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 iron 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 9k 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.

Key words: bio-oxidation, ferrous iron, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferooxidans*, operational pH, packed-bed bioreactor, biofilm.

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 [1-4] and in the ferrous ion removal in processes for the purification of liquid effluents [5-8].

The interest generated by these applications is still relevant, as witnessed by the numerous recent publications on the subject [9-14], some of which show results of research carried out in pilot plants [15, 16].

Among the tried and tested devices which perform bio-oxidation [5, 17-20], 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 [15, 16]. 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 [15, 21].

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 [22-26], although siliceous stone remains one of the most attractive for its availability and price.

Mazuelos et al, [24] 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 *Acidophilium*. The liquid medium feed was an aqueous ferrous sulphate solution at pH 1.25.

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 *Acidithiobacillus ferrooxidans* and in the range 1.5 to 2.0 for *Leptospirillum ferrooxidans* [27-29].

On the other hand, several authors demonstrate that jarosite plays a key role in the formation and stability of biofilm [30-36]; 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 [33], 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 [26], 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 et al, [34] 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 [37]:

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

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 [38, 39]:

- 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 [40-43]. These difficulties are accentuated when precipitation processes interact with bio-oxidation processes [39, 44] 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 (*Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*) and fed with a modified version with respect to the pH of the 9k 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 *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*, and some heterotrophs (*Ferrimicrobium spp.* and A*cidiphilium spp*). Identification of bacterial microorganisms is carried out by a molecular culture-independent method [45] whereby the DNA is purified from the sample and used in PCR with 16S 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 9K nutrient medium. The culture L is routinely maintained on a modified Silverman and Lungren 9K nutrient medium at pH 1.25 (adjusted with concentrated H_2SO_4).

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

2.2. Liquid medium.

All the tests are performed in 9k 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 continuously fed, rising through the bed and filling all the hollow space (flooding the bed). The liquid is flushed out by overflowing.

These bioreactors have a diameter of 4.2 cm and a height of 10 cm. In all cases the bed consists of siliceous stone particles, between 6 and 8 mm in size and randomly packed. The porosity of the beds is 0.42.

The bioreactors are placed in a thermostatically controlled room, maintained at 31ºC.

Depending on the culture used for inoculation and the pH of the feed both in the biofilm formation process and in continuous operation, these bioreactors are named as:

A-1.90: inoculum A, pH of feed =1.90.

A-1.25: inoculum A, pH of feed = 1.25.

L-1.90: inoculum L, pH of feed = 1.90.

L-1.25: inoculum L, pH of feed =1.25.

A-v: inoculum A, pH of feed varies from1.90 to 0.82.

2.4. Biofilm formation procedures.

In reactor L-1.25, the biofilm formation procedure follows the protocol proposed by Mazuelos et al, [24], which is described in Section 1.

For the other reactors, this procedure is modified as follows:

- The pH of the medium is adjusted to 1.90 instead of1.25 (reactors L-1.90, A-1.90 and A-v).

- Culture A is used as the inoculum instead of the culture adapted to pH 1.25 (reactors A-1.90 and A-1.25 and A-v).

2.5. Continuous bio-oxidation assays.

The liquid medium is fed to the reactor by a peristaltic pump through an 8 mm hole. The liquid flow rate is 100 mL/h. It is measured in the outlet stream with a 250-mL probe for two hours (standard deviation: 4.4 mL/h).

Air is supplied under a pressure of 0.5 bar and through a 1 mm nozzle. As the performance of this type of reactor is often limited by the conditions of aeration that are modified by medium composition, two air flow rates are used in order to detect possible interference of mass transport phenomena. The air-flow rates selected for the continuous operation are 250 and 500 mL/min. Air-flow rate is controlled using a P/N 10420 Cole Palmer, standard glass tube flow-meter, calibrated for air (accuracy ± 7 cm^3min^{-1}).

The composition of the liquid medium is constant in the continuous operation of all bioreactors except for reactor A-v. During reactor A-v operation, the pH of feed is gradually decreased from 1.90 to 0.82; the pH values tested are 1.90, 1.50, 1.25, 1.10, 1.00, 0.96 and 0.82. In these tests, the pH of feed is modified only when the bioreactor is in steady state. The operation is considered in steady state if the ferrous iron concentration in the outlet stream varies less than 5% with respect to the mean value for a time exceeding 50 h.

2.6. Batch bio-oxidation assays

Three batch bio-oxidation tests are conducted in 250 mL Erlenmeyer flasks containing: 80 mL of 9K medium, 80 mL of 9K medium at pH 1.25, and 80mL of 9k medium at pH 0.82, respectively. In each of the three flasks, as inoculum, 20 mL of effluent of bioreactor A-v operated at pH 0.82 is added. The flasks are stirred at 180 rpm in an orbital stirrer in a thermostatic chamber. Temperature is set to 31ºC. Ferrous iron concentration is measured during the culture growth as a function of time.

2.7. Concentration of Fe(II).

The Fe^{2+} concentration is determined by standard potassium dichromate solution in an automatic titrator with 0.05N $K_2Cr_2O_7$.

2.8. pH

The pH is measured with a Sartorius pT-10 pH meter and a WTW pH electrode which is calibrated with Panreac pH buffers between 1 and 3.

3. RESULTS

Figure 1 shows the productivities of ferric ion, calculated by the formula $([Fe^{2+}]_{inlet} - [Fe^{2+}]_{outlet}) \cdot liquid\ flow\ rate$, and the outlet pH in the operation of reactors L-1.90 and L-1.25 as a function of time and of the air-flow feed (250 and 500 mL/min). This graph can be divided into three zones:

Zone 1: After the cell fixation on the support particles (procedure explained in Section 2.4), the biofilm continues to grow during continuous operation. This occurs for the reactor L-1.25 in the time range 0 to 100 hours and for the reactor L-1.90 in the range 0 to 217 hours.

Zone 2: Steady operation at 250 mL/min air-flow rate. This takes place in the interval 100 to 337 hours for the reactor L-1.25 and in the range 217 to 337 hours for the reactor L-1.90

Zone 3: Operation at 500 mL/min air-flow rate, in the interval 337-433 hours for both reactors.

The sharp increase of ferric ion productivity as air-flow rate increases from 250 to 500 mL/min indicates an oxygen limitation. This limitation justifies that, in steady state at 250 mL/min air-flow rate (zone 2), reactor L-1.25 productivities are lower than in reactor L-1.90. Although fluid dynamic conditions in both reactors are the same (identical air-flow and liquid-flow rates and geometry), since the pH in the reactor L-

1.25 is lower than in reactor L-1.90, the ionic strength in the first reactor is higher, and the solubility of oxygen is lower due to the salting-out effect [46-49].

The situation is reversed when the flow is doubled (500 mL/min). In the third zone, the ferric iron productivities in reactor L-1.25 are greater than those in reactor L-1.90. Furthermore, while the reactor L-1.25 shows a stable response, the productivity of ferric in L-1.90 reactor decreases with time. This trend may be due to the following phenomena:

- The pH of liquor enables the intensive precipitation of Fe, and the deposition of precipitates on the bed particles modifies the flow of substances in the reactor. The trend of decreasing productivity in reactor L-1.90 is observed when the pH of the liquor which leaves the reactor exceeds the value 2.3, since at this pH Fe (III) is not significantly soluble.
- Once the restriction of aeration has been overcome, culture L, originally adapted at pH 1.25, suffers from a worse adaptation to an environment whose pH is higher.

To resolve doubts in interpreting these results, the reactors A-1.25 and A-1.90 are operated.

Regarding the starting up of the bioreactor, while it was possible to form stable biofilms in reactors A-1.90, L-1.90 and L-1.25, the formation of biofilm in reactor A-1.25 was unsuccessful, after three attempts it was impossible to support the cells on the inert solid. That is, the process of biofilm formation at pH 1.25 requires an inoculum previously adapted to this pH, although the process of biofilm formation is independent of the culture used as inoculum at pH 1.9.

Ferric ion productivity and pH in reactor A-1.90 operation as a function of air-flow rate and time are shown in Figure 2.

The ferric ion productivities obtained demonstrate the instability of the reactor, since it is impossible to maintain the reactor in a steady state, despite the strict control of the remaining operation conditions. A cyclic behaviour is noted in the instability of ferric ion productivity:

- The outlet pH increases as ferric ion productivity increases.
- When outlet pH is higher than 2.3, the system response is a fall in ferric ion productivity.
- The fall in ferric ion productivity is followed by a pH decrease.
- The response after the pH decrease is an increase in ferric ion productivity.

This cyclic behaviour shows that the effects of pH on the reactor performance must be reversible.

In spite of this instability, it is observed that reactor A-1.90 can achieve ferric ion productivities higher than those achieved with reactors L-1.90 and L-1.25, both with 250 mL/min and 500 mL/min air-flow rate. If it were possible to stabilize the operation of reactor A-1.90 in the value of maximum productivity, it could be stated that, as inoculum, culture A is more advantageous than culture L.

On the other hand, the cause of instability in the operation of bioreactors A-1.90 and L-1.90 appears to be associated with a pH in the outflow higher than 2.3, regardless of the culture used as inoculum. The stability of the reactor requires that the outlet pH does not exceed this value. In order to test two possible improvements in the performance of flooded packed-bed reactors, that is, to use culture A as inoculum, to set aeration at 500 mL/min, and to set an upper limit of pH=2.3 at the outlet of the bioreactor, the reactor A-v is operated.

The operation of the reactor A-v begins in a similar way to that of reactor A-1.90; the process of biofilm formation is the same, and the first pH tested in the liquor fed is 1.90.

The only difference is the aeration flow: at the beginning of operation of reactor A-1.90, this is 250 mL/min while it is 500 mL/min for reactor A-v.

Figure 3 shows ferric ion productivity and outlet pH as a function of inlet pH and time.

It is noted that the response of reactor A-v against outlet pH is similar to that given by the reactor A-1.90 (Figure 2); when the pH exceeds the value 2.3 then the productivity of ferric ion decreases. This effect is reversible, as shown by the immediate recovery of ferric productivity once the pH at the exit falls below this value. In the case of reactor A-v, the recovery of ferric iron productivity is triggered by a decrease in inlet pH from 1.90 to 1.50.

Moreover, Figure 3 shows that the targets proposed with reactor A-v experimentation are achieved, that is, ferric ion productivities are attained in the order of the maximum obtained with reactor A-1.90, but in stationary operation.

The influence of pH, in the range 1.91 to 1.39, upon flooded packed-bed reactors was discussed earlier, whereby a culture adapted to pH 1.25 is used as inoculum [24]. To extend that study to inocula adapted to a higher pH (culture A) and to a wider pH range, a similar study is conducted with the reactor A-v. Table 1 shows the mean ferric ion productivities obtained at the various pHs in steady state.

In the pH range from 1.00 to 2.3, the obtained ferric ion productivities are similar, which show that the culture adapts to conditions of increasing acidity up to pH=1 in the course of continuous operation.

It should be noted that the adaptation of culture A is not possible when reactor A-1.25 is operated. The causes put forward in justification of these results are:

- The abrupt change of pH, to which the culture A is subjected when reactor A-1.25 is inoculated, could cause irreversible damage to cells. However, in reactor A-v, pH starts with a value similar to that of culture A, and gradually decreases.

- The cells from culture A must require more time to adapt to new conditions of acidity in reactor A-1.25. In reactor A-v, it took more than 200 hours of continuous operation to reach pH 1.22.

From pH 1 to pH 0.82 in the feed, ferric productivity plummets, reaching a value of 0 g/h.

The productivity drop to zero can be due to the loss of biomass or to the loss of biomass activity. To establish whether there is loss of bacterial activity, the effluent of reactor A-v after operation at pH 0.82 is added, as inoculum, in three flasks containing 9k medium, 9k medium at pH 1.25 and 9k medium at pH 0.82, respectively. Figure 4 shows the percentage of ferrous iron oxidation versus time for these cultures. Despite a long lag phase caused by low initial cell concentration, it can be seen that the three cultures have grown, which signifies no loss of bacterial activity.

Thus, the productivity fall must be due to the loss of biomass. Biomass is lost since at this pH the precipitates disappear and the precipitates are the true support of bacteria. Figure 5 shows the appearance of bed particles after the operation of reactors A-1.90 and A-v. Particles from reactor A-1.90 are covered by precipitates while particles from reactor A-v are similar in appearance to the particles before use.

This means that at pH 0.82, iron precipitates have been completely dissolved by the continuous flow of liquid medium. The disappearance of these precipitates implies the destruction of the biofilm. In this case the effect on reactor performance caused by a drop in pH to 0.82 is irreversible.

The identification of microorganisms in the effluent of reactor A-v shows that the major species is *Leptospirillum ferrooxidans* instead of *Acidithiobacillus ferrooxidans,* which is the major species in the inoculum (culture A). These results are in accordance with those found in the cultures used as inocula. As in Section 2.1, *Acidithiobacillus*

ferrooxidans is the predominant species in culture A (grown in 9k medium) and *Leptospirillum ferrooxidans* predominates in culture L (grown in 9k medium at pH 1.25).

During the continuous operation of reactor A-v, the pH changes from 2.3 to 0.82, and a clear evolution of the bacterial population from being mainly *Acidithiobacillus ferrooxidans* to being mostly *Leptospirillum ferrooxidans* is observed. This finding suggests that the use of a mixed culture (*Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) enables the reactor operation in a wide pH range.

4. CONCLUSIONS.

The stability in the operation of flooded packed-bed reactors requires the pH to remain within the limit values 1.0 and 2.3; similar productivities are obtained throughout the entire interval. The effects on the reactor performance of pH when it exceeds the upper limit are reversible. The effects are irreversible, however, when the pH falls bellow the lower limit.

The limitations in operational pH appear to be linked to the chemistry of precipitation of ferric compounds and not to biological phenomena. This is because the cultures used as inoculum, mixed cultures *Leptospirillum ferrooxidans* - *Acidithiobacillus ferrooxidans*, showed a very versatile performance with respect to the pH; the continuous operation with a gradual lowering of pH enables its adaptation from pH 2.3 to pH 0.82.

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Table 1: Mean ferric ion productivities in steady state at different pH for reactor A-v

Inlet pH	1.90	1.50	1.25	1.10	1.00	0.96	0.82
Outlet pH	2.30*	1.81	1.45	1.22	1.02	1.00	0.82
Productivity (g/h)	0.55*	0.55	0.50	0.57	0.51	0.42	0.00
Standard deviation	-	0.011	0.011	0.014	0.005	0.009	-

* Values obtained in unsteady state.

FIGURE CAPTIONS

Figure 1: Operation of reactors L-1.25 and L-1.90: Ferric iron productivities and outlet pH versus time. Liquid-flow rate: 100mL/h. Air-flow rates: 250 and 500 mL/min.

Figure 2: Operation of reactor A-1.90: Ferric iron productivities and outlet pH versus time and air-flow rate. Liquid-flow rate: 100mL/h. Air-flow rates: 250 and 500 mL/min.

Figure 3: Ferric ion productivity and outlet pH as a function of time for reactor A-v. Inlet pHs: 1.9 and 1.5. Liquid-flow rate: 100 mL/h. Air-flow rates: 250 and 500 mL/min.

Figure 4: Percentage of oxidized Fe (II) versus time for batch tests inoculated with the effluent of reactor A-v when it is operated at pH=0.82. Media: 9k, 9k pH=1.25 and 9k pH=0.82.

Figure 5: Appearance of bed particles **a)** before operation, **b)** after reactor A-1.90 operation and **c)** after reactor A-v operation at pH=0.82.

Figure 1

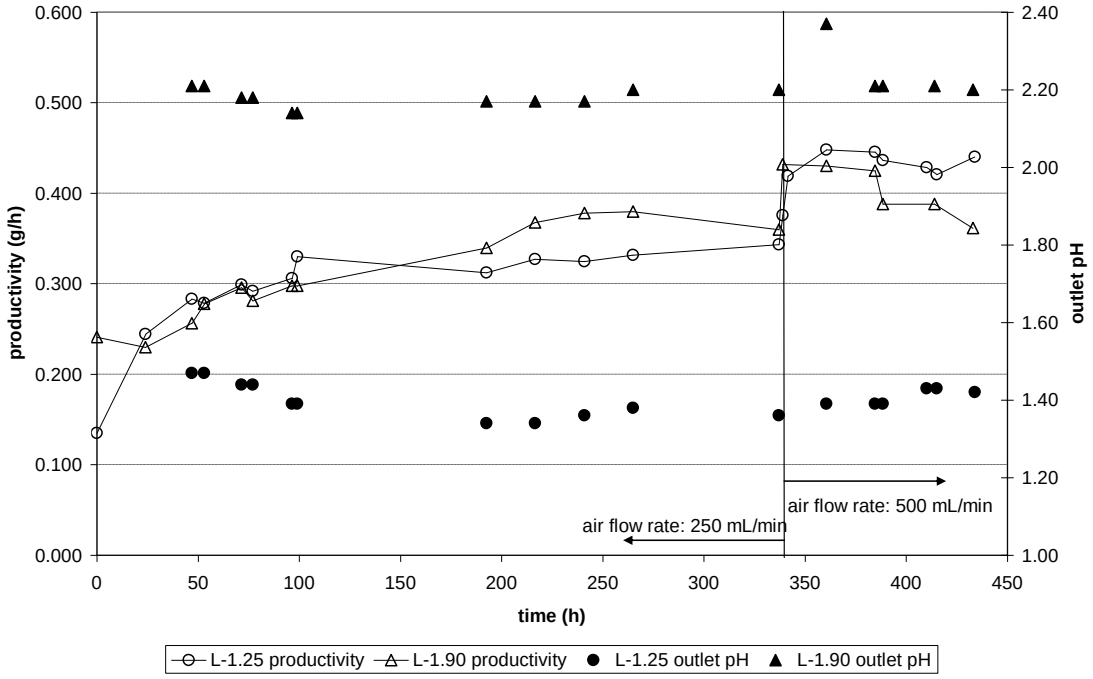
0.600
0.500
0.400
0.300
0.200
0.100
0.000
productivity (g/h)
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
outlet pH
0
50
100
150
200
250
300
350
400
450
time (h)
air flow rate: 250 mL/min
air flow rate: 500 mL/min
L-1.25 productivity
L-1.90 productivity
L-1.25 outlet pH
L-1.90 outlet pH

Figure 2

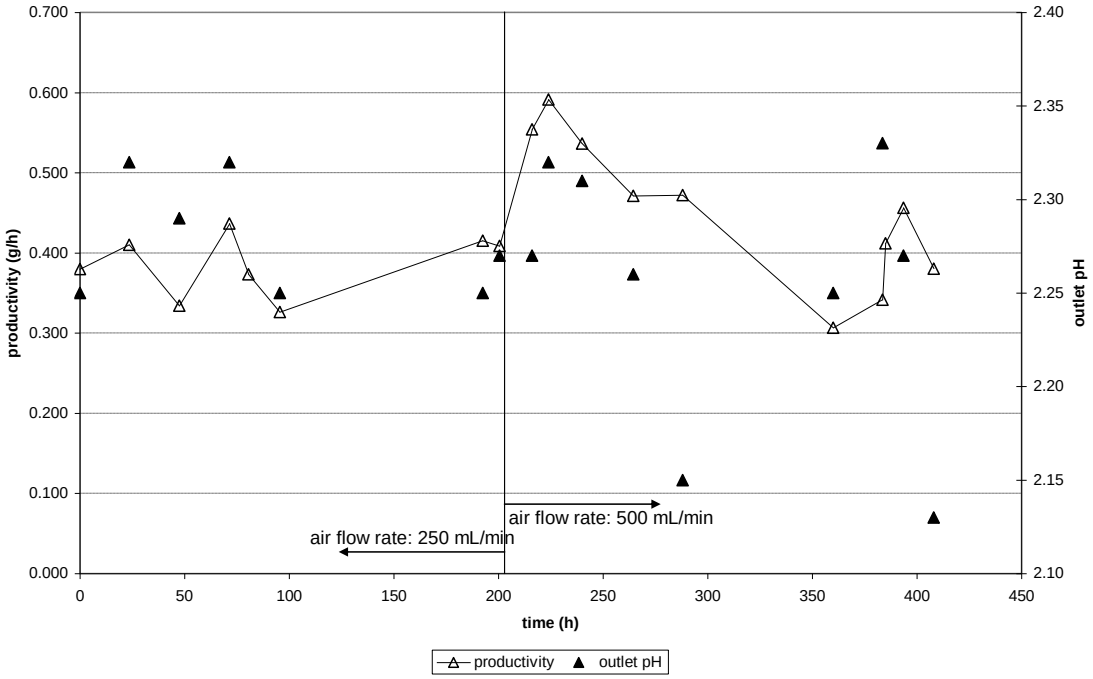
productivity (g/h)
outlet pH
time (h)
air flow rate: 250 mL/min
air flow rate: 500 mL/min
productivity
outlet pH

Figure 3

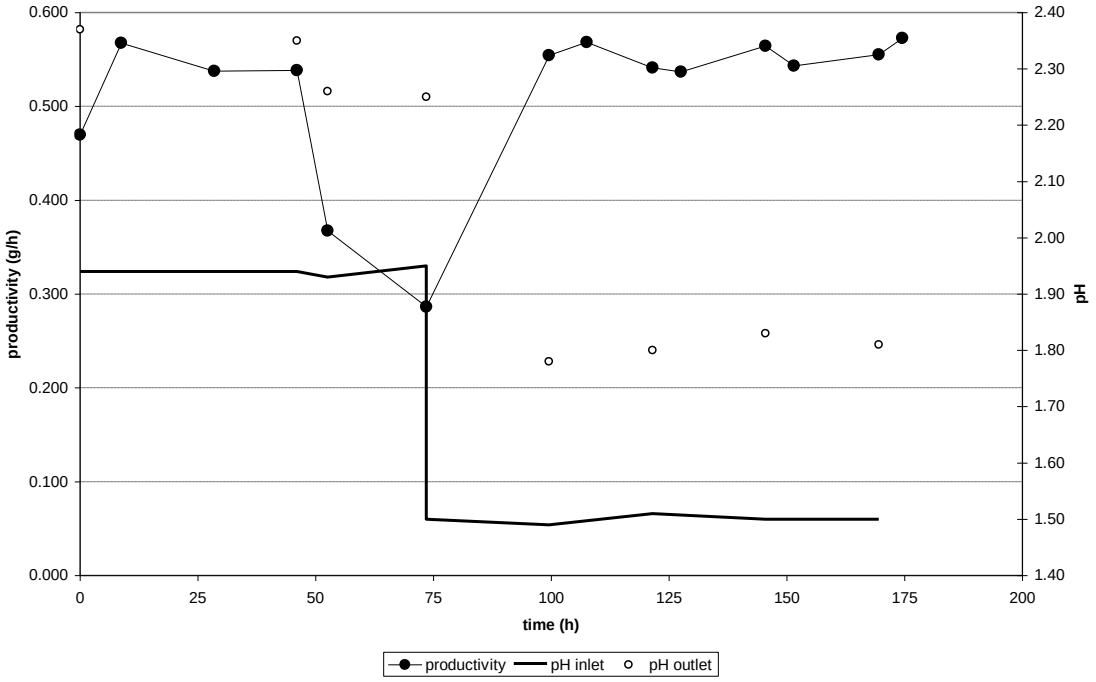
0.600
0.500
0.400
0.300
0.200
0.100
0.000
productivity (g/h)
2.40
2.30
2.20
2.10
2.00
1.90
1.80
1.70
1.60
1.50
1.40
pH
0
25
50
75
100
125
150
175
200
time (h)
productivity
pH inlet
pH outlet

Figure 4

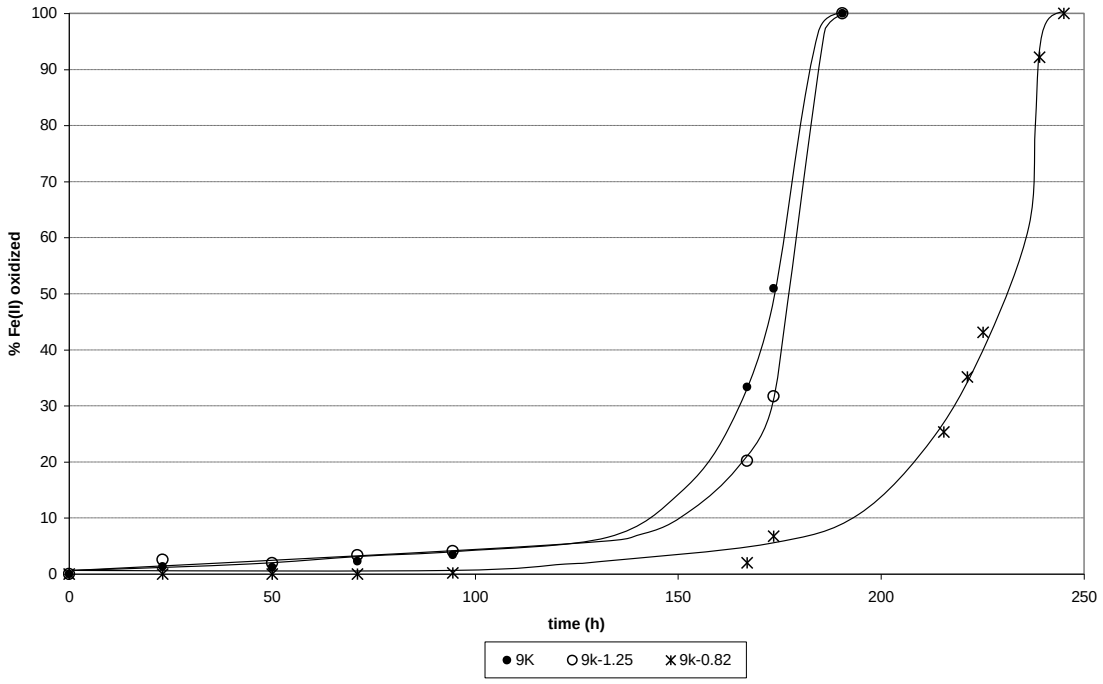
100
90
80
70
60
50
40
30
20
10
0
% Fe(II) oxidized
0
50
100
150
200
250
time (h)
9K
9k-1.25
9k-0.82

Figure 5