bed bioreactors are used (Mazuelos et al., 1999). In flooded packed-bed reactors, the medium is fed in at the bottom and the ‘dead zones’ are unlikely at high liquid flow rates through the bed, thus leading to higher ferric iron productivities and so, to smaller bioreactors for a given ferric iron productivity.

The present work studies the influence of some basic parameters on the ferrous iron biooxidation process in flooded packed-bed bioreactors such as particle size of the bacterial support material, nature of the support, pH, temperature, and type of air distributor.

EXPERIMENTAL

Inoculum

The culture used as inoculum was originally isolated from Riotinto mine drainage waters. It consisted mainly of *Thiobacillus ferrooxidans, Leptospirillum ferrooxidans* and some heterotrophic bacteria of the genus *Acidophilium: A. organovorum, A. cryptum* and A. *facilis.* It was routinely maintained on a modified Silverman and Lundgren 9K nutrient medium at pH 1.25. In order to get the inoculum volume required for biofilm formation successive

transfers into a fresh medium were performed by mixing 20% (vol/vol) inoculum of a spent, iron-grown culture wíth 80% (vol/vol) of nutrient medium at pH 1.25.

Inert support

Eight materials were tested: activated carbon, volcanic lava, siliceous stone, glass, polyethylene, polyvinylchloride, extruded polystyrene and expanded polystyrene. All materials were washed with 1 M sulphuric acid in order to remove the soluble compounds. Siliceous stone was previously screened to the desired particle size and washed with water in order to remove slimes.

Bioreactors

The bioreactors consisted of polymethyl metacrylate (PMMA) tubes of either 10 cm or 30 cm in height and 8.4 cm in diameter packed with inert particles for bacterial support. Sets of 10 cm-high bioreactors were used when different beds were required for the study of one variable -such as type and particle size of the inert support material-. The study of the rest of variables (pH, temperature and type of air distributor) was accomplished in 30 cm-high bioreactors. Air and ferrous sulphate solution were fed in at 30 mm above the bottom of the column from where they flooded the reactor. Solution outlet was placed at the top of the column (by overflow). Air was supplied under low pressure, 0.5 kg cm^2. The experimental equipment, with the exception of the air compressor, was placed in a 31ºC thermostatic room.

Biofi1m formation procedure

The bacterial fixed film was formed on the solid support according to the following procedure: the support particles were randomly placed in the column which was then filled

with 30% (vol/vol) of a spent, iron-grown culture as inoculum and 70% (vol/vol) of culture medium. The culture medium was an aqueous ferrous sulphate solution (7 g l^{-1} of ferrous iron) at pH 1.25. The flow rate of air was set at 500 ml min~' and the bioreactor operated in batch mode. Once 95% conversion of ferrous iron was achieved, a continuous-recycling flow mode of operation was initiated by passing a culture medium volume of around twelve times the void volume of the bed (Figure 1). Once 95% conversion of ferrous iron was achieved, the cells were found out to be immobilised on the support particles.

Analysis and control

The solution flow rate was controlled with peristaltic pumps and was measured in the outlet stream. The air flow rate was measured with rotameters. Ferrous iron concentration was determined by automatic titration with $K_2Cr_2O_7$.

Continuous operation

Once the biofilm had been formed, the continous operation of the reactor was initiated with the same liquid flow rate than in the continuous-recycling operation carried out for the biofilm formation. The liquid flow rate was increased stepwise keeping constant the rest of the experimental conditions. The liquid flow rate was changed after steady-state conditions were achieved (steady-state was considered when ferric iron productivity varied less than 5% for a period of time of 50 times the mean residence time). In all cases, an increase in the liquid flow rate resulted in an increase of the ferric iron productivity until a maximum value was achieved, afier which a decrease in productivity was observed.

Unless otherwise stated, the bioreactors were aerated at 500 ml min^{-1}.

Air distributors. Bubble size determination

Air was introduced in the biorreactor through three different kind of distributors: nozzle, ceramic difusser and wood diffuser. These diffusers were selected taking into consideration its stability in oxidant and acid medium conditions. The nozzle had an inner diameter of 7 mm. The ceramic difusser consisted of a 28 mm-lenght and 14 mm-diameter cylinder. The wood diffuser consisted of a wood prism (43 x 15 x 15 mm) that provided small air bubbles emerging ftom the wood pores. The bubble size distribution was determined by filming by a video camera the bubbles inside the bioreactor. The diameter of 200 air bubbles randomly selected were measured using a TV-monitor. Figure 2 shows the bubble size distribution for the ceramic and the wood diffusers. Air was flowing from the nozzle as large non-spheric air pockets which size could not be measured.

RESULTS AND DISCUSSION

Effect of the support particle size

Several tests were performed in a 10 cm-high bioreactor packed with glass spheres of different particle size as bacterial support. The ferrous sulphate solution was fed in at increasing flow rates from 100 ml h^{-1} to 600 ml h^{-1} and had a ferrous iron concentration of 7.5 g l^{-1}. Air was introduced through diffusers with the aim of getting air bubbles of small size and avoiding the observed formation of air pockets and flow channelling or maldistribution when nozzle was used. A preliminary set of runs with glass spheres of 1 to 7 mm of particle size was carried out. Ferric iron productivity increased with an increase of the particle size. In the course of the experiments, bioreactors packed with glass spheres smaller than 4 mm

underwent a rearrangement of the bed that produced flow channelling resulting in low ferric iron productivities. This circumstance hindered the biofilm development and consolidation, as biofilm is preferentially formed in the space between the particles (Nikolov et al., 1988). A second set of experiments was carried out with glass spheres of higher particle size. Air was introduced through a wood diffuser at two air flow rates, 250 and 500 ml min^{-1}, and glass spheres of 5, 7, 9 and 14 mm of particle size respectively were used. Maximum ferric iron productivities at steady-state conditions are shown in Table 1. The kighest ferric iron productivity was achieved with 7 mm of particle size. In ah cases, the increase of the air flow rate from 250 to 500 ml min^{-1} leaded to an increase of the ferric iron productivity. This effect suggests that oxygen could be the limiting reagent in these experiments.

Éffect of the inert support material

Several tests were carried out to determine the influence of the nature of the material used as bacterial support on the ferrous iron biooxidation. Tests were performed in bioreactors of 10 cm in height packed with particles of each material. Air was supplied through a wood diffuser at a flow rate of 250 ml min^{-1}. The ferrous sulphate solution was fed in at increasing flow rates from 100 ml h^{-1} to 600 ml h^{-1} and had a ferrous iron concentration of 7 g l^{-1}. Preliminary experimental runs were carried out in order to find suitable matenais for bacterial adhesion. Volcanic lava was rejected because of its fragile character that resulted in the fracture of particles during the operation. Extruded polystyrene was also rejected because of the considerable permanent strain that particles underwent during the operation. On the other hand, the biofilm did not form on polyethylene particles and it was also excluded.

Geometric and physical characteristics of the five suitable materials are shown on Table 2. Figure 3 shows the maximum ferric iron productivity at steady-state conditions for the five materials used. The siliceous stone packed-bed bioreactor exhíbited the highest ferric iron

productivity. Siliceous stone was the most attractive material for bacterial support because of its high ferric iron productivity, availability and low price.

Effect of pH

The biooxidation of ferrous iron ($Fe^{2}+ + ¼\ 0_2 + H^+ \longrightarrow Fe^{3+} + ½\ H_20$) involves a consumption of H^+. It is obvious that the range of pH inside the reactor depends basically on the pH and the ferrous iron concentration of the inlet solution, and on the ferrous iron conversion achieved. The pH must be within the range 1-1.6 inside the bioreactor, because bacterial growth is inhibited at pH values lower than 1 and, on the other hand, values higher than 1.6 promote considerable ferric iron precipitation as jarosite. Ferric iron precipitates formation inside the bioreactor can lead to the reduction of the bed porosity. The outlet solution should have the highest pH value in the practicable range (1-1.6) mainly for two reasons: Firstly, in the treatment of acid mine drainage, neutralization and elimination of iron from biooxidation are needed. Secondly, in hydrometallurgical processes based in indirect bioleaching (IBES and BRISA) (Carranza et al., 1993) metals are obtained as soluble sulphates and are recovered from the biooxidation outlet solution by solvent extraction (and electrowinning), whose efficiency increases as pH increases.

Several tests were carried out to study the effect of the fed solution pH over the range 1.15 - 1.5 on the ferrous iron biooxidation. Tests were performed in a 30 cm-high bioreactor packed with siliceous stone of 4-5 mm in diameter particles as bacterial support. Air was supplied through a 7 mm-diameter nozzle. The ferrous sulphate solution was fed in at a flow rate of 400 ml h^{-1} and had a ferrous iron concentration of 8 g l^{-1}. Table 3 shows the corresponding ferric iron productivities at the steady-state conditions (conversion higher than 80%). As can be observed, the ferric iron productivity does not depend on the pH in the studied range. A pH of 1.25 was chosen for subsequent experiments.

Effect of temperature

The effect of temperature on the ferrous iron biooxidation was studied over the range 6-31ºC. *Thiobacillus ferrooxidans* is a mesophilic bacterium with an optimum temperature of 31ºC (Carranza, 1983). The decrease of the bacterial activity is very pronounced at temperatures higher than this. The upper temperature limit is 40^0C (Carranza, 1983), whereas ferrous iron oxidation has been demonstrated to occur at temperatures as low as 4ºC (Ahonen and Tuovinen, 1989). Tests were performed in a 30 cm-high bioreactor packed with siliceous stone of 4-5 mm in diameter particles as bacterial support. Air was supplied through a 7 mm-diameter nozzle at a flow rate of 500 ml min^{-1}. The ferrous sulphate solution was fed in at a flow rate of 2300 ml h^{-1} and had a ferrous iron concentration of 3 g l^{-1}. Temperature was gradually decreased stepwise from 31 ºC to 6 ºC. Figure 4 shows the ferric iron productivity at the steady-state conditions against temperature. As can be seen, temperature has a noticeable effect on the ferric iron productivity, which linearly decreases as temperature decreases. This result is different from that found in literature for discontinuous experiments, in which an exponential decay has been observed (Ahonen and Tuovinen, 1989).

Effect of the type of air distributor

Several tests were carried out to determine the influence of the type of air distributor on the ferrous iron biooxidation. Tests were performed in 30 cm-high bioreactors packed with siliceous stone particles of 6-7 mm of size as bacterial support with a bed porosity of 0.42. The ferrous sulphate solution was fed in at a flow rate of 2000 ml h^{-1} and had a ferrous iron concentration of 7 g l^{-1}. Air flow rate was 1000 ml min^{-1}. Three types of air distributors were studied: a 7 mm-diameter nozzle, a ceramic diffuser and a wood díffuser. Results are illustrated in Figure 5. The highest ferric iron productivity was attained when a wood diffuser

was used which is the distributor that generates the smallest size bubbles (section 2.7). Fine bubbles result in higher gas-liquid interfacial area than large bubbles: both the specific gas-liquid interfacial area and the gas hold-up increase as the bubble size decreases.

The strong influence of the gas-liquid interfacial area on the ferric iron productivity implies that in this type of reactors and in the experimental conditions tested, the oxygen is the limiting reagent.

The highest ferric iron productivity was 7.7 g h^{-1}, which is equivalent to a productivity per unit of void volume of 11 g l^{-1} h^{-1}.

CONCLUSIONS

The flooded packed-bed bioreactor is a feasible alternative for ferrous iron biooxidation in continuous operation. It display a reliable performance at pH 1.25 and 3 1 ºC with activated carbon, siliceous stone, expanded polystyrene, PVC, and glass particles as bacterial support material. Siliceous stone was the most suitable material for bacterial support in terms of ferric iron productivity and price. The air bubble size has a significant influence on the productivíty achieved. It can be concluded that the oxygen is the limiting reagent of the ferrous iron biooxidation in this type of bioreactor and in the experimental conditions tested.

The highest ferric iron productivity achieved in this study was 11 g l^{-1} h^{-1}. This ferric iron productivity is very high compared to that achieved in other bioreactors found in the literature, leading to smaller size bioreactors for the ferric iron demand of industrial processes; this fact is very important from an economic point of view, increasing the viability of the processes in which it is integrated.

ACKNOWLEDGEMENTS

We gratefully ackNowledge financial support for this work from CICYT (Comisión Interministerial de Ciencia y Tecnología, Spain), project B1095 0482.

REFERENCES

Ahonen, L. and Touvinen, 0. H., Microbiological oxidation of ferrous iron at low temperatures. Applied and Enviromental Microbiology, 1989, **55(2)**, 312-316.

Armentia, H. and Webb, C., Ferrous sulphate oxidation using *Thiobacillus ferrooxidans* cells immobilised in polyurethane foam support particles. Applied Microbiology and Biotechnology, 1992, **36,** 697-700.

Carranza, F., Lixiviación microbiológica de pirita en discontinuo: estudio de variables e investigación del mecanismo. Ph. D. Thesis, 1983, University of Sevilla, Sevilla.

Carranza, F. and García, M.J., Kinetic comparison of support materials in the bacterial ferrous iron oxidation in packed-bed colunms, 1990, Biorecovery, **2**, 15-27.

Carranza, F., Iglesias, N., Romero, R. and Palencia, I., Kinetics improvement of high-grade sulphides bioleaching by effects separation. FEMS Microbiology Reviews, 1993, **11,** 129-138.

García, M.J., Palencia, I. and Carranza, F., Biological ferrous oxidation in packed bed columns with low grade sulphide mineral as support. Process Biochemistry, 1989, **24**, 84 -87.

Grishin, S.I., Tuovinen, 0.H., Fast kinetics of Fe^{2+} oxidation in packed-bed reactors. Applied and Environmental Microbiology, 1988, **54(12)**, 3092-3100.

Mazuelos, A., Romero, R., Palencia, 1., Iglesias, N. and Carranza, F., Continuous ferrous iron biooxidation in flooded packed bed reactors. Minerals Engineering, 1999, **12(5)**, 559-564.

Nikolov, L., Karamanev, D., Experimental study of the inverse fluidized bed biofilm reactor. The Canadian Journal of Chemical Engineering, 1987, **65,** 214-217.

Nikolov, L., Mehochev, D. and Dimitrov, D., Continuous bacterial ferrous iron oxidation by *Thiobacillus ferrooxidans* in rotating biological contactors. Biotechnology Letters, 1986,

8(10), 707-710.

Nikolov, L., Valkova-Valchanova, Mehochev, D., Oxidation of high ferrous iron concentration by chemolithotrophic T. ferrooxidans in packed bed biorreactors. Joumal of Biotechnology, 1988, **7**, 87-94.

Nyavor, K., Egiebor, N.0., Fedorak, P.M., Bacterial oxidation of sulfides during acid mine drainage formation: A mechanistic study. In *EPD Congress 1*996, ed. W. G. Warren. TMS, 1996, PP. 269-287.

Palencia, 1., Romero, R. and Carranza, F., Silver catalyzed IBES process: Application to a spanish copper-zinc sulphide concentrate. Part 2. Biooxidation of the ferrous iron and catalyst recovery. Hydrometallurgy, 1998, **48**, 101 - 112.

TABLE 1. MAXIMUM FERRIC IRON PRODUCTIVITY ($g\ h^{-1}$)

Particle size (mm)	(250 ml min^{-1} air)	(500 ml min^{-1} air)
5	1.05	2.46
7	1.37	2.72
9	1.09	1.84
14	0.87	1.80

TABLE 2. GEOMETRIC AND PHYSICAL CHARACTERISTICS OF THE BED

Material	Bed mass (g)	Shape and size of particles (mm)	Void fraction of the bed (%)
Activated carbon	398	Cylinders (4.5 x 7-9)	0.460
Siliceous stone	849	Granulated (7-9)	0.400
Expanded polystyrene	4	Spheres (7-9)	0.360
PVC	501	Cylinders (4.3 x 6-8.5)	0.347
Glass	801	Spheres (9)	0.452

TABLE 3. EFFECT OF pH ON THE STEADY STATE FERRIC IRON PRODUCTIVITY

pH inlet solution	1.49	1.33	1.25	1.15
pH outlet solution	1.91	1.73	1.55	1.39
Ferric iron productivity (g h^{-1})	2.64	2.75	2.67	2.73

FIGURE CAPTIONS

Figure 1. Procedure of the biofilm formation.

Figure 2. Bubble size distribution for the ceramic and wood diffusers.

Figure 3. Effect of the nature of the support material on the maximum steady-state ferric iron productivity. Bioreactor: 10 cm-high; Inlet liquor: 7 g l^{-1} Fe^{2+}. Air: 250 ml min^{-1}, wood diffuser.

Figure 4. Effect of temperature on the steady-state ferric iron productivity. Bioreactor: 30 cm-high packed with siliceous stone particles of 4-5 mm of size. Inlet liquor: 3 g l^{-1} Fe^{2+} at a flow rate of 2300 ml min^{-1}. Air: 500 ml min^{-1}, nozzle.

Figure 5. Effect of the type of air distributor on the maximum steady-state ferric iron productivity. Bioreactor: 30 cm-high packed with siliceous stone particles. Inlet liquor: 7 g l^{-1} Fe^{2+} . Air: 1000 ml min^{-1}.

Figure 1

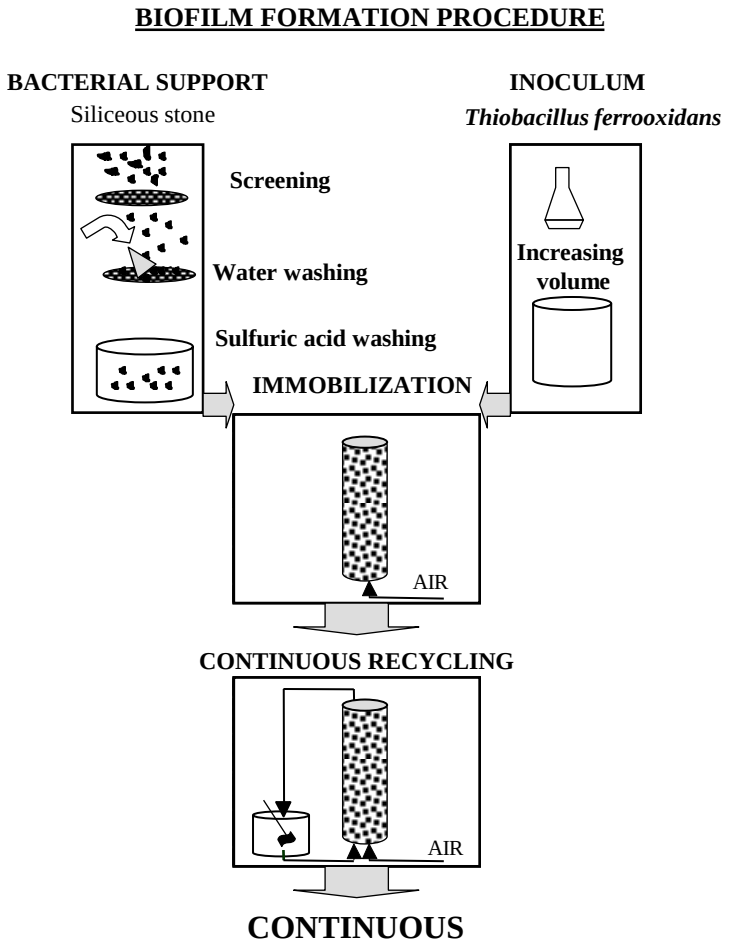


Figure 2

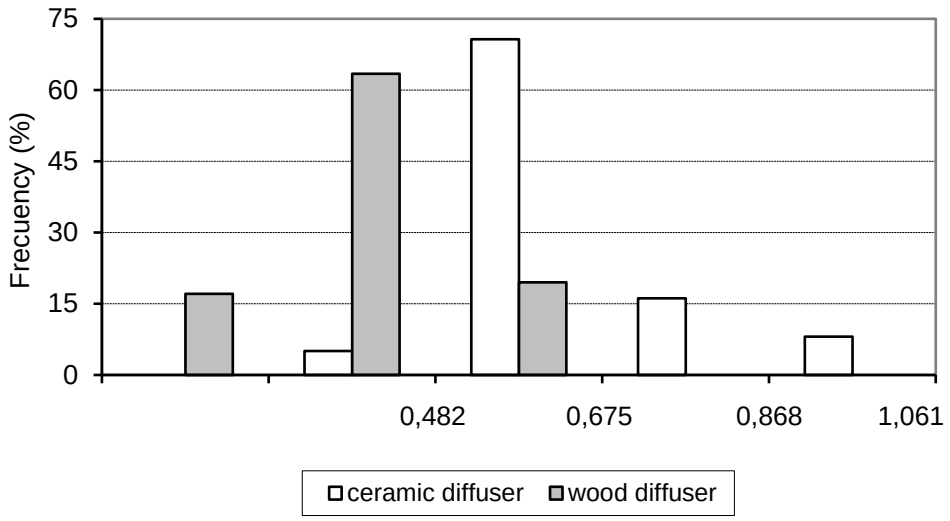


Figure 3

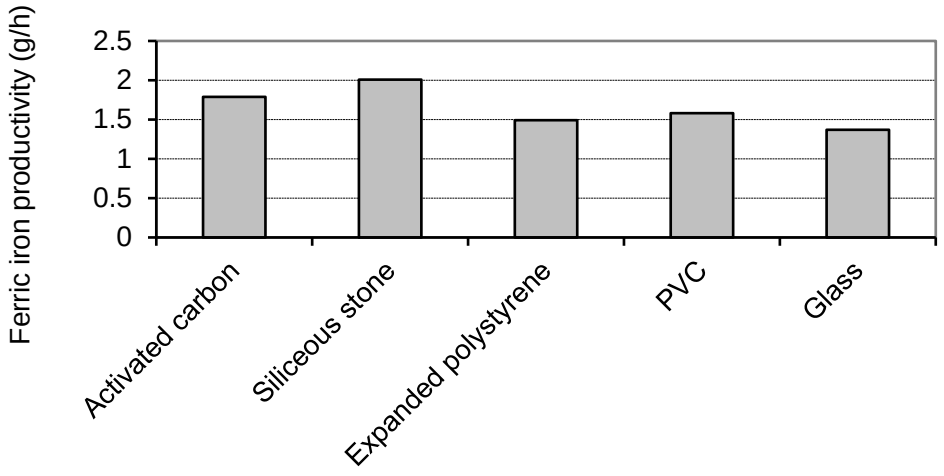


Figure 4

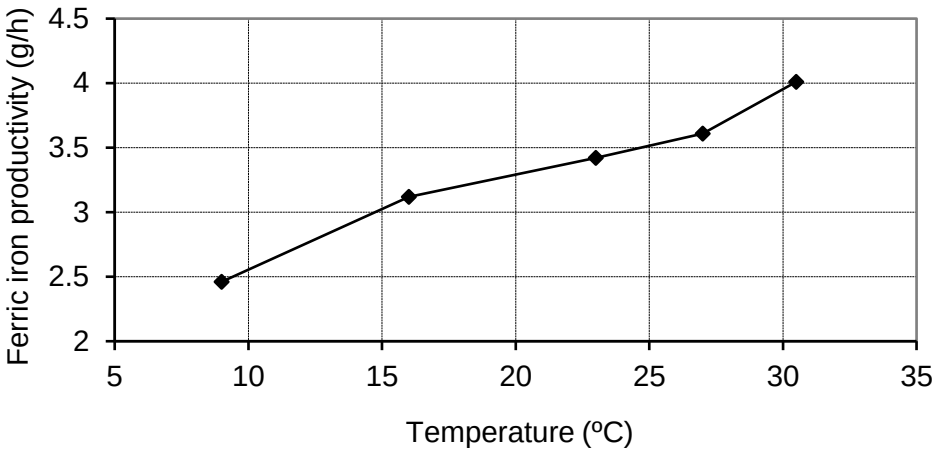
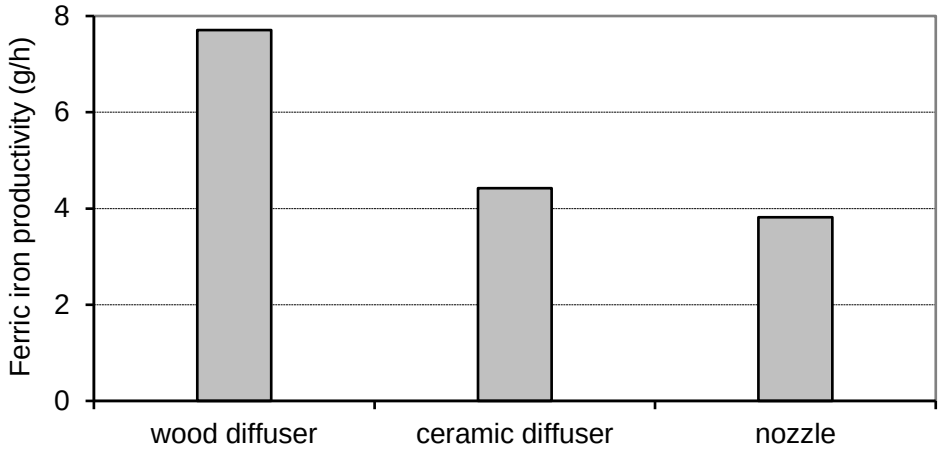


Figure 5


Ferric iron productivity (g/h)
8
6
4
2
0
wood diffuser
ceramic diffuser
nozzle