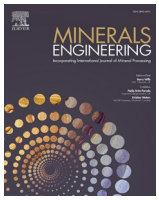
Minerals Engineering 204 (2023) 108408

Contents lists available at ScienceDirect

Minerals Engineering

journal homepage: www.elsevier.com/locate/mineng



Ferrous iron biooxidation in a flooded packed-bed bioreactor at extreme conditions of iron concentration and acidity

Alfonso Mazuelos [a,*], Martin Moreno-Perez [a], Blanca Perdigones [a], Pablo Ramirez [a], Nieves Iglesias-Gonzalez [a]

[a] Chemical Engineering Department, Faculty of Chemistry, University of Seville, C/ Profesor Garcia Gonzalez, Edificio de Quimica, Planta 1, 41012 Seville, Spain

ARTICLE INFO

Keywords:
Ferrous iron biooxidation
Acidithiobacillus ferrooxidans
Leptospirillum ferrooxidans
Flooded packed-bed bioreactor
Low pH
High iron concentration

ABSTRACT

The utility of ferrous iron biooxidation is to regenerate Fe(III) at required rates and conditions in specific hydrometallurgical contexts, such as metal extraction from ores/concentrates and mining and electronic waste. In these applications, considerable kinetics improvements can be achieved by increasing [Fe(III)], but pH must be decreased to avoid precipitation of this oxidant. Information about continuous biooxidation operation is limited to [Fe] < 20 g/L and 2.3 > pH > 1, therefore, it is interesting to test wider ranges for these parameters.

A 1L flooded packed-bed bioreactor (30 cm in height), inoculated with *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans,* was operated 60 days in continuous mode without interruptions at [Fe] up to 57 g/L and pH up to 0.44.

Total Fe(II) conversion was achieved when operating at [Fe] = 57 g/L and pH = 1.2. The maximum biooxidation rate reached was 3.5 g/L·h for [Fe] = 40 g/L and 1 h of hydraulic retention time. Biooxidation rate decreases by 32 % when pH decreases from 1.2 to 0.44. Nevertheless, the biofilm remained stable at this low pH and steady state was achieved. When comparing the relative decreases in biooxidation rate and oxygen solubility, the drop of efficiency can be explained by aeration limitations and salting out effect.

1. Introduction

The term "ferrous iron biooxidation" refers to the rapid oxidation of Fe(II) to Fe(III) with oxygen catalyzed by microorganisms (Reaction 1).

$$Fe(II) + H^+ + \frac{1}{4}O_2 \xrightarrow{microorganisms} Fe(III) + \frac{1}{2}H_2O \qquad \text{(Reaction 1)}$$

The microorganisms capable of catalyzing this reaction are known as iron-oxidizing microorganisms (Nemati et al., 1998; Norris et al., 2000; Johnson, 2008). Among them, the species of the genera *Acidithiobacillus* and *Leptospirillum* stand out.

The applications of Fe(II) biooxidation are depuration and valorization of acid mine drainage (AMD) (Sandström and Mattsson, 2001; Song et al., 2022), desulfurization of combustible gases (Malhotra et al., 2002; Lin et al., 2013), valorization of copper slags (Carranza et al., 2009; Kaksonen et al., 2017), valorization of e-wastes (Hubau et al., 2020; Iglesias-González et al., 2021; Iglesias-Gonzalez et al., 2022) and metal extraction from ores and concentrates. All of them can be considered clean technologies because they are based on circularity and sustainability. With exception of the first one, they are inspired by indirect bioleaching with separation of effects chemical and biological (Carranza et al., 1993).

Indirect bioleaching consists of two simultaneous stages (Nemati et al., 1998; Schippers & Sand, 1999; Mishra et al., 2023):

- A chemical leaching stage, in which the oxidizing agent Fe(III) takes electrons from the metal sulfide (MeS), oxidizing it to the metal (Me) and S°, and modifying its oxidation state to Fe(II) (Reaction 2).

$$MeS + Fe(III) \rightarrow Me(II) + S^o + Fe(II) \qquad \text{(Reaction 2)}$$

Abbreviations: a, Ratio C_{X0}/Y_{XS}; AMD, Acid Mine Drainage; ε, Gas hold-up; EW, Electrowinning; $[Fe(II)]_{0\ ino}$, Initial ferrous iron concentration of the inoculum; FPBB, Flooded Packed-Bed Bioreactor; Me, Metal; q_s, Specific rate of substrate; C_X, Biomass concentration; C_{X0}, Initial biomass concentration; $C_{Xf\ ino}$, Final biomass concentration of the inoculum; μ_{max}, Specific growth rate; ORP, Oxidation Reduction Potential; S, Oxygen solubility; S^0, Oxygen solubility in water; SX, Solvent Extraction; V_{ino}, Volume of the inoculum; V_{cul}, Volume of the culture; Y_{XS}, Yield of biomass from substrate.

* Corresponding author.
E-mail address: mazuelos@us.es (A. Mazuelos).

https://doi.org/10.1016/j.mineng.2023.108408
Received 16 June 2023; Received in revised form 31 July 2023; Accepted 23 September 2023
Available online 6 October 2023
0892-6875/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

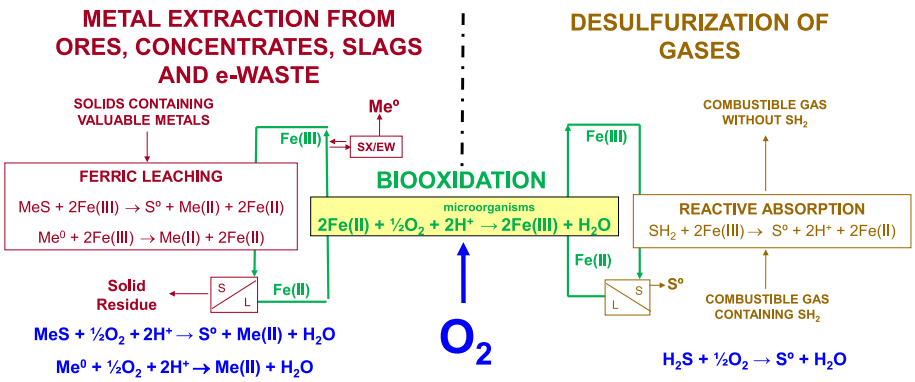


Fig. 1. Conceptual engineering of metal extraction from ores and concentrates, slags and e-waste, and desulfurization of gases.

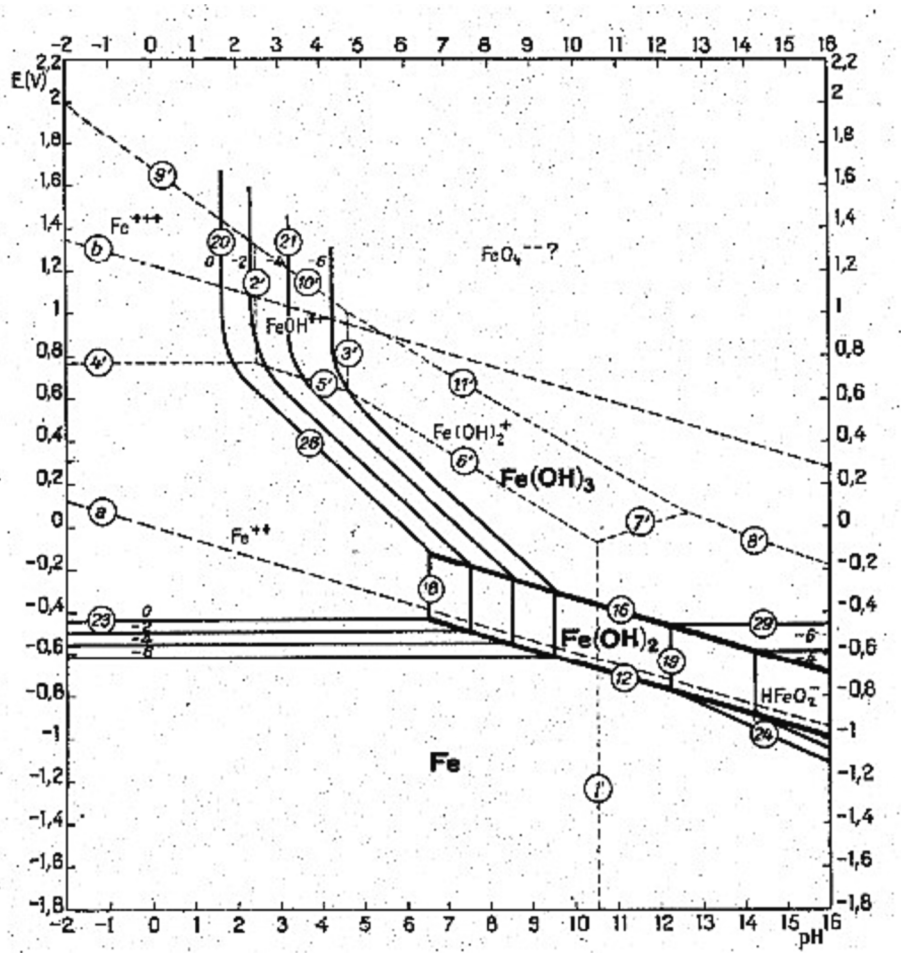


Fig. 2. Iron Pourbaix diagram (Pourbaix, 1974).

- A biological stage, the biooxidation of Fe(II) regenerating the oxidizing agent Fe(III) (Reaction 1).

It is possible to accelerate indirect bioleaching by carrying out the chemical leaching and biological Fe(II) oxidation stages separately, allowing the optimization of each independently (Carranza et al., 1993).

With the exception of depuration of acid mine drainage (AMD), in the applications mentioned above a sulfide, a metal sulfide, H_2S or an elemental metal is oxidized by O_2, with the redox couple Fe(II))/Fe(III) acting as an intermediate in electron transfer, and microorganisms catalyzing the regeneration of the oxidizing agent Fe(III) (Fig. 1).

In all these processes, the chemical leaching state can be accelerated by increasing the concentration of Fe(III). Along with this increase in Fe (III) concentration, sufficient acidic conditions are required to prevent the precipitation of this oxidizing reagent. In the Pourbaix diagram (Fig. 2) it can be seen that a pH lower than 1.5 is required for a Fe(III) concentration of 1 M (56 g/L) (Pourbaix, 1974).

In order to achieve an accurate control in the operation of continuous bioreactors, this thermodynamic interdependence between acidity and iron concentration must be assumed since it conditions critical operational aspects such as microorganism performance, oxygen supply and physicochemical stability of biofilms.

As far as the authors know, biooxidation carried out at iron concentrations higher than 25 g/L has only been tested in batch

experiments. Kawabe et al. (2003) informed that biooxidation rate drops by more than 80 % in the presence of Fe(III) at a concentration of 25 g/L when strains of *Acidithiobacillus ferrooxidans* are used as inoculum. Pyrite bioleaching performance with *A. ferrooxidans* is considerably depressed when Fe(III) concentration is 35 g/L, being slightly affected by Fe(II) concentration up to 27 g/L (Battaglia et al., 1994). Nevertheless, tolerance of *A. ferrooxidans* to Fe ions can be increased to 36 g/L of total iron by an adaptation process based on subculturing (Saavedra et al., 2020). In fact, there is no mechanism of inhibition caused by Fe (III) described in the existing literature (Amouric et al., 2009; Moinier et al., 2017; Ponce et al., 2012). Rawlings et al. (1999) reported that *Leptospirillum* species are more resistant to Fe(III) than *Acidithiobacillus* species. In continuous operation, only liquors with an iron concentration lower than 25 g/L have been biooxidized (Mousavi et al., 2007; Frias et al., 2008).

pH has a great influence in the metabolism of the iron-oxidizing microorganisms and their ability to oxidize Fe(II). *A. ferrooxidans* typically grows at a pH $>$ 1.5 with an optimum pH between 1.8 and 2.5 whereas *L. ferrooxidans* typically grows at pH $>$ 1 and its optimum pH is between 1.3 and 2 (Torma, 1977; Karamanev & Nikolov, 1988; Smith et al., 1988; Breed & Hansford, 1999; Oren, 2010). Using a mixed culture of these microorganisms allows continuous Fe(II) biooxidation in a range of pH between 1 and 2.3 (Mazuelos et al., 2010, 2012).

Fe(II) biooxidation is a heterogeneous reaction, in which microorganisms, Fe(II) and oxygen are in different phases. Therefore, in addition to the biochemical reaction itself, the transport phenomena of matter must be considered. As a result, the transport of oxygen from the gas to the liquid significantly influences the overall kinetics of the biooxidation reaction, and aeration is usually a key design factor (Savic et al., 1998; Mazuelos et al., 2002). Oxygen solubility in aqueous solutions depends on ion concentration due to salting out effect (Mazuelos et al., 2017). When the iron concentration is increased and pH is decreased (increasing SO_4^{2-}, HSO_4^- concentrations), a decrease in oxygen solubility must be assumed and, as a consequence, a lower oxygen concentration in the liquid medium is available for the bacteria.

Understanding the structure that allows the microorganisms to attach themselves to surfaces is essential to operate continuous bioreactors based on biofilms. Jarosite provides iron-oxidizing microorganisms with a porous structure in which they can attach (Karamanev, 1991). Jarosite formation depends on the Fe(III) concentration and pH (Dutrizac, 1983; Kaksonen et al., 2014). pH has been mentioned as a key design factor (Pogliani & Donati, 2000; Kinnunen & Puhakka, 2004; van der Meer et al., 2007; Kahrizi et al., 2009; Mazuelos et al., 2010) because it affects the biofilm stability. pH must be low enough to avoid precipitation of these iron compounds but not so low as to compromise the structure of the biofilm due to the dissolution of the inorganic matrix. At a low pH ($<$1), an excessive dissolution of these precipitates occurs, resulting in the destabilization of the biofilm and in a cell wash-out. Precipitation of Fe(III) compounds can lead to an accumulation of precipitates, causing clogging of the channels meant for the liquid and the air, hindering nutrients diffusion to the microorganisms (Curutchet et al., 1992; Mazuelos et al., 2010).

The utility of biooxidation is to regenerate Fe(III) at demanded rates and conditions in the above mentioned industrial contexts. On a commercial scale, only continuous operation is of interest. Continuous biooxidation can be carried out using bioreactors designed specifically for this purpose. In the last two decades, several types of reactors have been studied such as stirred tank reactors operating in both batch and continuous mode (Ojumu et al., 2008; Candy et al., 2009), airlifts (Kaksonen et al., 2014; He et al., 2022) and packed-bed bioreactors (Mazuelos et al., 2000; Chowdhury & Ojumu, 2014; Abbasi et al., 2021). Of the bioreactors found in the literature, those that have shown the highest efficiency are the flooded packed-bed bioreactors (FPBBs), which can obtain biooxidation rates higher than 3.5 kg/h·m^3 (Mazuelos et al., 2000). This bioreactor has been successfully tested at pilot plant scale, integrated in both hydrometallurgical and environmental processes (Frias et al., 2008; Avila et al., 2011).

In FPBBs, the inlet liquor and air are continuously fed at the bottom, where an efficient mixing must be promoted between the gas and liquid phases. Both fluids ascend through the packed-bed occupying all the hollow volume between the particles on which the cells are supported. The liquid overflows from the top of the bioreactor (Mazuelos et al., 1999).

The advantages associated with FPBBs are:

- Ease of operation as no mechanical elements are required and low associated energy costs, as mechanical agitation systems are not required.
- Stability of the biofilm. The cells are fixed on immobile particles.
- Mean residence time and residence time distribution for the liquid can be controlled during operation from the inlet flow.
- Gas-liquid interfacial area can be controlled during operation from gas injection conditions.
- High gas–liquid interfacial areas can be achieved, due to the retention of the gas phase in the packed-bed.

The objective of this work is to study continuous Fe(II) biooxidation at wider ranges of iron concentration and pH than those reported in literature ([Fe] $<$ 25 g/L and 2.5 $>$ pH $>$ 1). For this purpose, a 1L FPBB was operated in continuous, thus testing the flexibility of this design regarding feed composition at extreme operational conditions. Control and robustness are also checked by planning more than 60 days of continuous operation without interruptions and reaching consecutives steady states for each operational condition tested.

2. Materials and methods

2.1. Microorganisms

The culture used as inoculum for the present study was originally obtained from the Rio Tinto Mine in Huelva, Spain. It consists mainly of the mesophilic acidophilic iron oxidizing species Acidithiobacillus *ferrooxidans* and *Leptospirillum ferrooxidans* (Mazuelos et al., 2012). This culture has been regularly maintained on a modified Silverman and Lundgren 9 K nutrient medium (Silverman & Lundgren, 1959) at pH 1.25 (adjusted with concentrated H_2SO_4) and 31 °C.

2.2. Batch tests

A set of preliminary batch Fe(II) biooxidation tests have been carried out in stirred Erlenmeyer flasks at 180 rpm and 31 °C. These cultures were monitored by oxidation–reduction potential (ORP) probes (reference to Ag/AgCl) (Atlas Scientific ®) connected to a PC with an Arduino UNO controller.

Influence of Fe(III) concentration and pH was studied in order to observe the tolerance and the adaptation of the culture to these parameters and its impact on biooxidation kinetics. All the tests were performed with 100 mL of 9 K nutrient medium, whose Fe(III) and Fe(II) concentrations and pH were modified to the set values for each case, and 10 mL of inoculum.

Specific growth rate (μ_{max}) was calculated from ln[Fe(III)] vs time curves.

The rate of Fe(II) consumption is given by:

$$\frac{d[Fe(II)]}{dt} = -q_s \cdot C_X \quad (1)$$

- where C_X is the biomass concentration and assuming exponential growth can be calculated as follows:

$$C_X = C_{X0} \cdot e^{\mu_{max} \cdot t} \quad (2)$$

where C_{X0} is the initial biomass concentration.

- and q_s is the specific rate of substrate uptake and, when neglecting substrate uptake for maintenance, can be expressed as:

$$q_s = \frac{\mu_{max}}{Y_{XS}} \quad (3)$$

where Y_{XS} is the yield of biomass from substrate and μ_{max} is the specific growth rate.

By substituting *Eqs.* 2 and 3 into *Eq.* 1 and integrating with initial condition: t = 0 and [Fe(II)] = $[Fe(II)]_0$, the following expression is obtained:

$$[Fe(II)] = [Fe(II)]_0 - \frac{C_{X0}}{Y_{XS}} \cdot (e^{\mu_{max} \cdot t} - 1) \quad (4)$$

In *Eq.* 4, C_{X0} can be referred to the final biomass concentration of the inoculum $C_{Xf\ ino}$:

$$C_{X0} = C_{Xf\ ino} \cdot \frac{V_{ino}}{V_{cul}} \quad (5)$$

where V_{ino} and V_{cul} are the volumes of the inoculum and the culture, respectively.

Moreover, $C_{Xf\ ino}$ can be calculated as:

$$C_{Xf\ ino} = Y_{XS} \cdot [Fe(II)]_{0\ ino} \quad (6)$$

where $[Fe(II)]_{0\ ino}$ is the initial ferrous iron concentration of the inoculum.

In *Eq. 6*, it is assumed that the initial cell concentration of the inoculum must be considerably lower than $C_{Xf\ ino}$ and, in consequence, was neglected. In addition, it is considered that the final Fe(II) concentration in the inoculum is 0 g/L.

Substituting *Eq.* 6 in *Eq.* 5 and separating variables, the ratio C_{X0}/Y_{XS} (a) can be obtained from known values as follows:

$$a = \frac{C_{X0}}{Y_{XS}} = [Fe(II)]_{0\ ino} \cdot \frac{V_{ino}}{V_{cul}} \quad (7)$$

Therefore *Eq.* 4 can be written as:

$$[Fe(II)] = [Fe(II)]_0 - a \cdot (e^{\mu_{max} \cdot t} - 1) \quad (8)$$

From the ORP vs time curves it can be obtained the time when [Fe(II)] ≈ 0 as the time when an ORP value of 605 mV is reached ($t_{ORP=605}$). At this time *Eq.* 8 reads:

$$[Fe(II)]_0 = a \cdot (e^{\mu_{max} \cdot t_{ORP=605}} - 1) \quad (9)$$

and from *Eq.* 9, the specific growth rate can be calculated as follows:

$$\mu_{max} = \frac{\ln(\frac{[Fe(II)]_0}{a} + 1)}{t_{ORP=605}} \quad (10)$$

2.3. Continuous operation

A FPBB for continuous Fe(II) biooxidation was operated for more than 60 days without interruptions in three campaigns planned with different objectives:

- Campaign 1 (553.5 h): to test the influence of total iron concentration at total conversion of Fe(II). Total iron concentration was increased (variable Fe(III) concentration and Fe(II) concentration close to 9 g/L) step by step from 9 to close to 60 g/L. pH had to be decreased from 2 to 1 to avoid precipitation of Fe. Air flow rate was 250 mL/min and liquid flow rate was about 125 mL/h. Air was injected by a ceramic diffuser.
- Campaign 2 (577.5 h): at total iron concentration of 40 g/L and pH 1.2, liquid and air flow rates were simultaneously and progressively increased for preventing Fe(II) and oxygen limitations, respectively.
- Campaign 3 (504 h): to test the influence of pH at total iron concentration of 40 g/L. In this campaign, pH was progressively decreased from 1.25 to 0.4, adjusting pH in feed with H_2SO_4. Air flow rate was 1500 mL/min and hydraulic retention time for the liquid was 3 h. A 2 mm open pipe was used as air diffuser.

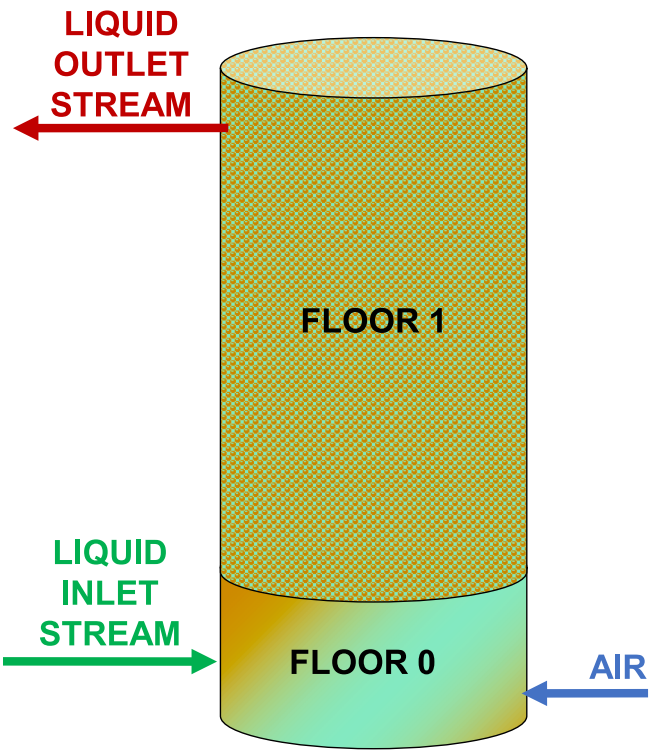


Fig. 3. Schematic of a flooded packed-bed bioreactor for continuous Fe(II) biooxidation.

Table 1
Dimensions of the FPBB operated.

Dimension	FPBB
Diameter (cm)	8.4
Floor 0 height (cm)	5
Floor 0 vol (mL)	277
Floor 1 height (cm)	28
Floor 1 vol (mL)	652
Overflow volume (mL)	0
Total volume (mL)	930

The FPBB consisted of an 8.4 cm diameter column containing a packed bed of 30 cm in height (floor 1 in Fig. 3). The bed consisted of siliceous stone particles ranging from 4 to 6 mm in diameter. Bed porosity was 0.42. The bed rested on a plastic grid of 5 mm mesh size. A ceramic diffuser and a 2 mm internal diameter pipe were used for injecting air. These diffusers were placed in floor 0 (Fig. 3) without support particles in order to promote turbulence by air injection.

The geometrical characterization of the FPBB used in this experimental work is presented in Table 1.

The start-up of the FPBB required 10 days, following the protocol described by Mazuelos et al. (2001). FPBB was operated at room temperature.

FPBB monitoring consisted of the control of flow dynamics: liquid flow rate, air flow rate, and density, and the measurement of chemical parameters: pH, Fe and Fe(II) concentrations.

Conversion of Fe(II) in the FPBB was calculated by *Eq.* 11:

$$\text{Conversion} = \frac{[Fe(II)]_{inlet} - [Fe(II)]_{outlet}}{[Fe(II)]_{inlet}} \quad (11)$$

Biooxidation rate was calculated with the *Eq.* 12:

Table 2
Tolerance (T) and adaptation (A) preliminary tests to high Fe(III) concentrations. Fe(II) initial concentration 9.09 g/L, pH 1.25, 180 rpm, 31 °C.

Culture	$[Fe(III)]_0$ (g/L)	$t_{[Fe(II)]\rightarrow 0}$ (h)	μ_{max} (h^{-1})
0Fe(III)-T	0.82	56	0.048
10Fe(III)-T	9.91	53	0.051
20Fe(III)-T	19.0	57	0.047
30Fe(III)-T	28.1	61	0.044
0Fe(III)-A	0.90	48	0.052
10Fe(III)-A	10.81	44	0.056
20Fe(III)-A	20.74	47	0.053
30Fe(III)-A	30.65	46	0.054

$$\text{Biooxidation rate} = \frac{([Fe(II)]_{inlet} - [Fe(II)]_{outlet})\cdot\text{Liquid flow rate}}{\text{Total volume of the FPBB}} \quad (12)$$

Oxygen solubility values were estimated by the model described in Mazuelos et al. (2017). And gas hold-up (ε) was calculated as follows:

$$\varepsilon = \frac{Volume\ of\ air}{Total\ volume\ of\ the\ FPBB} \quad (13)$$

To determine the volume of air for each flow rate the following procedure was carried out:

- The bioreactor was slowly filled with liquid medium (9 K medium) avoiding bubble accumulation in the packed-bed.
- Air stream was fed at the set flow.
- The liquid displaced by the air, which overflowed by the outlet pipe, was gathered and weighted.

2.4. Analytics

Total Fe concentration was determined by atomic absorption spectrophotometry at 248.33 nm in air-acetylene flame.

Fe(II) concentration was determined by automatic potentiometric titration with $K_2Cr_2O_7$ 0.05 N.

Fe(III) concentration was determined by the sulfosalicylic acid method (Karamanev et al., 2002).

3. Results and discussions

3.1. Batch tests

A set of preliminary batch biooxidation tests was carried out in order to study the influence of total iron concentration and pH on biooxidation kinetics.

3.1.1. Influence of Fe(III) concentration

Tolerance and adaptation of the inoculum to different Fe concentrations were studied. In tolerance (T) tests, the inoculum was exposed for the first time to different Fe(III) concentrations (Table 2). Subcultures from the previous tolerance tests were carried out in the same conditions to study the adaptation (A) of the microorganisms to Fe(III) (Table 2). All the tests were conducted at pH 1.25 and an initial Fe(II) concentration of 9.09 g/L. Table 2 shows the initial Fe(III) concentration, the Fe(II) depletion time ($t_{[Fe(II)]\rightarrow 0}$) and the specific growth rate for both tolerance and adaptation tests. Fig. 4 shows ORP vs time curves for the tolerance cultures.

Slight differences for Fe(II) depletion times ($t_{[Fe(II)]\rightarrow 0}$) and maximum

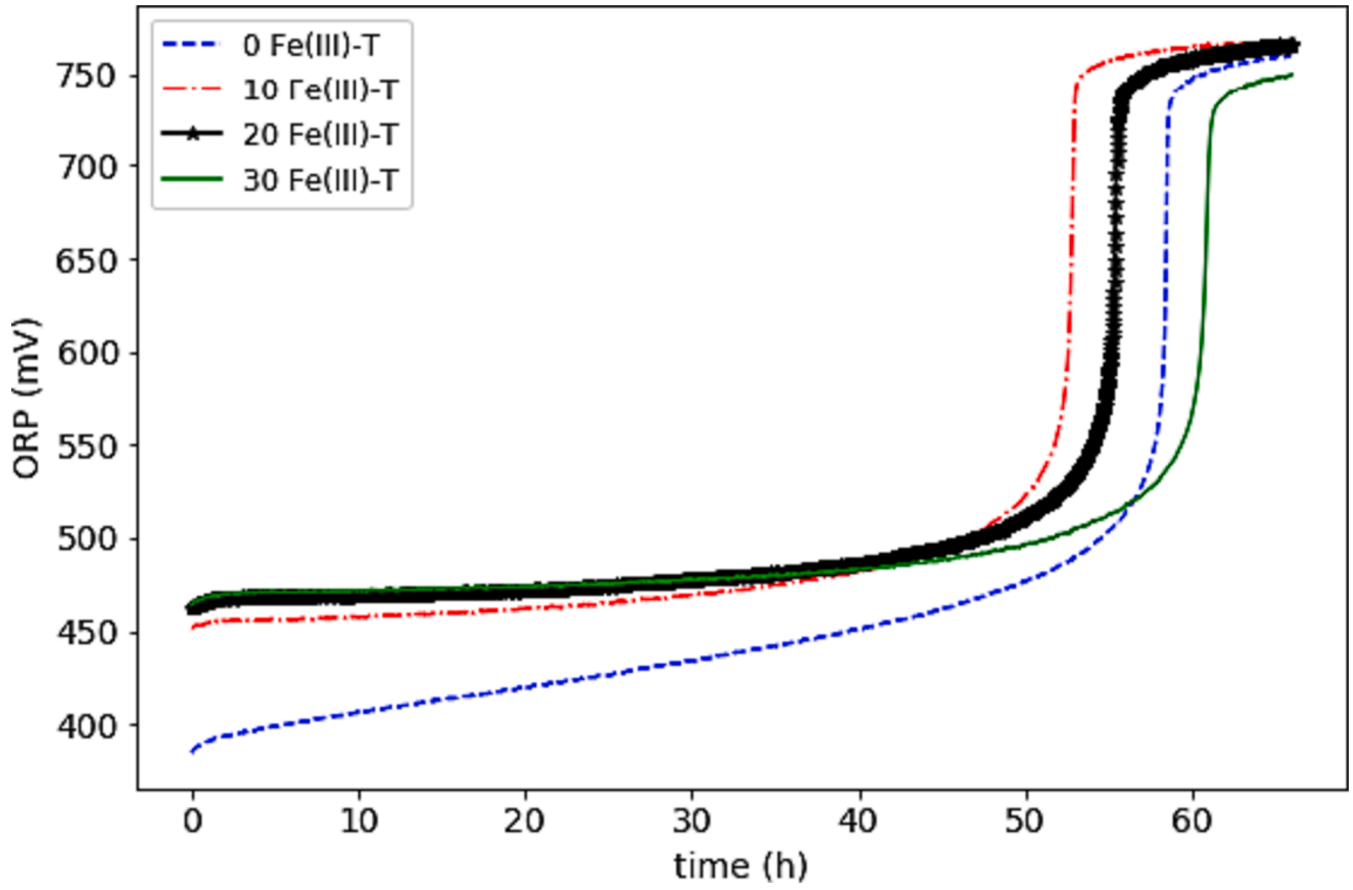


Fig. 4. ORP vs time curves for the tolerance tests to Fe(III). (---) 0Fe(III)-T; (-·-) 10Fe(III)-T; (-▲-) 20Fe(III)-T;(—) 30Fe(III)-T. Fe(II) initial concentration 9.09 g/L, pH 1.25, 180 rpm, 31 °C.

Table 3
Tolerance (T) and adaptation (A) preliminary tests to low pH. Initial Fe(II) and Fe(III) concentrations 9.09 and 28.1 g/L, respectively, 180 rpm, 31 °C.

Culture	pH	$t_{[Fe(II)]\rightarrow 0}$ (h)	μ_{max} (h^{-1})
1.25pH-T	1.25	61	0.044
1.00pH-T	1.00	60	0.045
0.75pH-T	0.75	73	0.037
0.50pH-T	0.50	129	0.021
1.25pH-A	1.25	46	0.054
1.00pH-A	1.00	45	0.055
0.75pH-A	0.75	50	0.049
0.50pH-A	0.50	73	0.034

specific growth rates were observed for the tolerance cultures (Table 2). These differences were even diminished in the adaptation study (Table 2). The small influence of the Fe(III) concentration on the bio-oxidation kinetic allows to affirm that the culture used as inoculum is easily adaptable to iron for concentrations up to 40 g/L.

3.1.2. *Influence of pH*

An analogous set of experiments was carried out for testing the influence of pH. Table 3 shows the initial pH, the Fe(II) depletion time ($t_{[Fe(II)]\rightarrow 0}$) and the maximum specific growth rate (μ_{max}) for the tolerance (T) and adaptation (A) tests. All tests were conducted with initial Fe(II) and Fe(III) concentrations of 9.09 and 28.1 g/L, respectively. ORP vs.

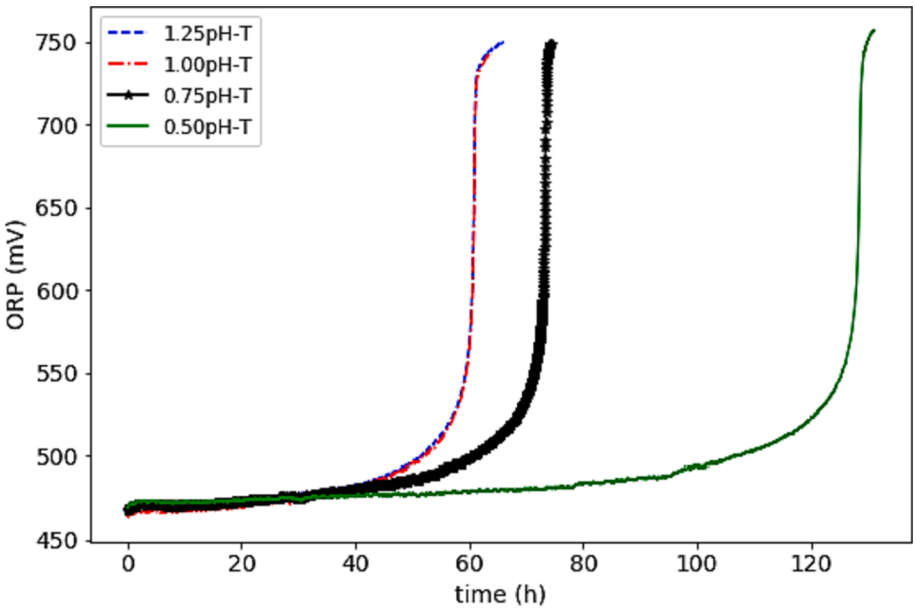


Fig. 5. ORP vs time curves for the tolerance tests to pH. (---) 1.25pH-T; (-·-) 1.00pH-T; (-▲-) 0.75pH-T;(—) 0.50pH-T. Initial Fe(II) and Fe(III) concentrations 9.09 and 28.1 g/L, respectively, 180 rpm, 31 °C.

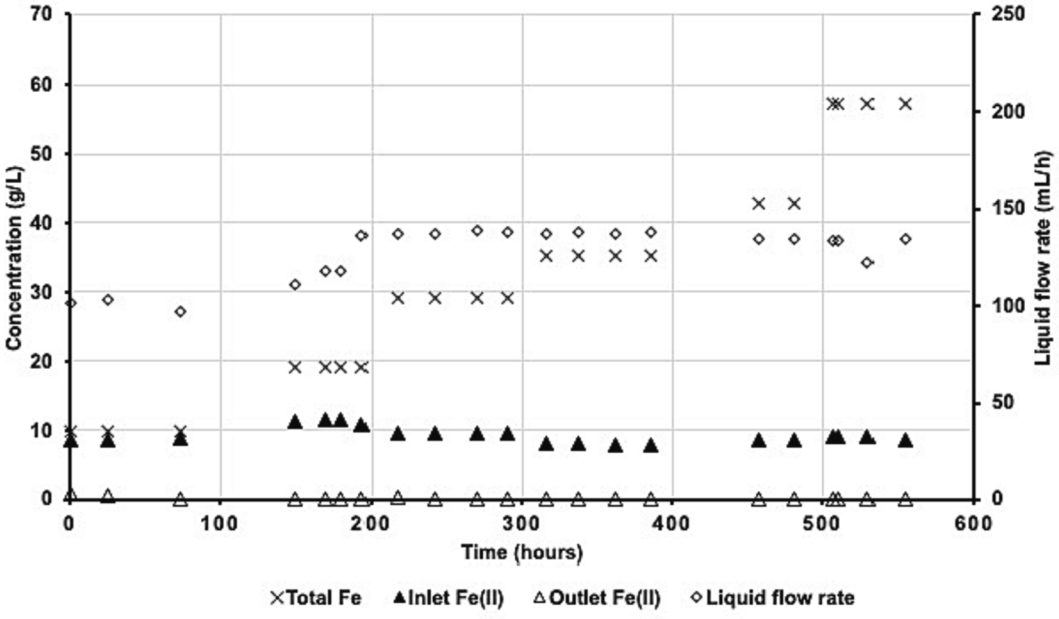


Fig. 6. Operating conditions to study the influence of Fe concentration. Total Fe concentration (×) and Fe(II) concentration in the inlet stream (▲) and outlet stream (△). Liquid flow rate (◊) values are also presented. Air flow rate was 250 mL/min. Aeration by a ceramic diffuser.

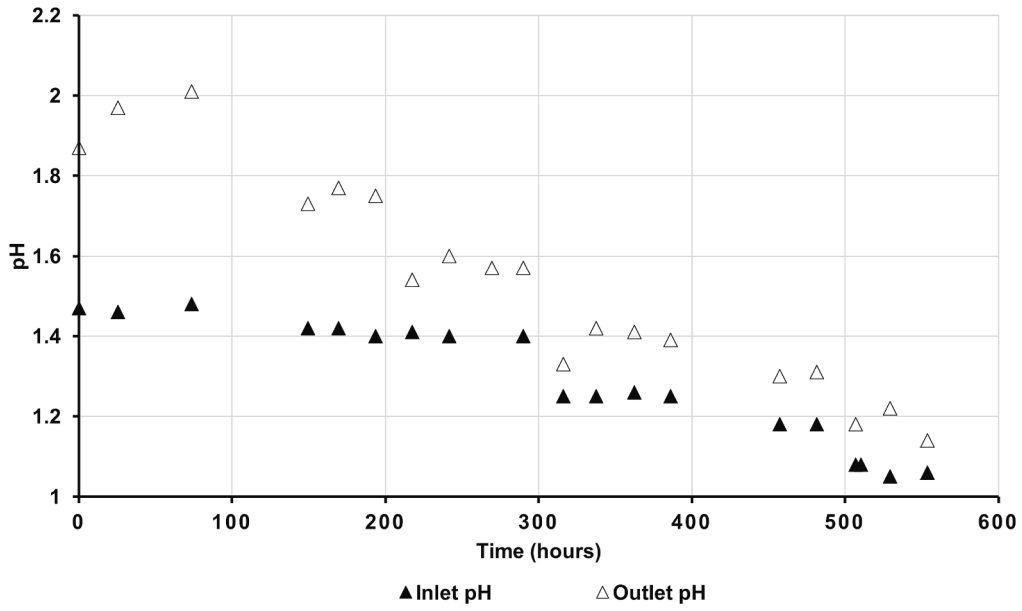


Fig. 7. Ph values in the inlet stream (▲) and outlet stream (△) of the fpbb during the time of operation for the study of the influence of fe concentration. air flow rate was 250 mL/min. Aeration by a ceramic diffuser.

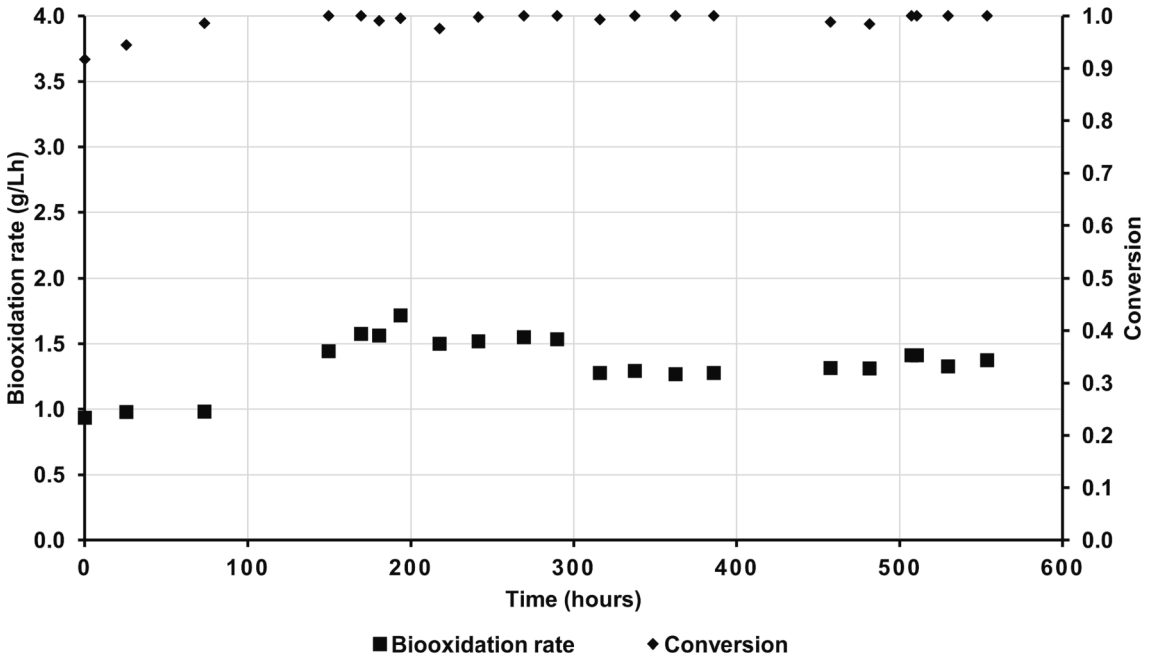


Fig. 8. Study of the influence of Fe concentration: biooxidation rate (■) and conversion (◆) calculated for the FPBB during the time of operation. Air flow rate was 250 mL/min. Aeration by a ceramic diffuser.

time curves for the tolerance cultures are shown in Fig. 5.

Similar biooxidation rates were obtained for the tolerance tests with initial pH values of 1.25 and 1.00 (Table 3). Nevertheless, for the tolerance cultures with initial pH values below 1, the biooxidation rate is greatly diminished (Table 3).

Similar results were obtained in the adaptation tests (Table 3). Bio-oxidation kinetics for the cultures with initial pH values of 1.25 and 1.00 are practically identical and the biooxidation rates were lower for the adapted cultures at pH below 1.00. However, the adapted cultures showed a kinetic improvement regarding the tolerance cultures (Table 3).

The Fe(II) depletion times ($t_{[Fe(II)]\rightarrow 0}$) and maximum specific growth rates (μ_{max}) for the adapted cultures at pH 1.25 and 1.00 are the similar that those obtained for the adapted culture to Fe(III) concentration of 30.65 g/L (Tables 2 and 3), showing that, after an adaptation step, the highest biooxidation rate is reached.

3.2. *Continuous operation*

Continuous biooxidation tests were carried out in three campaigns to study the influence of Fe concentration, liquid and air flow rates and pH.

3.2.1. *Influence of Fe concentration*

Figs. 6 and 7 show the history of operating conditions for testing the

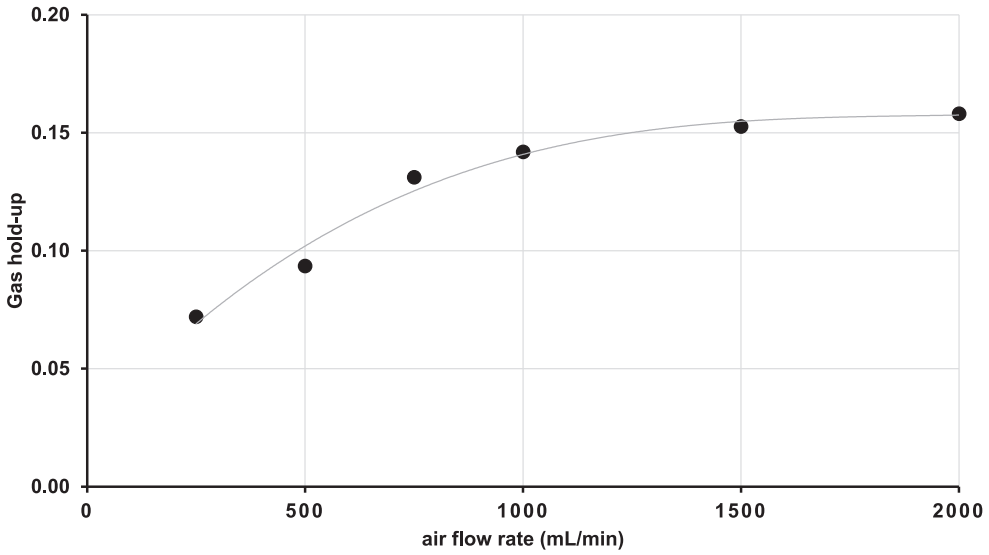


Fig. 9. Gas hold-up (•) values calculated for the FPBB at different air flow rates.

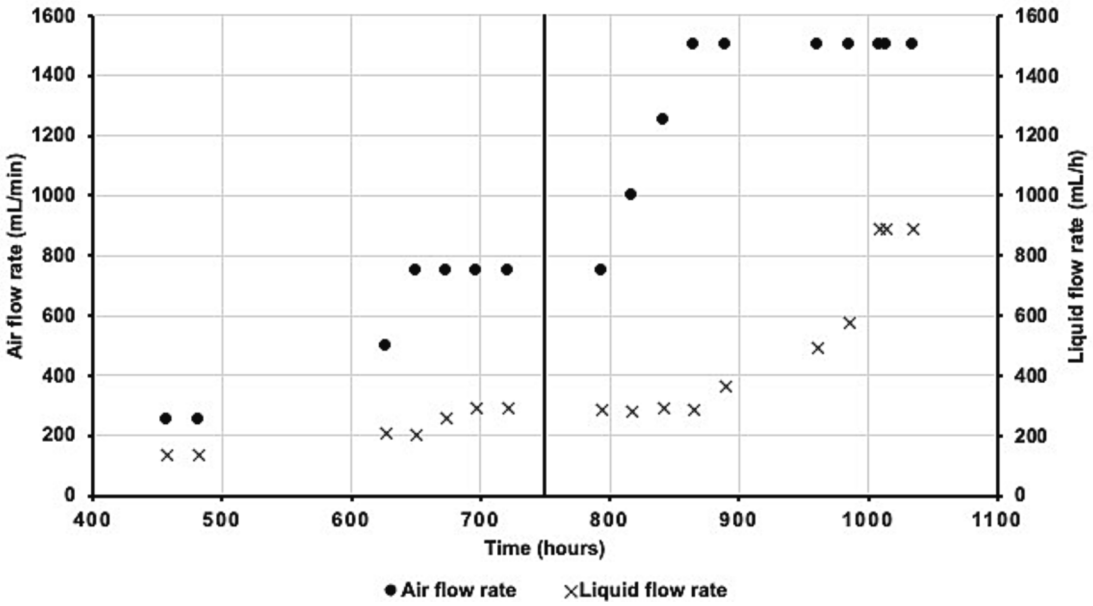


Fig. 10. Operating conditions in the study of the influence of air and liquid flows rates. Air (•) and liquid (×) flow rates of the FPBB during the time of operation. Total Fe concentration 40 g/L, Fe(II) concentration 10 g/L and pH 1.10. At 750 h (vertical line), the ceramic diffuser was changed by a 2 mm open pipe.

influence of iron concentration. In the inlet liquid stream Fe(III) concentration was progressively increased while Fe(II) concentration was kept at values close to 9 g/L (Fig. 6).

Iron can precipitate in the boundary layers that form close to the solids (support particles, air diffusers, walls, and pipes). Biofilm growth in these locations. As the biofilm is a sink of protons, pH close to the biofilm can be expected to be different from the pH in the bulk liquid. To prevent iron precipitation, pH was decreased from 1.5 to 1.1 (Fig. 7).

From the results shown in Fig. 6, Fe(II) conversion and biooxidation rate were calculated by equations 11 and 12, respectively (Fig. 8).

Fe(II) conversion was 100 % at practically all times except for the first 72 h, which were attributed to the period of growth and maturation of the biofilm after the start-up process (Fig. 8). Fe(III) concentration and pH did not affect the performance of the reactor in the range tested. These results are in accordance with those obtained in the batch tests (Tables 2 and 3).

In this reactor, biooxidation rates were close to 1.5 g/L·h, which is lower than the reported one in the literature (Mazuelos et al., 2000). This can be explained by substrate (Fe(II)) limitation. It is worth noting the stability of the bioreactor for more than 500 h of continuous operation without interruptions, reaching steady state for each Fe(III) concentration studied.

3.2.2. *Influence of liquid and air flow rates*

Under non Fe(II) limiting conditions, the productivity of the bioreactor should be very sensitive to changes in the aeration conditions, especially those regarding to the flow rate at which air is injected. An increase of this variable usually has the positive effect of increasing the gas–liquid interfacial area through the increase of gas hold-up (Mazuelos et al., 2002). The air flow rate not only modifies gas hold-up but also introduces turbulence and mixing in the liquid phase. Fig. 9 shows gas hold-up values calculated by Equation 13 as a function of the air flow rate for the FPBB operated.

The FPBB was continuously operated for 25 days without

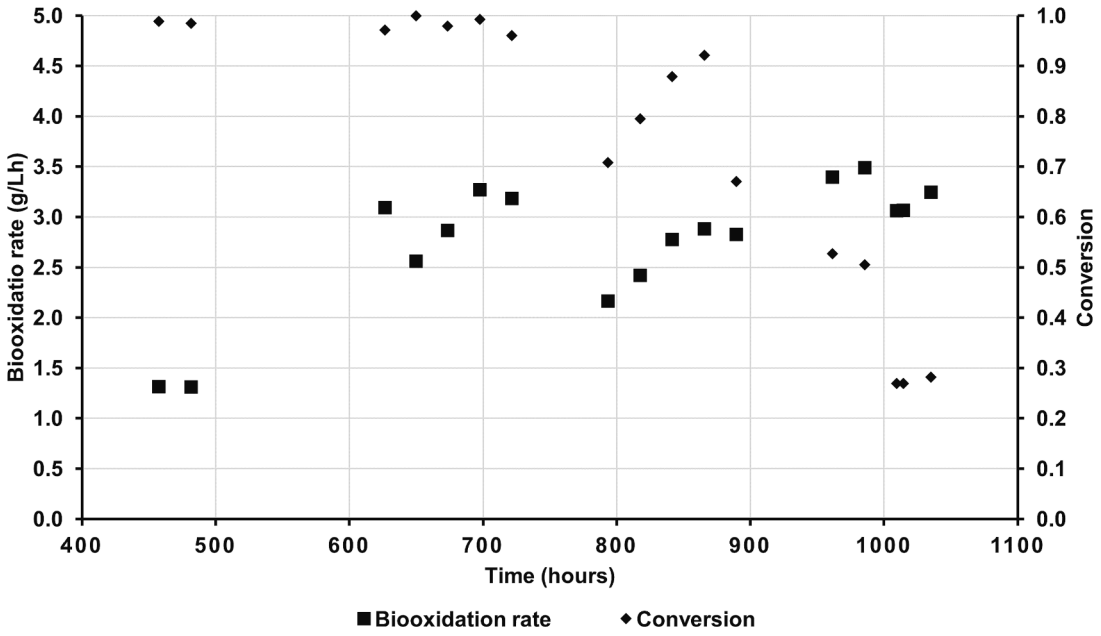


Fig. 11. Study of the influence of air and liquid flow rates: biooxidation rate (■) and conversion (◆) calculated for the FPBB during the time of operation. Total Fe concentration 40 g/L, Fe(II) concentration 10 g/L and pH 1.10.

interruptions in order to study the influence of air and liquid flow rates. To prevent substrate and oxygen limitations, the liquid and air flow rates were increased progressively from 134 to 886 mL/h and from 250 to 1500 mL/min, respectively (Fig. 10). A ceramic diffuser was used for the first 750 h. The air passage channels in the ceramic diffuser were progressively closing due to the deposition of ferric precipitates and biofilm, requiring more air pressure to maintain the desired flow rate. As a result, during the FPBB operation it was necessary to replace the ceramic diffuser with a 2 mm open pipe. The inlet liquors had the following set point values: total iron concentration 40 g/L, Fe(II) concentration 10 g/L and pH 1.10.

Fig. 11 shows biooxidation rate and conversion as a function of time. Increasing the air flow rate from 250 to 750 mL/min allowed the liquid flow rate to be increased from 134 to 291 mL/h maintaining the complete conversion of Fe(II) and increasing the biooxidation rate up to 3.27 g/L·h. Figs. 10 and 11 include the data obtained for similar operational conditions at 457.5 h and 481.5 h in the campaign 1 for the study of the influence of Fe concentration..

The change of the ceramic diffuser to a 2 mm open pipe (at 750 h) brought a decrease in conversion and biooxidation rate (Figs. 10 and 11). In this low height reactor, the influence of the diffuser plays an important role in the generation of gas–liquid interfacial area (Mazuelos et al., 2002). However, the progressive increase in air flow rate from 750 to 1500 mL/min is associated with an increase in biooxidation rate and conversion that corrects the aforementioned decrease in the FPBB performance due to the diffuser change.

After 890 h, the conversion decreased drastically as a consequence of the increase in liquid flow rate up to 886 mL/h, maintaining the

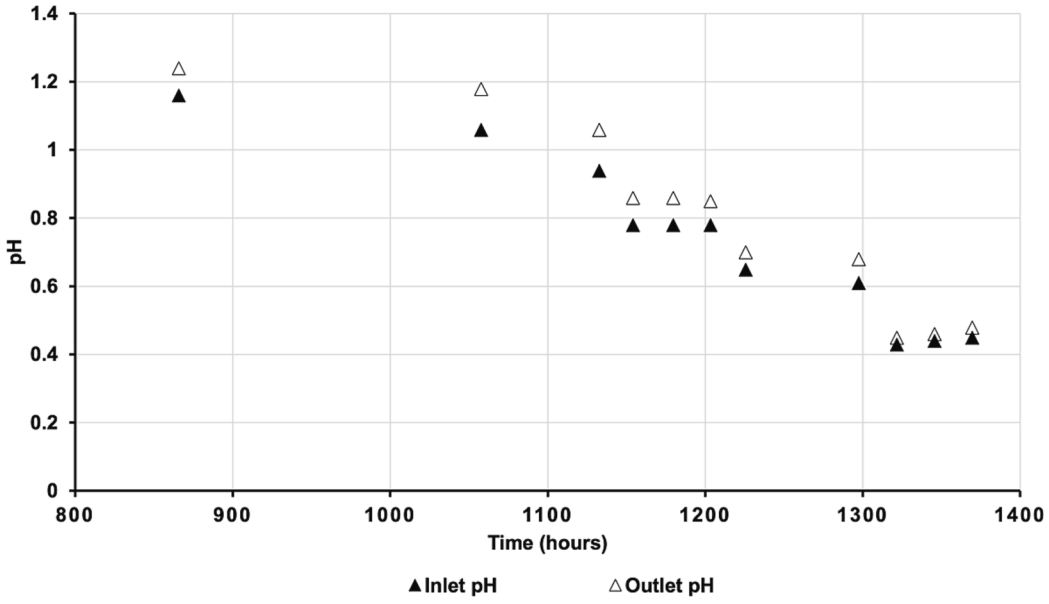


Fig. 12. Operating conditions in the study of the influence of acidity. pH values in the inlet stream (▲) and outlet stream (Δ) of the FPBB during the time of operation. Total Fe concentration 40 g/L, Fe(II) concentration 10 g/L, liquid flow rate 290 mL/h and air flow rate 1500 mL/min. 2 mm open pipe as air diffuser.

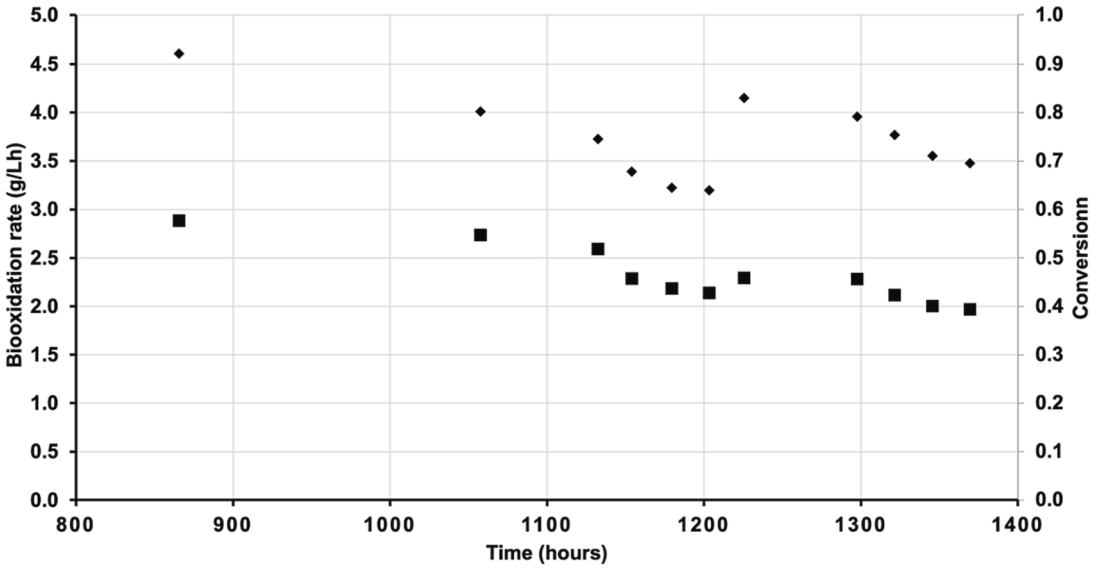


Fig. 13. Study of the influence of acidity: biooxidation rate (■) and conversion (◆) calculated for the FPBB during the time of operation.

biooxidation rate above 3 g/L·h, and even reaching 3.5 g/L·h (Fig. 11). Under these operating conditions, conversions in the order of 30 % are obtained, so that to achieve complete conversion of Fe(II) it would be necessary to operate a reactor 3 times higher than the one used in the present study (Mazuelos et al., 2000).

3.2.3. *Influence of acidity*

In this campaign, the influence of the inlet pH on continuous bio-oxidation was studied. The operation had the following set point values: total Fe concentration 40 g/L, Fe(II) concentration 10 g/L, liquid flow rate 290 mL/h and air flow rate 1500 mL/min with a 2 mm open pipe as air diffuser. The inlet pH was progressively decreased from 1.16 to 0.44 (Fig. 12). Fig. 13 shows the obtained biooxidation rates and Fe(II) conversions. Figs. 12 and 13 include the data obtained for similar operational conditions at 865.5 h in the campaign 2 for the study of the influence of liquid and air flow rates.

Decreasing pH in the inlet fed from 1.16 to 0.44 results in a 32 % decrease in the biooxidation rate (Fig. 13). Although pH limits described in literature for the growth of iron-oxidizing microorganisms have been thoroughly exceeded, the biofilm remained stable at very low pH values, below 1, with biooxidation rates above 2 g/L·h. In order to explain this result, it can be postulated two different scenarios inside the bioreactor with different acidity and Fe(III) concentrations: the surrounding of biofilm, which is attached to the particles of solid support, and the liquid bulk. Biofilm is a sink of protons and the place for Fe(III) generation. Therefore, at the boundary layer which surrounds the support particles, pH and Fe(III) concentration must be higher than at the liquid bulk. The presence of Fe(III) at concentrations above 30 g/L limits the extent to which the redissolution of Fe precipitates from the biofilm matrix takes place, preventing their massive washing out. Although not so extreme conditions can be postulated for attached cells, it is interesting to highlight the astonishing tolerance and adaptation abilities of the inoculum to pH lower than 1 at Fe concentration of 40 g/L for more than 10 days (75 times the hydraulic retention time).

The drop of the biooxidation rate due to pH decrease, also observed in batch tests (Table 3), could be also attributed to oxygen limitations

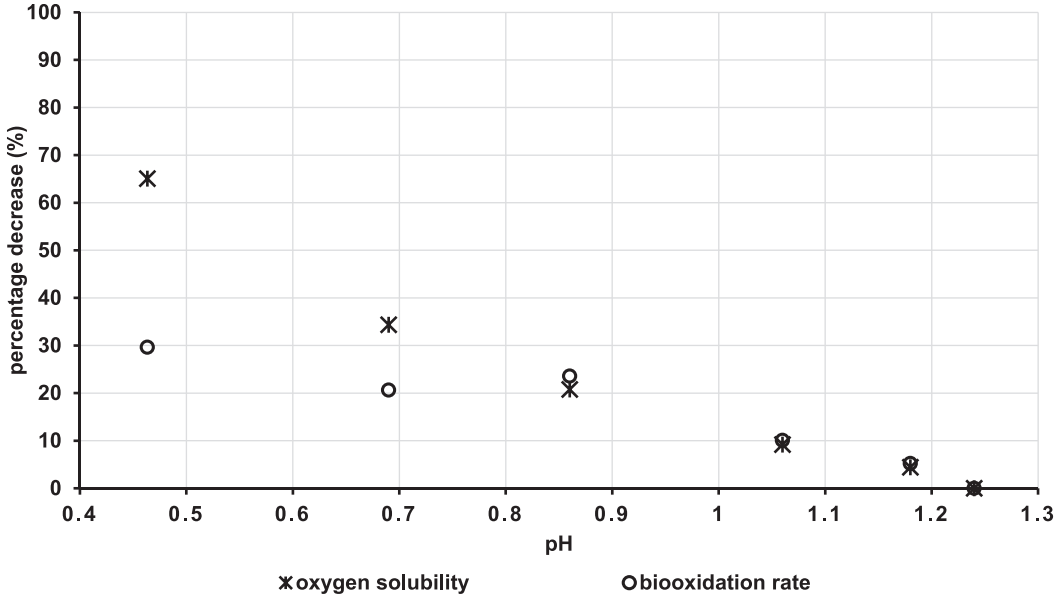


Fig. 14. Percentage decrease of oxygen solubility (×) and biooxidation rates (■) referred to those calculated at pH 1.25.

caused by salting out effect. Oxygen solubility (S) can be estimated by the model of Mazuelos et al. (2017) for bioleaching systems:

$$S = S^0 - 12.698 \cdot 10^{-pH} - 0.0555[Fe(II)] - 0.0290 \cdot [Fe(III)] - 0.0265 \cdot [Cu(II)] \quad (14)$$

where S^0 is the oxygen solubility in water.

For every pH tested mean ferrous iron biooxidation rate was calculated. The relative decrease of biooxidation rate referred to pH 1.25 can be calculated by dividing the mean biooxidation rate obtained at each pH by the biooxidation rate obtained at pH 1.25. An analogous procedure can be applied for calculating the relative decrease of oxygen solubility. These two relative parameters in percentage versus pH are plotted in Fig. 14.

Although the accuracy of this model is limited to a pH range between 1 and 2, and [Fe(II)] and [Fe(III)] lower than 20 g/L, and deviations due to extrapolation ([Fe] = 40 g/L and 0.44 1.25 > pH > 0.44) are expected, similar results can be observed at pH higher than 0.85.

4. Conclusions

A FPBB inoculated with a mixed culture of *A. ferrooxidans* and *L. ferrooxidans* was operated in continuous mode for 1370 h without interruptions. Progressive changes in feed composition allowed to successfully test Fe(II) biooxidation at an iron concentration of 57 g/L and a pH of 0.43. The results obtained allow to affirm that this bioreactor design is robust and versatile regarding feed composition.

An accurate control of the operation resulted on the stability of the FPBB, thus achieving steady states for each operational condition tested, even at pH lower than 0.5.

Astonishing tolerance and adaptation abilities of the inoculum to extreme operational conditions were observed and maintained during the entire continuous operation (~60 days). These results were consistent in both batch and continuous biooxidation tests.

No inhibitory effects were observed at high Fe concentrations (57 g/L) when pH was higher than 1. With a 30 cm bed height and feeding 40 g/L of total Fe at pH 1.1, it is possible to achieve a biooxidation rate higher than 3 g/L·h for complete Fe(II) conversion, hence FPBB can be considered as an efficient design for Fe(II) biooxidation processes at very extreme operational conditions.

When Fe(II) is not limiting, oxygen becomes the limiting reagent. In this case, air flow rate and air diffuser play an essential role in the FPBB performance, and control of aeration is critical. Taking into account these factors, it is possible to achieve a biooxidation rate of 3.5 g/L·h feeding 40 g/L of Fe at pH 1.1.

Biooxidation rate decreases (30 %) as pH decreases from 1.25 to 0.44. This may be due not only to biological effects, but also to physicochemical effects (chemical equilibrium of iron precipitation and oxygen solubility drop by salting out effect).

CRediT authorship contribution statement

Alfonso Mazuelos: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Martin Moreno-Perez:** Validation, Writing – original draft, Writing – review & editing. **Blanca Perdigones:** Validation, Writing – original draft, Writing – review & editing. **Pablo Ramirez:** Methodology, Validation, Investigation, Writing – original draft. **Nieves Iglesias-Gonzalez:** Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This research was funded by the European Union's Horizon 2020 research and innovation program under grant agreement N° 958252 in the framework of collaborative research project RAWMINA.

References

Abbasi, M., Aminian-Dehkordi, J., Mousavi, S.M., 2021. A novel computational simulation approach to study biofilm significance in a packed-bed biooxidation reactor. Chemosphere 262. https://doi.org/10.1016/j.chemosphere.2020.127680.

Amouric, A., Appia-Ayme, C., Yarzabal, A., Bonnefoy, V., 2009. Regulation of the iron and sulfur oxidation pathways in the acidophilic Acidithiobacillus ferrooxidans. Adv. Mat. Res. 71–73, 163–166. https://doi.org/10.4028/www.scientific.net/AMR.71-73.163.

Avila, M., Grinbaum, B., Carranza, F., Mazuelos, A., Romero, R., Iglesias, N., Lozano, J.L., Perez, G., Valiente, M., 2011. Zinc recovery from an effluent using Ionquest 290: From laboratory scale to pilot plant. Hydrometall. 107 (3–4), 63–67. https://doi.org/10.1016/j.hydromet.2011.01.009.

Battaglia, F., Morin, D., Ollivier, P., 1994. Dissolution of cobaltiferous pyrite by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*: factors influencing bacterial leaching efficiency. J. Biotechnol. 32 (1), 11–16. https://doi.org/10.1016/0168-1656(94)90115-5.

Breed, A.W., Hansford, G.S., 1999. Effect of pH on ferrous-iron oxidation kinetics of *Leptospirillum ferrooxidans* in continuous culture. Biochem. Eng. J. 3 (3), 193–201. https://doi.org/10.1016/S1369-703X(99)00018-2.

Candy, R.M., Blight, K.R., Ralph, D.E., 2009. Specific iron oxidation and cell growth rates of bacteria in batch culture. Hydrometall. 98 (1–2), 148–155. https://doi.org/10.1016/j.hydromet.2009.04.013.

Carranza, F., Iglesias, N., Romero, R., Palencia, I., 1993. Kinetics improvement of high-grade sulphides bioleaching by effects separation. FEMS Microbiol. Rev. 11 (1–3), 129–138. https://doi.org/10.1111/j.1574-6976.1993.tb00276.x.

Carranza, F., Romero, R., Mazuelos, A., Iglesias, N., Forcat, O., 2009. Biorecovery of copper from converter slags: Slags characterization and exploratory ferric leaching tests. Hydrometall. 97 (1–2), 39–45. https://doi.org/10.1016/j.hydromet.2008.12.012.

Chowdhury, F., Ojumu, T.V., 2014. Investigation of ferrous-iron biooxidation kinetics by *Leptospirillum ferriphilum* in a novel packed-column bioreactor: Effects of temperature and jarosite accumulation. Hydrometall. 141, 36–42. https://doi.org/10.1016/j.hydromet.2013.09.011.

Curutchet, G., Pogliani, C., Donati, E., Tedesco, P., 1992. Effect of iron (III) and its hydrolysis products (jarosites) on *Thiobacillus ferrooxidans* growth and on bacterial leaching. Biotechnol. Lett 14 (4), 329–334. https://doi.org/10.1007/BF01022333.

Dutrizac, J.E., 1983. Factors Affecting Alkali Jarosite Precipitation. Metall. Trans. B 14 (4), 531–539. https://doi.org/10.1007/BF02653939.

Frias, C., Carranza, F., Sanchez, F., Mazuelos, A., Frades, M., Romero, R., Diaz, G., Iglesias, N., 2008. New development in indirect bioleaching of Zinc and Lead sulfide concentrates. In: Hydrometallurgy'2008, Sixth International Symposium, pp. 497–505.

He, S., Yang, J., Fan, X., Lu, D., Zhang, S., Yan, L., 2022. Magnetosome yield characteristics modeling of *Acidithiobacillus ferrooxidans* in airlift bioreactor using response surface methodology. J. Biomater. Appl. 37 (7), 1325–1338. https://doi.org/10.1177/08853282221133647.

Hubau, A., Minier, M., Chagnes, A., Joulian, C., Silvente, C., Guezenne, A.-G., 2020. Recovery of metals in a double-stage continuous bioreactor for acidic bioleaching of printed circuit boards (PCBs). Sep. Purif. Technol. 238, 116481 https://doi.org/10.1016/j.seppur.2019.116481.

Iglesias-González, N., Carranza, F., Mazuelos, A., Romero, R., Lorenzo-Tallafigo, J., Romero-García, A., Ramírez, P., 2021. The BRISA process as a path for efficient copper recovery from waste PCBs. Hydrometall. 205, 105750 https://doi.org/10.1016/j.hydromet.2021.105750.

Iglesias-Gonzalez, N., Ramirez, P., Lorenzo-Tallafigo, J., Romero-Garcia, A., Mazuelos, A., Romero, R., Carranza, F., 2022. Copper recovery from unground printed circuit board by biogenic ferric at high solid/liquid ratio. Miner. Eng. 180, 107471 https://doi.org/10.1016/j.mineng.2022.107471.

Johnson, D.B., 2008. Biodiversity and interactions of acidophiles: Key to understanding and optimizing microbial processing of ores and concentrates. Trans. Nonferrous Met. Soc. Chin. 18 (6), 1367–1373. https://doi.org/10.1016/S1003-6326(09)60010-8.

Kahrizi, E., Alemzadeh, I., Vossoughi, M., 2009. Biooxidation of ferrous ions by *Acidithioobacillus ferrooxidans* in a monolithic bioreactor. J. Chem. Technol. Biotechnol. 84 (4), 504–510. https://doi.org/10.1002/jctb.2069.

Kaksonen, A.H., Morris, C., Hilario, F., Rea, S.M., Li, J., Usher, K.M., Wylie, J., Ginige, M. P., Cheng, K.Y., Du Plessis, C., 2014. Iron oxidation and jarosite precipitation in a two-stage airlift bioreactor. Hydrometall. 150, 227–235. https://doi.org/10.1016/j.hydromet.2014.05.020.

Kaksonen, A.H., Särkijärvi, S., Puhakka, J.A., Peuraniemi, E., Junnikkala, S., Tuovinen, O.H., 2017. Solid phase changes in chemically and biologically leached copper smelter slag. Miner. Eng. 106, 97–101. https://doi.org/10.1016/j.mineng.2016.08.029.

Karamanev, D.G., 1991. Model of the biofilm structure of *Thiobacillus ferrooxidans*. J. Biotechnol. 20 (1), 51–64. https://doi.org/10.1016/0168-1656(91)90034-S.

Karamanev, D.G., Nikolov, L.N., 1988. Influence of some physicochemical parameters on bacterial activity of biofilm: Ferrous iron oxidation by *Thiobacillus ferrooxidans*. Biotechnol. Bioeng. 31 (4), 295–299. https://doi.org/10.1002/bit.260310403.

Karamanev, D.G., Nikolov, L.N., Mamatarkova, V., 2002. Rapid simultaneous quantitative determination of ferric and ferrous ions in drainage waters and similar solutions, Minerals Engineering, 15(5), 2002. ISSN 341–346, 0892–6875. https://doi.org/10.1016/S0892-6875(02)00026-2.

Kawabe, Y., Inoue, C., Suto, K., Chida, T., 2003. Inhibitory Effect of High Concentrations of Ferric Ions on the Activity of *Acidithiobacillus ferrooxidans*. J. Biosci. Bioeng. 96 (4), 375–379. https://doi.org/10.1016/S1389-1723(03)90140-X.

Kinnunen, P.H.M., Puhakka, J.A., 2004. High-Rate Ferric Sulfate Generation by a *Leptospirillium ferriphilum*-Dominated Biofilm and the Role of Jarosite in Biomass Retainment in a Fluidized-Bed Reactor. Biotechnol. Bioeng. 85 (7), 697–705. https://doi.org/10.1002/bit.20005.

Lin, W.-C., Chen, Y.-P., Tseng, C.-P., 2013. Pilot-scale chemical–biological system for efficient H_2S removal from biogas. Bioresour. Technol. 135, 283–291. https://doi.org/10.1016/j.biortech.2012.10.040.

Malhotra, S., Tankhiwale, A.S., Rajvaidya, A.S., Pandey, R.A., 2002. Optimal conditions for biooxidation of ferrous ions to ferric ions using *Thiobacillus ferrooxidans*. Bioresour. Technol. 85 (3), 225–234. https://doi.org/10.1016/S0960-8524(02)00148-7.

Mazuelos, A., Romero, R., Palencia, I., Iglesias, N., Carranza, F., 1999. Continuous ferrous iron biooxidation in packed bed reactors. Miner. Eng. 12 (5), 559–564. https://doi.org/10.1016/S0892-6875(99)00037-0.

Mazuelos, A., Carranza, F., Palencia, I., Romero, R., 2000. High efficiency reactor for the biooxidation of ferrous iron. Hydrometall. 58 (3), 269–275. https://doi.org/10.1016/S0304-386X(00)00141-9.

Mazuelos, A., Palencia, I., Romero, R., Rodríguez, G., Carranza, F., 2001. Ferric iron production in packed bed bioreactors: Influence of pH, temperature, particle size, bacterial support material and type of air distributor. Miner. Eng. 14 (5), 507–514. https://doi.org/10.1016/S0892-6875(01)00038-3.

Mazuelos, A., Romero, R., Palencia, I., Carranza, F., Borjas, F.J., 2002. Oxygen transfer in ferric iron biological production in a packed-bed reactor. Hydrometall. 65 (1), 15–22. https://doi.org/10.1016/S0304-386X(02)00061-0.

Mazuelos, A., Carranza, F., Romero, R., Iglesias, N., Villalobo, E., 2010. Operational pH in packed-bed reactors for ferrous ion biooxidation. Hydrometall. 104 (2), 186–192. https://doi.org/10.1016/j.hydromet.2010.06.002.

Mazuelos, A., Moreno, J.M., Carranza, F., Palomino, C., Torres, A., Villalobo, E., 2012. Biotic factor does not limit operational pH in packed-bed bioreactor for ferrous iron biooxidation. J. Ind. Microbiol. Biotechnol. 39 (12), 1851–1858. https://doi.org/10.1007/s10295-012-1187-9.

Mazuelos, A., García-Tinajero, C.J., Romero, R., Iglesias, N., Carranza, F., 2017. Oxygen solubility in copper bioleaching solutions. Hydrometall. 167, 1–7. https://doi.org/10.1016/j.hydromet.2016.10.023.

Mishra, S., Panda, S., Akcil, A., Dembele, S., 2023. Biotechnological Avenues in Mineral Processing: Fundamentals, Applications and Advances in Bioleaching and Bio-beneficiation. Miner. Process. Extr. Metall. Rev. 44 (1), 22–51. https://doi.org/10.1080/08827508.2021.1998043.

Moinier, D., Byrne, D., Amouric, A., Bonnefoy, V., 2017. The Global Redox Responding RegB/RegA Signal Transduction System Regulates the Genes Involved in Ferrous Iron and Inorganic Sulfur Compound Oxidation of the *Acidophilic Acidithiobacillus ferrooxidans*. Front. Microbiol. 8, 1277. https://doi.org/10.3389/fmicb.2017.01277.

Mousavi, S.M., Yaghmaei, S., Jafari, A., Vossoughi, M., Ghobadi, Z., 2007. Optimization of ferrous biooxidation rate in a packed bed bioreactor using Taguchi approach. Chem. Eng. Process. 46 (10), 935–940. https://doi.org/10.1016/j.cep.2007.06.010.

Nemati, M., Harrison, S.T.L., Hansford, G.S., Webb, C., 1998. Biological oxidation of ferrous sulphate by *Thiobacillus ferrooxidans*: A review on the kinetic aspects. Biochem. Eng. J. 1 (3), 171–190. https://doi.org/10.1016/S1369-703X(98)00006-0.

Norris, P. R., Burton, N. P., & Foulis, N. A. M. (2000). Acidophiles in bioreactor mineral processing. In *Extremophiles* (Vol. 4, Issue 2, pp. 71–76). https://doi.org/10.1007/s007920050139.

Ojumu, T.V., Petersen, J., Hansford, G.S., 2008. The effect of dissolved cations on microbial ferrous-iron oxidation by *Leptospirillum ferriphilum* in continuous culture. Hydrometall. 94 (1–4), 69–76. https://doi.org/10.1016/j.hydromet.2008.05.047.

Oren, A. (2010). Acidophiles. In *Encyclopedia of Life Sciences* (pp. 192–206). https://doi.org/10.1002/9780470015902.a0000336.pub2.

Pogliani, C., Donati, E., 2000. Immobilisation of *Thiobacillus ferrooxidans*: Importance of jarosite precipitation. Process Biochem. 35 (9), 997–1004. https://doi.org/10.1016/S0032-9592(00)00135-7.

Ponce, J.S., Moinier, D., Byrne, D., Amouric, A., Bonnefoy, V., 2012. *Acidithiobacillus ferrooxidans* oxidizes ferrous iron before sulfur likely through transcriptional regulation by the global redox responding RegBA signal transducing system. Hydrometall. 127–128, 187–194. https://doi.org/10.1016/j.hydromet.2012.07.016.

Pourbaix, M., 1974. Atlas of electrochemical equilibria in aqueous solutions (2nd, English ed. National Association of Corrosion Engineers, Houston, Tex.

Rawlings, D.E., Tributsch, H., Hansford, G.S., 1999. Reasons why "Leptospirillum"-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology 145, 5–13. https://doi.org/10.1099/13500872-145-1-5.

Saavedra, A., Aguirre, P., Gentina, J.C., 2020. Climbing the hill: The implications of a two-step adaptation on biooxidation of ferrous ion at high total iron concentrations by *At. Ferrooxidans*. *Hydrometallurgy* 197, 105486. https://doi.org/10.1016/j.hydromet.2020.105486.

Sandström, A., Mattsson, E., 2001. Bacterial ferrous iron oxidation of acid mine drainage as pre-treatment for subsequent metal recovery. Int. J. Miner. Process. 62 (1–4), 309–320. https://doi.org/10.1016/S0301-7516(00)00062-4.

Savic, D.S., Veljković, V.B., Lazić, M.L., Vrvić, M.M., Vučetić, J.I., 1998. Effects of the oxygen transfer rate on ferrous iron oxidation by *Thiobacillus ferrooxidans*. Enzyme Microb. Technol. 23 (7–8), 427–431. https://doi.org/10.1016/S0141-0229(98)00071-4.

Schippers, A., Sand, W., 1999. Bacterial Leaching of Metal Sulfides Proceeds by Two IndirectMechanisms via Thiosulfate or via Polysulfides and Sulfur. Appl. Environ. Microbiol. 65 (1), 319–321. https://doi.org/10.1128/AEM.65.1.319-321.1999.

Silverman, M.P., Lundgren, D.G., 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*: I. An improved medium and a harvesting procedure for securing high cell yields. J. Bacteriol. 77 (5), 642–647. https://doi.org/10.1128/jb.77.5.642-647.1959.

Smith, J.R., Luthy, R.G., Middleton, A.C., 1988. Microbial ferrous iron oxidation in acidic solution. J. Water Pollut. Control Fed. 60 (4), 518–530. https://www.jstor.org/stable/25043528.

Song, Y., Guo, Z., Wang, R., Yang, L., Cao, Y., Wang, H., 2022. A novel approach for treating acid mine drainage by forming schwertmannite driven by a combination of biooxidation and electroreduction before lime neutralization. Water Res. 221 (1), 118748 https://doi.org/10.1016/j.watres.2022.118748.

Torma, A. E. (1977, January). The role of *Thiobacillus ferrooxidans* in hydrometallurgical processes. In T.K., Ghose, A., Fiechter, & N., Blakebrough (Eds), *Advances in Biochemical Engineering, vol 6* (pp.1-37). Springer, Berlin, Heidelberg.

van der Meer, T., Kinnunen, P.H.M., Kaksonen, A.H., Puhakka, J.A., 2007. Effect of fluidized-bed carrier material on biological ferric sulphate generation. Miner. Eng. 20 (8), 782–792. https://doi.org/10.1016/j.mineng.2007.02.002.