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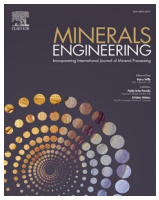

Adaptation of an iron oxidising culture to extremely high Fe concentration by a programmed fed-batch bioreactor

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ABSTRACT

Ferric iron plays a key role as a leaching agent. In ferric leaching, the generated ferrous iron can be biologically regenerated by biooxidation. In commercial applications, microbial growth usually limits operational conditions such as iron concentration and pH. Therefore, design and operation are often conditioned by strict restrictions of pulp density and need of addition of alkaline reagents. In the present work a mixed culture consisting mainly of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* has been adapted to extremely high iron concentrations in comparison with the reported information in literature. For this purpose, a programmed fed-batch bioreactor has been used. A continuous stirred tank reactor was operated for testing the adapted culture. As a result, no biological inhibition was observed with iron concentrations up to 50 $g{\cdot}L^{-1}$ and pH of 0.7. By this methodology, an adapted inoculum can be continuously generated in order to assist industrial operations.

1. Introduction

The scarcity of metals is one of the greatest challenges facing society in the 21st century. Due to the rapidly increasing global demand, the current extraction and supply chain for metals will not be able to satisfy the entire population. Against this backdrop of need, the independence on the supply of essential raw materials, including many of the metals, has become a global geo-political instrument (European Commission, 2022).

This is the context for biohydrometallurgy-based extractive processes, a "green" technology where ferric ion (Fe^{3+}) is the reagent used on an industrial scale as a leaching agent in a process called bioleaching (r1) (Carranza et al., 1993; Schippers & Sand, 1999).

$$MS + 2Fe^{3+} \rightarrow M^{2+} + S^0 + 2Fe^{2+} \quad \text{(r1)}$$

Where *MS* is a sulphide of the metal *M* and M^{2+} is the oxidised metal in solution.

The consumed ferric ion during the bioleaching reaction(r1) can be regenerated by oxidising the ferrous iron (Fe^{2+}) with oxygen. This process is very slow at the pH values required to avoid precipitation of Fe^{3+} (pH < 2) (Pourbaix, 1974). It is possible to multiply the rate of this reaction by several orders of magnitude when catalysed by bacteria in a process known as biooxidation, consisting of the transfer of electrons from Fe^{2+} to oxygen in an acidic medium with the generation of water and Fe^{3+} (r2) (Nemati et al., 1998):

$$2\,Fe^{2+} + \frac{1}{2}O_2 + 2\,H^+ \rightarrow 2\,Fe^{3+} + H_2O \quad \text{(r2)}$$

Bioleaching by the indirect contact mechanism is based on the oxidation of metal sulphides and the biooxidation of Fe^{2+} simultaneously, where leaching takes place through an electrochemical corrosion process induced by the positive redox potential generated by the oxidation of Fe^{2+} (Rawlings et al., 1999). The process of bioleaching can be optimised by separating these two steps: chemical leaching and biological biooxidation, as these processes can be enhanced separately (IBES and BRISA processes) (Carranza et al., 1993, 1997).

Nowadays, bioleaching is used worldwide for commercial purposes to improve the extraction of gold from ores and mineral concentrates in which the precious metal is bound inside a sulphide ore, and, to a lesser extent, to extract copper from secondary copper ores and to leach non-copper base metals from ores and concentrates (Brierley, 2008; Petersen,

Abbreviations: *a*, specific area; C_L^*, and are, respectively, the saturation concentration of oxygen and the; C_L, measurable oxygen concentration in the liquid medium; cSTR, Continuous Stirred Tank Reactors; *D*, dilution rate; k_L, individual mass transfer coefficient referred to the liquid phase; k_La, volumetric mass transfer coefficient; ORP, Oxidation Reduction Potential; PCBs, Printed Circuit Boards; Q_i, inlet liquid flow rate; Q_o, outlet liquid flow rate; Q_{O_2}, oxygen transfer rate; $r_{Fe^{2+}}$, ferrous iron biooxidation rate; τ, average residence time..

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2010).

The bioleaching process is currently carried out in the industry through technologies such as Heap leaching or Dump leaching, and in Continuous Stirred Tank Reactors (cSTRs) (Rawlings et al., 2003). Although it stands out mainly as a pre-treatment of Au ores (Rawlings et al., 2003), bioleaching has been successfully applied to obtain copper, nickel and cobalt (Panda et al., 2015; Petersen, 2010).

The biooxidation of Fe^{2+} is catalysed by extremophilic microorganisms, among which *Acidithiobacillus ferrooxidans*, an autotrophic gram-negative bacterium, stands out (Vera et al., 2013). It is an aerobic, mesophilic and acidophilic bacterium that obtains energy by oxidising Fe^{2+}, elemental sulphur and various reduced forms of sulphur (Drewniak and Sklodowska, 2013; Johnson and Hallberg, 2008; Rawlings, 2002; Saavedra et al., 2020b). Its combination with other acidophilic bacteria, such as *Acidithiobacillus thiooxidans*, allows reduced sulphur compounds generated in the leaching of sulphide minerals (r1) to be oxidised to sulphuric acid (H_2SO_4), thus maintaining a low pH essential for acidophiles and for the solubility of Fe^{3+} (Domic, 2007; Johnson, 2014; Rawlings, 2005).

Besides *Acidithiobacillus* genus, species of the genus *Leptospirillum* have been widely exploited in indirect bioleaching processes, with the most common species being *L. ferrooxidans* and *L. ferriphilum*. These iron-oxidising bacteria seem to be dominant in cases in which access to energy is more restricted, as they have a greater affinity for Fe^{2+} (Norris, 2007; Rawlings et al., 1999). Furthermore, *Leptospirillum* species have shown to be highly resistant to extreme acidic conditions, high temperatures and metals concentration in the medium, becoming dominant over species of other bacterial genera in these situations (Giaveno et al., 2007; Harrison & Norris, 1985; Rawlings et al., 1999; van Aswegen and Marais, 1999).

The liquors produced in biooxidation have been successfully employed in numerous processes of economic, social and environmental utility. Among these processes is the recovery of metals such as copper, gold, silver, nickel, palladium and aluminium from PCBs (Printed Circuit Boards) (Hubau et al., 2018; Iglesias-González et al., 2021, 2022; Yazici and Deveci, 2014).

Likewise, the biooxidation of Fe^{2+} to Fe^{3+} is a biological process used in the treatment of acid mine water, in the copper slags valorisation and in the desulphurisation of fuel gases such as the biogas generated in wastewater treatment plants (Johnson and Hallberg, 2005; Mazuelos et al., 1999, 2000; Nemati et al., 1998; Song et al., 2020).

Therefore, Fe^{2+} biooxidation is a Fe^{3+} regeneration process with huge industrial potential (Mazuelos et al., 2000; Song et al., 2020). Optimisation of this process to obtain a Fe^{3+} flow at the rate demanded by industry has become a major issue due to the growing interest in these sustainable processes (Chowdhury and Ojumu, 2014).

Among the variables that condition the industrial applicability of biooxidation are iron concentration and pH, as they can lead to inhibition of bacterial growth and a decrease in the rate of biooxidation (Kawabe et al., 2003; Lizama and Suzuki, 1989).

It has been observed that the extraction of metals from PCBs is strongly dependent on the Fe^{3+} concentration in the feed liquor: the higher the Fe^{3+} concentration, the higher the metals extraction (Iglesias-Gonzalez et al., 2022; Yazici and Deveci, 2014). Similarly, metal extraction has been observed to increase with the acidity of the liquor (Yazici and Deveci, 2014). Likewise, several studies have shown that the use of biogenic Fe^{3+} at low pH in bioleaching can save process costs, as it reduces the use of reagents (Lorenzo-Tallafigo et al., 2022). Therefore, Fe concentration is a key factor for the success of the subsequent biogenic iron applications. However, despite the fact that there is no mechanism of inhibition caused by Fe^{3+} described in the bibliography (Amouric et al., 2009; Moinier et al., 2017; Ponce et al., 2012), most studies find difficulties in biooxidation process when Fe^{3+} reaches concentrations of 25 $g{\cdot}L^{-1}$ (Battaglia et al., 1994; Kawabe et al., 2003). As far as the authors know, maximum iron concentration in discontinuous operation found in the literature is 36 $g{\cdot}L^{-1}$ (Saavedra et al., 2020b). Nevertheless, the biooxidation process for industrial applications only makes sense in continuous operations. In this case, maximum iron concentrations do not exceed 25 $g{\cdot}L^{-1}$ (Mousavi et al., 2007; Frias et al., 2008).

Regarding pH, the industrial applicability of biooxidation liquors is quite dependent on the acidity, as the high iron concentrations at which most processes operate require very acidic conditions for iron solubility (Pourbaix, 1974). At these extreme conditions, the pH range supported by the bacteria usually limits the biooxidation process, with the minimum reported being 1.00 (Mazuelos et al., 2012). However, most studies use pH values no lower than 1.25 (Chowdhury and Ojumu, 2014; Iglesias-Gonzalez et al., 2022).

The efficiency of biooxidation is strongly conditioned by the design of the bioreactor. Continuous biooxidation is usually performed in Packed-Bed Bioreactors (PBBs) (Chowdhury and Ojumu, 2014; Mazuelos et al., 2010; Song et al., 2020). This methodology allows cell concentration to be independent of the liquid flow rate as the cells are attached to the surface of the bed particles forming a biofilm (Karamanev, 1991; Mazuelos et al., 2000;). Increasing the flow rate therefore results in higher biooxidation rates than in other systems (Chowdhury and Ojumu, 2014; Mazuelos et al., 1999). However, attachment of the bacteria to the particle surface limits the operational conditions of this type of bioreactor, as it depends on different factors such as pH (necessarily higher than 1.08), aeration rate, Fe^{2+} and Fe^{3+} concentration, etc. (Karamanev & Nikolov, 1988; Mazuelos et al., 2001, 2010; Song et al., 2020).

On the other hand, cSTRs do not have these limitations as cells are in suspension. This involves a strong dependence of the cell concentration on the liquid flow rate, as cells are evacuated in the outlet stream, which is the main issue of these reactors. A solution to this inconvenience may be cell recirculation. In addition, cSTRs show a more homogeneous distribution of substrate and air due to their design, avoiding mass transfer problems (Doran, 2013b).

However, although STRs have an easier start-up than PBBs, there is a lack of a start-up procedure to ensure fast and reliable operation. In the same way, the lack of standardised control systems makes it difficult to monitor a complex process such as biooxidation (Molchanov et al., 2007).

The present work studies the biooxidation of ferrous iron in a continuous stirred tank bioreactor with the aim of testing the adaptability of iron-oxidising cells to high concentrations of Fe^{3+}. The main objective is to adapt cells to extremely high iron concentrations in comparison with the information reported in the literature by using a programmed fed-batch bioreactor system in order to bring this biotechnology closer to the conditions required by industrial processes. It is also intended to operate at extreme iron concentrations and pH in a chemostat, prolonging the condition of balanced growth over time, so that adapted inocula can be continuously generated to assist industrial operations in the event of technical stops, perturbations, or operational failures.

2. Materials and methods

2.1. Cultures and liquid medium

A mixed culture originated from drainage waters of Rio Tinto Mine was used as inoculum. This culture mainly consisted of the species *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans,* and associated heterotrophs of *Acidiphilium* genus (Mazuelos et al., 2012). The inoculum was grown in 9 K medium (Silverman and Lundgren, 1959) (9 $g{\cdot}L^{-1}$ Fe^{2+}) modified at pH 1.25. The inoculum was maintained by routinely subculturing in an orbital shaker incubator at 180 rpm and 30 °C.

Additionally, a modified 9 K medium at pH 1.25 and 71 $g{\cdot}L^{-1}$ Fe^{2+} was used for the fed-batch bioreactor operation.

Finally, another modified 9 K medium at pH 0.5 and 40 $g{\cdot}L^{-1}$ Fe^{2+}

Table 1
Dimensions of the STR bioreactor and the inclined blade turbine.

Dimension (cm)	
STR external diameter	12
STR internal diameter	10
STR total height	17
STR overflow height	14.1
Jacket height	1.1
Baffle width	1
Turbine diameter	6.5
Blade length	2.5
Blade width	0.3
Blade angle	50º

was used as feed for the cSTR.

2.2. Bioreactor

For fed-batch and continuous tests, a 1 L glass jacketed stirred tank reactor with fluid inlet and outlet and 3 baffles of 1 cm was used. Dimensions are given in Table 1. The working volume according to the overflow height and the agitation used was 0.8 L. The reactor was mechanically stirred at 500 rpm with a 6.5 cm diameter pitched blade turbine located 1 cm from the bottom.

A thermostatically regulated circulating bath was used to maintain a temperature of 30 °C.

A 4 mm internal diameter glass open pipe centred under the turbine was used for aeration. The air flow rate was 3 L·min^{-1}. To prevent evaporation, air was previously humidified.

Inlet medium flow rate was programmed and controlled by an Arduino EZO-PMP™ dosing pump.

Liquid level was controlled at 0.8 L with a peristaltic pump LLG Labware.

2.3. Oxygen transfer coefficient

The oxygen transfer coefficient was determined to check fulfilment of aeration requirements for avoiding oxygen limitations, as the availability of oxygen in the medium is usually a limiting factor in biooxidation (Mazuelos et al., 2000).

Gas-liquid oxygen transfer rate (Q_{O_2}) can be calculated by the following equation:

$$Q_{O_2} = k_L \cdot a \cdot (C_L^* - C_L) \quad (1)$$

Where k_L is the individual mass transfer coefficient referred to the liquid phase, a is the specific area, and C_L^* and C_L are, respectively, the saturation concentration of oxygen and the measurable oxygen concentration in the liquid medium.

The product$k_L \cdot a$, the volumetric mass transfer coefficient, must be determined experimentally. For this purpose, the sulphite method was applied (Cooper et al., 1944).

2.4. Fed-Batch

A fed-batch operation was programmed so that, when the potential of the bioreactor reached 605 mV, 1 mL of modified 9 K medium at pH 1.25 and 71 g·L^{-1} Fe^{2+} was dosed. This loop was finished when total working volume (0.8 L) was reached. Previously, the bioreactor was inoculated with 385 mL of a finished culture to assure that Fe^{2+} concentration was 0 g·L^{-1}.

This system was programmed and monitored using an AtlasScientific® ENV-40-ORP potential electrode and an EZO-PMP™ dosing pump connected to an Arduino digital system.

Table 2
Inlet flow rate (Q_i), outlet flow rate (Q_o), residence time (τ), dilution rate (D) and biooxidation rate ($r_{Fe^{2+}}$) of cSTR.

Q_i (mL·h^{-1})	Q_o (mL·h^{-1})	τ(h)	D (h^{-1})	$r_{Fe^{2+}}$ (g· L^{-1}·h^{-1})
10.00	*7*	114.29	0.009	0.50
15.00	*12*	66.67	0.015	0.75
19.00	*16*	50.00	0.020	0.96
24.00	*21*	38.10	0.026	–

2.5. Continuous tests

Continuous biooxidation was carried out in the cSTR for more than 900 h.

The inlet stream was the modified 9 K medium at pH 0.5 and 40 g·L^{-1} Fe^{2+}. Inlet liquid flow rate (Q_i) was programmed by an EZO-PMPTM dosing pump connected to an Arduino digital system. The set points were 10, 15, 19 and 24 mL·h^{-1} (Table 2).

2.6. Analytics

ORP (Oxidation Reduction Potential) was continuously measured by an AtlasScientific® ENV-40-ORP portable electrode (Ag/AgCl reference) connected to an Arduino digital system.

The pH was measured periodically by an HORIBA Scientific LAQUA PH1200 electrode.

The densities of inlet and outlet liquid streams were measured using a Mettler Toledo 30PX densimeter.

Fe^{2+} concentration was determined by redox titration with potassium dichromate ($K_2Cr_2O_7$). Fe^{3+} concentration was determined spectrophotometrically at 500 nm by sulphosalicylic acid method.

Cell concentration was estimated by turbidimetry at 600 nm using a calibration curve obtained by cell counting chamber.

2.7. Kinetic parameters

2.7.1. Residence time and dilution rate

The characteristic parameters of continuous reactor operation, residence time (τ) and dilution rate (D), can be calculated as the relation between the liquid flow rate(Q) and the working reactor volume (V) (Eq. (2)).

$$\tau = \frac{1}{D} = \frac{V}{Q} \quad (2)$$

2.7.2. Specific growth rate

In a cSTR, the specific microbial growth rate (μ) can be obtained by mass balance applying steady state and chemostat conditions. Postulating that the inlet flow is sterile and that the specific cell death constant is negligible, the specific growth rate is equal to the dilution rate (D) (Eq.(3)) (Doran, 2013b).

$$\mu = D \quad (3)$$

2.7.3. Productivity and biooxidation rate

Productivity can be calculated as shown in Equation (4):

$$Productivity = \left[Fe^{2+}\right]_i \cdot Q_i - \left[Fe^{2+}\right]_o \cdot Q_o \quad (4)$$

Where $[Fe^{2+}]_i$ and $[Fe^{2+}]_o$ are the Fe^{2+} concentration in the inlet and outlet streams respectively and Qi and Q_o are the liquid flow rates in the inlet and outlet streams.

The biooxidation rate ($-r_{Fe^{2+}}$) can be calculated dividing productivity by the working volume (Eq. (5)):

$$-r_{(Fe^{2+})} = \frac{Productivity}{V} \quad (5)$$

3. Results and discussion

3.1. Aeration

Sulphite method was carried out to determine the oxygen transfer rate under the established hydrodynamic conditions. In the presence of Cu(II) or Co(II), the oxidation of the sulphite ion to sulphate ion is immediate so that oxygen concentration in the liquid medium is 0 ($C_L = 0$). Then oxygen transfer rate can be calculated from controlling sulphite concentration as a function of time (Cooper et al., 1944).

With an agitation speed of 500 rpm, a 6.5 cm diameter pitched blade turbine and an air flow rate of 3 L·min^{-1}, the oxygen transfer rate was 0.072 mol·L^{-1}·h^{-1}.

A C_L^* of 6.56 mg·L^{-1} was estimated by applying correction of temperature and solutes (Doran, 2013a).

Therefore, substituting in Eq. (1), a $k_L{\cdot}a$value of 350.98 h^{-1} was calculated.

3.2. Protocol for the start-up of biooxidation bioreactors: Fed-Batch

A protocol for the start-up of biooxidation bioreactors was carried out based on a programmed fed-batch.

Continuous and automated measurements of ORP using the Arduino-controlled electrode allowed for the regulated addition of ferrous iron by the peristaltic pump, so that no more medium was added until the bacteria had exhausted the previous ferrous iron. The evolution of ORP during the loading stage of the bioreactor is shown in Fig. 1.

In each cycle ORP dropped with the addition of Fe^{2+} and after quickly increased when the added Fe^{2+} was consumed by bacteria (Fig. 1). With this programmed operation Fe^{2+} is dossed according to

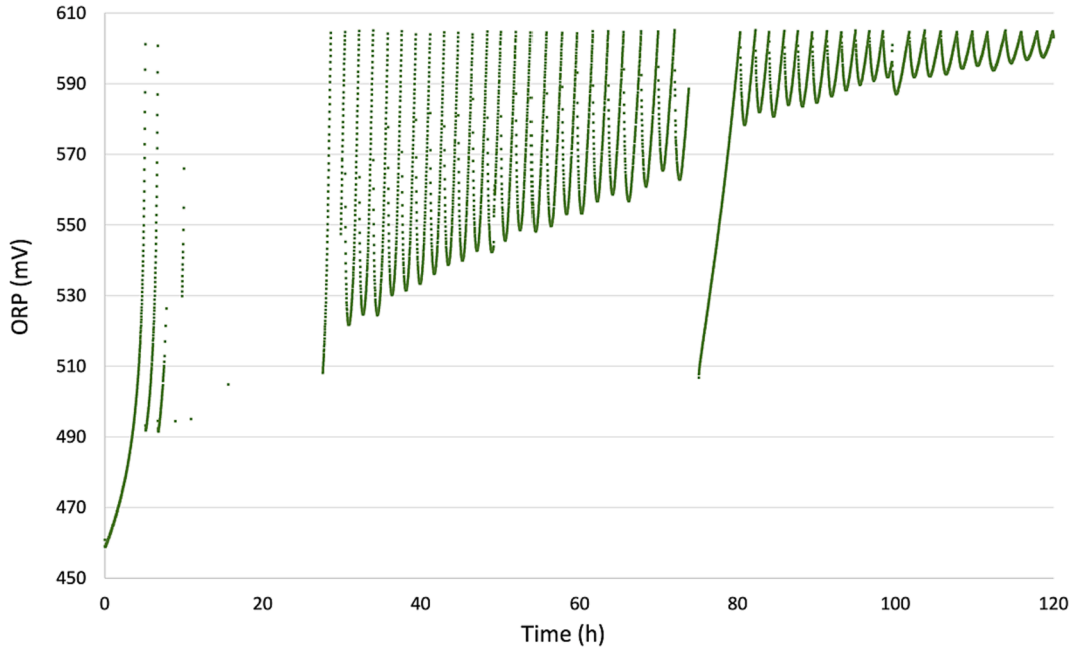

Fig. 1. Online ORP measurements during the fed-batch configuration. Inlet medium containing 71 g Fe^{2+}·L^{-1} at pH 1.25. Air flow rate of 3 L·min^{-1}, 500 rpm, 30 °C.

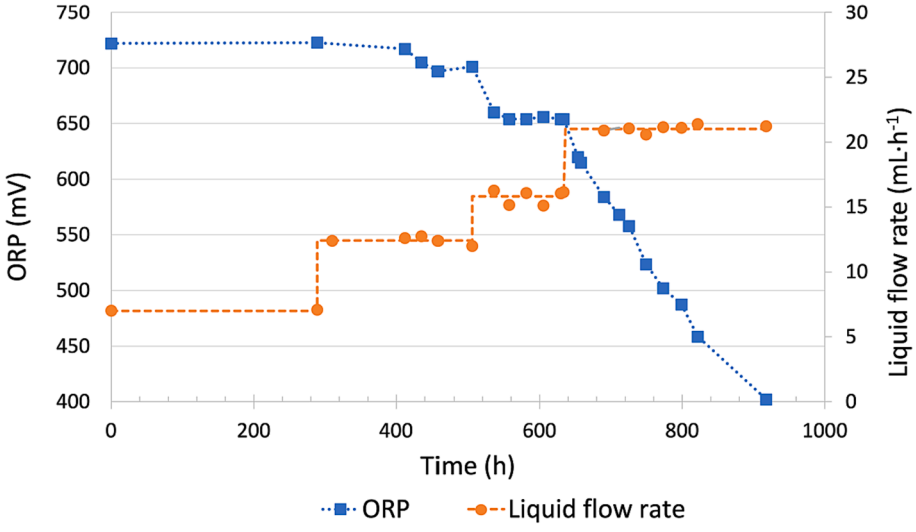

Fig. 2. ORP (··■··) evolution and outlet liquid flow rate (--●--) of the cSTR.

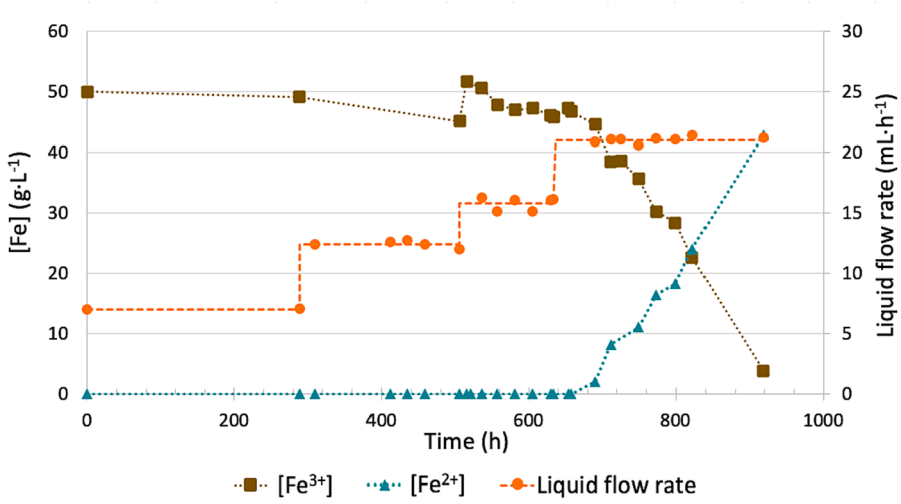

Fig. 3. Fe^{3+} (..■..) and Fe^{2+} (..▲..) concentration over time and outlet liquid flow rate of the cSTR (--●--).

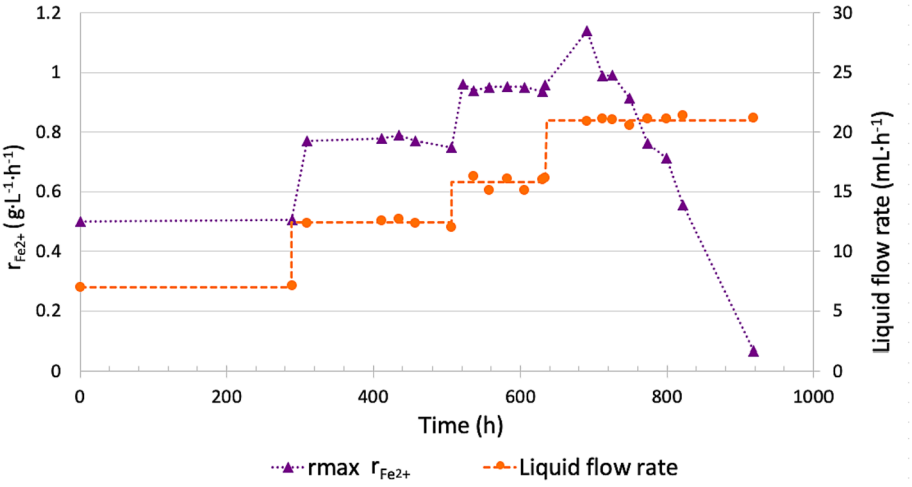

Fig. 4. Biooxidation rate (..▲..) and outlet liquid flow rate (--●--) during the operation of the cSTR.

bacterial activity, achieving a gradual increase in iron concentration and avoiding inhibition phenomena and thus promoting bacterial growth. As a consequence, cell concentration increased from 1.24×10^8 cells per millilitre to 1.13×10^9 cells per millilitre while Fe concentration was increased from 9 $g{\cdot}L^{-1}$ to 50 $g{\cdot}L^{-1}$.

3.3. Stable physicochemical conditions and transient states

After finishing the programmed fed-batch operation, continuous biooxidation was carried out in the cSTR for more than 900 h.

The outlet flow rates (Q_o) obtained for each inlet flow rate (Q_i) are shown in Table 2. The evaporation flow rate was calculated as the difference between Q_i and Q_o, this was 3 $mL{\cdot}h^{-1}$.

The average residence time (τ) and dilution rates (D) calculated for the outlet flow rates are given in Table 2.

The evolution of potential and flow rates versus time is plotted in Fig. 2.

ORP decreases as the flow rate increases. For outlet liquid flow rates of 7 $mL{\cdot}h^{-1}$, 12 $mL{\cdot}h^{-1}$ and 16 $mL{\cdot}h^{-1}$ it is observed that ORP values are almost constant for each flow rate tested and higher than 650 mV, indicating that stable physicochemical conditions are reached.

However, at a liquid flow rate of 21 $mL{\cdot}h^{-1}$ it is not possible to reach stable physicochemical conditions, and a drop in ORP is observed. Stable physicochemical conditions have been achieved for three times the average residence time. The evolution of the Fe^{3+} and Fe^{2+} concentrations, as well as the liquid flow rates, are shown in Fig. 3.

Fig. 3 shows a total oxidation of Fe^{2+} at 7 $mL{\cdot}h^{-1}$, 12 $mL{\cdot}h^{-1}$ and 16 $mL{\cdot}h^{-1}$ and a progressive increase of Fe^{2+} at 21 $mL{\cdot}h^{-1}$. These results are consistent with those shown in Fig. 2 for ORP values.

Fig. 4 shows the calculated biooxidation rates for each liquid flow rate tested.

Fig. 4 shows that biooxidation rate increases with liquid flow rate when Fe^{2+} is limiting. Stable physicochemical conditions are achieved for liquid flow rates of 16 $mL{\cdot}h^{-1}$ and lower, and similar values of cell evacuation and cell growth rates can be assumed. However, although increasing the flow rate to 21 $mL{\cdot}h^{-1}$ leads to an initial increase in the

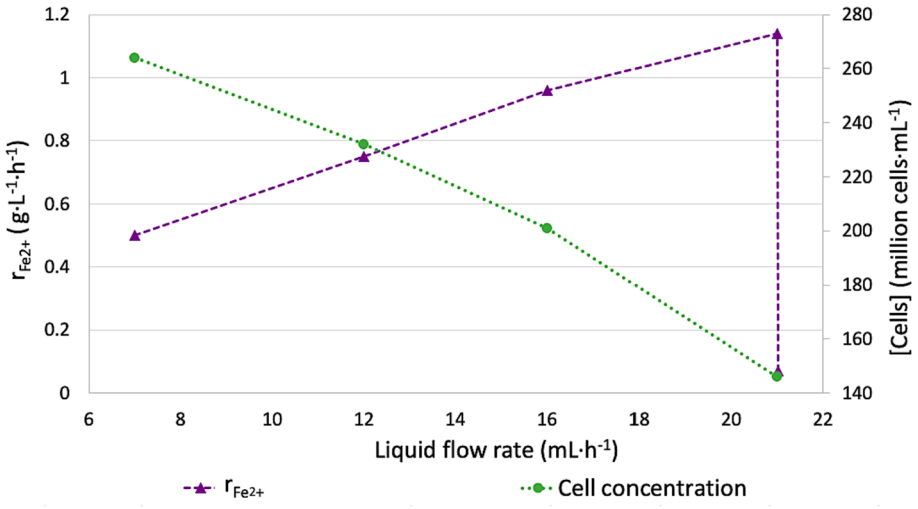

Fig. 5. Relationship between outlet liquid flow rate, biooxidation rate (-- ▲ --) and cell concentration (·· ● ··) in the cSTR.

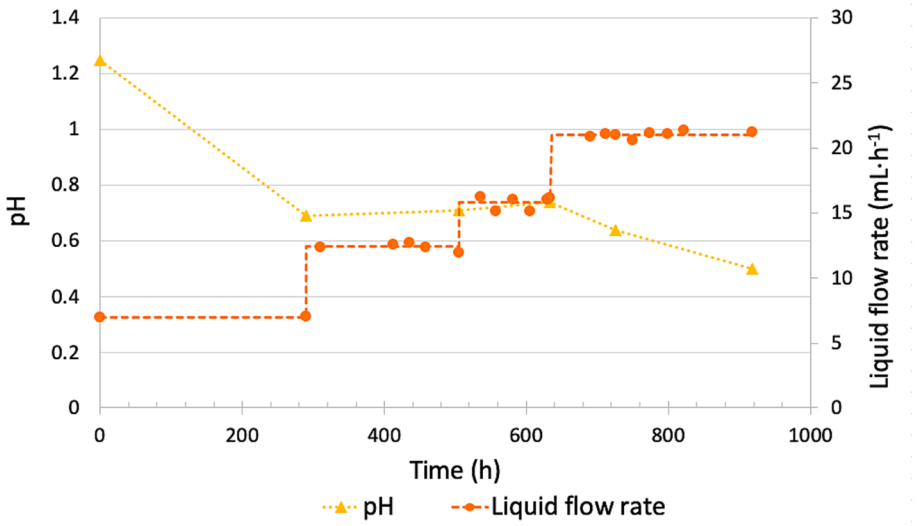

Fig. 6. Evolution of the pH (·· ▲ ··) and outlet liquid flow rate (-- ● --) of the cSTR.

biooxidation rate (1.14 g·L^{-1}·h^{-1}), it starts to fall steadily with time which suggests that cells evacuation rate is higher than the bacterial growth rate. These results are consistent with cell concentration for each liquid flow rate (Fig. 5).

The biooxidation rates reached for each flow rate are given in Table 2. The maximum biooxidation rate under stable physicochemical conditions was 0.96 g·L^{-1}·h^{-1}.

Knowing the maximum rate of biooxidation, the maximum rate of oxygen consumption can be calculated with reaction (2) (r2), resulting in a value of 0.0043 mol O_2·L^{-1}·h^{-1}. Thus, the oxygen transfer rate obtained under the operating conditions of the STR (0.072 mol·L^{-1}·h^{-1}, *3.1. Aeration*) was about 17 times higher than the maximum oxygen consumption rate. Therefore, it can be affirmed that the oxygenation conditions were not limiting and that oxygen was in excess at all times.

The biooxidation rates obtained are higher than most of those found in the literature for cSTRs, around 0.2–0.84 g·L^{-1}·h^{-1} (Chowdhury and Ojumu, 2014; Saavedra et al., 2020a,b; Penev and Karamanev, 2010). However, some authors have achieved higher biooxidation rates (in the range of 1.2 to 6 g·L^{-1}·h^{-1}), either in batch trials (Mousavi et al.,2007) or by attaching and fixing cells to inert support particles (Mazuelos et al., 2010, 2012) and under more moderate conditions regarding iron concentration and acidity.

On the other hand, adaptation of the culture used as inoculum to extreme pH values and iron concentration, 0.7 and 50 g·L^{-1} respectively, was achieved (Figs. 3 and 6). It can be considered that the fed-batch protocol carried out for starting-up of continuous stirred tank reactors and subsequent continuous operation in cSTR not only promotes high cell concentrations, and in consequence high ferrous iron biooxidation rates, but it also allows to fast adaptation by progressively increasing the Fe^{2+} concentration and decreasing the pH.

Iron concentration and acidity reached are higher than those found in the literature. The pH values used in most studies are in the range of 1.25–2.3 (Chowdhury and Ojumu, 2014; Mousavi et al.,2007; Penev and Karamanev, 2010; Saavedra et al., 2020a,b), being the lowest value 1 (Mazuelos et al., 2012).

From Eq. (3) and the results shown in Table 2, a specific growth rate

(μ) close to 0.020 h^{-1} was obtained at pH 0.7 and 50 $g{\cdot}L^{-1}$ Fe. This specific growth rate is similar of that found in the literature for cultures at high iron concentration (36 $g{\cdot}L^{-1}$ Fe) (Saavedra et al., 2020b).

The methodology presented in this work can be potentially applied for supplying active iron-oxidising bacteria to commercial-scale bioreactors for requirements at the start-up process or in case of interruptions caused by operational failures.

4. Conclusions

From the results obtained in this work, the following conclusions can be drawn:

- A simple method for fast starting-up of ferrous iron biooxidation bioreactors has been tested, consisting of a programmed fed-batch operation for avoiding undesired latency and inhibitory phenomena.
- Complete conversion of iron was achieved in continuous operation for iron concentrations in feed higher than 50 $g{\cdot}L^{-1}$.
- Extreme pH values of around 0.7 have been maintained during the operation time without observing inhibition.
- The maximum ferrous iron biooxidation rate achieved under stable physicochemical conditions for the continuous stirred tank reactor was close to 1 $g{\cdot}L^{-1}{\cdot}h^{-1}$.
- The maximum specific growth rate for the tested culture has been calculated experimentally under non-oxygen limiting conditions and was in the range of 0.019–0.024 h^{-1}.
- As potential application of this work, adapted inoculum can be continuously generated by this methodology and thus assisting industrial bioreactors when required.

CRediT authorship contribution statement

Blanca Perdigones: Conceptualization, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Pablo Ramírez:** Conceptualization, Methodology, Software, Supervision, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Alfonso Mazuelos:** Conceptualization, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

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.

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