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Operational pH in packed-bed reactors for ferrous ion bio-oxidation

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ABSTRACT

The flooded packed-bed bioreactor plays a major role in the field of applications of ferrous ion bio-oxidation. The pH is an important variable in the control of this type of reactor, upon which the functionality of biofilm depends. In the present work, five continuous flooded packed-bed reactors have been inoculated with mixed cultures (*Acidithiobacillus ferrooxidans* and *Leptospirillum ferooxidans*) and fed with 9 k medium in the pH range 0.82 to 1.90. It has been experimentally tested that the operation of these reactors is stable at the maximum productivity levels when the pH varies within the interval 1.00 to 2.30 inside the reactor.

It has been observed that the negative effect on the productivity when the upper pH limit is exceeded is reversible. No such reversibility occurs when the pH goes below the lower limit.

The results of our experiments indicate that the limitations in operational pH are linked to the chemistry of precipitation ferric compounds and not to biological phenomena.

1. Introduction

Potential applications of ferrous ion bio-oxidation in the field of the mining and metallurgy industry are well known; in the regeneration of ferric ion as a leaching agent in indirect bioleaching processes (Carranza et al., 1996; Palencia et al., 2002; Romero et al., 2003; Carranza et al., 2004) and in the ferrous ion removal in processes for the purification of liquid effluents (Olem and Unz, 1977; Umita, 1996; Sandstrom and Mattsson, 2001; Wood et al., 2001).

The interest generated by these applications is still relevant, as witnessed by the numerous recent publications on the subject (Giaveno et al., 2007; Carranza et al., 2009a; Carranza et al., 2009b; Johnson and Hallberg, 2005; Natarajan, 2008; Nurmi et al., 2010), some of which show results of research carried out in pilot plants (Frías et al., 2008; Valiente et al., 2008).

Among the tried and tested devices which perform bio-oxidation (Olem and Unz, 1977; Livesey-Goldblatt et al., 1977; Nikolov and Karamanev, 1987; Grishin and Tuovinen, 1988; Mazuelos et al., 1999), the device with the greatest potential with respect to industrial application is the flooded packed-bed bioreactor, due to its efficiency, simplicity, stability, low price and the fact that it has already been successfully tested in pilot-scale plants (Frías et al., 2008; Valiente et al., 2008). Productivities in excess of 4000 g of ferric per hour and per m^2 of base area can be achieved with this type of bioreactor. The height of tested reactors did not exceed two metres (Frías et al., 2008; Mazuelos et al., 2000).

The flooded packed-bed bioreactor is a column consisting of randomly arranged particles upon which cells are supported. Cells are fed by two currents that rise through and flood the bed: a liquid stream (continuous phase) carrying ferrous iron and H^+, and a gas stream (discrete phase) bearing O_2 (air).

It is possible to achieve high bio-oxidation rates with any of a number of materials as biomass support (Carranza and Garcia, 1990; Armentia and Webb, 1992; Mazuelos et al., 2001; Giaveno et al., 2008; van der Meer et al., 2007), although siliceous stone remains one of the most attractive for its availability and price.

Mazuelos et al. (2001) propose a protocol to fix the cells to siliceous stone particles. This procedure considers the following two stages:

Stage 1 — batch operation. The bioreactor, packed with support particles, is filled with 30% (vol/vol) of culture as the inoculum and 70% (vol/vol) of liquid medium.

Stage 2 — recirculating flow. Once 95% conversion of ferrous iron is achieved in Stage 1, the bioreactor is connected with a deposit, thereby establishing a loop for the liquid stream.

Finally, once 95% conversion of ferrous iron is achieved, then the bioreactor can be operated in continuous mode.

As the inoculum, Mazuelos et al., used a mixed culture adapted to pH 1.25 consisting of *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and some heterotrophic bacteria of the genus *Acidiphilium*. The liquid medium feed was an aqueous ferrous sulphate solution at pH 1.25.

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The choice of this pH value appears to be inconsistent with information gathered in the literature regarding the optimal conditions for the growth of these micro-organisms and their adherence on solid supports.

In the first place, various authors agree on locating the optimum pH for growth in the range 2.0 to 2.5 for *A. ferrooxidans* and in the range 1.5 to 2.0 for *L. ferrooxidans* (Nemati et al., 1998; Gomez et al., 1999; Breed and Hansford, 1999).

On the other hand, several authors demonstrate that jarosite plays a key role in the formation and stability of biofilm (Grishin et al., 1988; Grishin and Tuovinen, 1989; Toro et al., 1989; Karamanev, 1991; Pogliani and Donati, 2000; Nikolov et al., 2002; Kinnunen and Puhakka, 2004); the jarosite precipitation becomes evident at pH higher than 1.5. Jarosite precipitates are the true support of biomass. In this vein, Karamanev's model (Karamanev, 1991), the most cited in the literature, suggests that biofilm principally consists of jarosite attached to the solid support and cells adsorbed on the pores. This model is still valid. Recently, van der Meer et al. (2007), through testing several carrier materials (activated carbon, diatomaceous earth and Al_2O_3) in fluidized bed reactors, observed, by scanning electron microscopy coupled with energy dispersive spectroscopy, that all of the materials were covered with jarosite precipitates and that the bacteria were mainly retained on the jarosite-covered areas.

Pogliani and Donati (2000) go further and suggest a direct relationship between precipitation of jarosite and the number of cells attached to the support (glass beads), which implies an advantage in selecting those conditions to promote the precipitation of jarosite in the process of biofilm formation.

The processes which take place in the above applications require bio-oxidation liquors whose pH is as high as possible within the range in which Fe (III) is mostly in solution, since:

- In indirect bioleaching, metals of commercial interest are extracted from the fertile leaching liquor by solvent extraction, which can be generically represented by the following equation (Davenport et al., 2002):

$$Me^{2+}_{(aq)} + H_2{-}R_{(org)} \rightarrow Me{-}R_{(org)} + 2H^{+}_{(aq)}$$

where Me^{2+} is a metal cation, $H_2{-}R_{(org)}$ is the extracting agent, and the subscripts (aq) and (org) signify aqueous and organic phases, respectively.

- In the purification of wastewater (acid liquor containing heavy metals in solution), the typical sequence of operations placed after the bio-oxidation includes a neutralization stage by addition of alkali.

Since the bio-oxidation process involves consumption of H^+, iron precipitates are generated and accumulated within the bioreactor. The intensive deposition of these precipitates on the support particles may involve (Jensen and Webb, 1995; Daoud and Karamanev, 2006):

- At a macroscopic level, the partial or total obstruction of the channels of passage of liquid and gas flows through the bed.
- At a microscopic level, the formation of solid structures that line the biofilm and impede, or even halt, the diffusion of substrates into cells.

These phenomena can become the cause of a significant decrease in the bioreactor performance, and can even end its operation, if they are irreversible.

This paper argues that the control of pH in the bioreactor is an essential aspect of its operation and, consequently, the need arises for scientific arguments which define the limits of this variable, which depend on the composition of the medium.

The thermodynamics and kinetics of iron precipitation in a sulphate ion medium have been addressed by several authors who emphasize the complexity of studying these processes and the difficulty in modelling a general application. These systems are very sensitive to composition (mainly Fe(III), monovalent cations, sulphate, bisulphate, and pH) and to temperature, and involve multiple simultaneous equilibria which give rise to different products, mainly jarosite, iron hydroxides and oxyhydroxides (Baron and Palmer, 1996; Smith et al., 2006; Welch et al., 2008; Leahy and Schwarz, 2009). These difficulties are accentuated when precipitation processes interact with bio-oxidation processes (Daoud and Karamanev, 2006; Jin-yan et al., 2009) and even more when biomass is immobilized in biofilms. The biofilm is a proton sink and a source of Fe (III), which leads to the existence of local concentration gradients in the bioreactor. Therefore, physical phenomena of mass transfer are involved in precipitation processes. Thus, measurable values of composition will not correspond to real values in the particular location where precipitation phenomena take place. This greatly limits the application of precipitation patterns for the establishment of criteria for pH control in industrial bioreactors, which necessarily require finding them in experimentation.

This paper shows the results obtained when testing bio-oxidation reactors (flooded packed-bed) which are inoculated with mixed cultures (*L. ferrooxidans* and *A. ferrooxidans*) and fed with a modified version with respect to the pH of the 9 k medium of Silverman and Lundgren. The objectives of these tests are:

- To define the pH range at maximum productivity.
- To evaluate both the consequences and the reversibility when the pH limits are exceeded.

2. Materials and methods

2.1. Cultures

Two mixed cultures, named A and L, obtained from Riotinto Mine acid drainage waters are used as inocula. These mainly consist of autotrophic bacteria such as *L. ferrooxidans* and *A. ferrooxidans*, and some heterotrophs (*Ferrimicrobium spp.* and *Acidiphilium spp*). Identification of bacterial microorganisms is carried out by a molecular culture-independent method (Steffan and Atlas, 1991) whereby the DNA is purified from the sample and used in PCR with 16 S rRNA primers. Amplicon is cloned and sequenced, and the nucleotide sequence obtained is compared against databases.

The culture A is routinely maintained on Silverman and Lungren 9 K nutrient medium. The culture L is routinely maintained on a modified Silverman and Lungren 9 K nutrient medium at pH 1.25 (adjusted with concentrated H_2SO_4).

A. ferrooxidans is the predominant species in culture A and *L. ferrooxidans* predominates in culture L.

2.2. Liquid medium

All the tests are performed in 9 k nutrient medium, whose pH is modified with concentrated sulphuric acid to the value set for the operation. The liquid medium is sterilized by pressure filtration (4 bar) using Millipore sterilized filter medium with 0.45 μm pore size. The filtration equipment, also Millipore, is made of stainless steel and is cylindrical with a diameter of 47 mm and a volume of 340 ml.

2.3. Bioreactors

Continuous biooxidation assays are carried out in five geometrically identical flooded packed-bed bioreactors. These bioreactors consist of columns filled with inert solid particles with inlets for liquid medium and for air at the bottom. The liquid and gas streams are