FLUORIDE ION EFFECTS ON THE KINETICS OF FERROUS IRON OXIDATION BY *SULFOBACILLUS THERMOSULFIDOOXIDANS*

Lázaro Chaves Sicupira, Tácia Costa Veloso, Isabel Cristina Braga Rodrigues, Victor Alvarenga Oliveira, Versiane Albis Leão[1]

Universidade Federal de Ouro Preto, Department of Metallurgical and Materials Engineering. Bio&Hydrometallurgy Laboratories, Campus Morro do Cruzeiro, s.n., Bauxita, Ouro Preto, MG, 35400-000, Brazil.

e-mail: lazaruschaves@yahoo.com.br and versiane@demet.em.ufop.br.

Abstract

High temperature bioleaching has become increasingly relevant as microorganism able to growth at high temperatures such as *Sulfobacillus thermosulfidooxidans* can improves metal extraction and leaching kinetics. Bacterial growth can however be severely impacted by the nature of the gangue minerals. Fluoride-containing minerals, such as fluorite and fluorapatite, for instance, when associated with metal sulfide ores are dissolved as the sulfide minerals are bioleached for metal recovery. There are strong evidences that some fluoride species can inhibit bacterial growth, therefore having adverse effects on iron biooxidation. In this work, the fluoride influence on the kinetics of ferrous iron biooxidation with *Sulfobacillus thermosulfidooxidans* (DSMZ 9293) was studied. The experiments were carried out in batch mode in a 2L bioreactor and the

[1] *Paper presented at 5th International Symposium on Bio-&Hydrometallurgy (BioHydromet '10), November 8-9, 2010, Cape Town, South Africa.*

effect of fluoride concentration (0-0.5mmol/L) on both iron oxidation and bacterial growth rates was assessed. In all experiments, the initial ferrous iron concentration was maintained at 2.5g/L; pH at 1.5; temperature at 50°C, air flow at 1L/min; and stirring speed at 300min^{-1}. It was also assessed the effect of aluminum (Al^{3+}) additions aiming to reduce HF concentration through the formation of stable aluminum-fluoride complexes, which cannot cross the bacterial cell membrane. The results show that 0.5mmol/L (10mg/L) total fluoride completely inhibits bacterial growth. Nevertheless, fluoride toxicity to *S. thermosulfidooxidans* can be minimized by controlling the ratio aluminum to fluoride in the system. At 2:1 aluminum to fluoride molar ratio, bacterial growth is similar to that observed in the absence of anion.

1. Introduction

Bioleaching is now a proven technology that can be applied to the processing of a series of metallic sulphides, specially ores or mining residues (marginal ores). Due to its relatively low cost, bio-heap-leaching seems to consolidate as the main biotechnology in the field of non-ferrous hydrometallurgy (Petersen and Dixon, 2007). Actually, sulphide heap-leaching and bio-heap-leaching are not dissociable since bacteria are always present in these environments (Rawlings and Johnson, 2007).

As far as ore bioleaching is concerned, the gangue nature can have a very important impact on leaching performance. This is because the gangue minerals can contain elements which are harmful to the bacteria. Ojumu et al. (2008) has shown the effect of increasing ionic strength on bacterial growth. In addition to the reducing effect on

dissolved oxygen concentrations, higher ionic strength reduces the concentration of free water and the bacteria looses water to the solution due to osmotic pressure effects (Suzuki et al., 1999). Anions have different effects on Fe(II) or sulphur oxidation, but nitrate and chloride are the most important inhibitors of mesophilic bacterial growth (Harahuc et al., 2000). Acidophilic bacteria have their membrane internal pH in the range 6 – 7 that is maintained, despite the much lower external pH, by a positive potential ($\Delta\psi$), which prevents H^+ from crossing the membrane. Weak base anions such as NO_3^- and Cl^- reduces this potential and enables H^+ crossing, lowering the internal membrane pH (Booth, 1985). Recently, it was reported a failure on a heap bioleaching operation due to presence of fluoride (Brierley and Kuhn, 2010) which affected the growth of *Acidithiobacillus ferrooxidans*. HF, which can penetrate cell membranes, is the main fluoride species at the pH of bioleaching. It dissociates to H^+ and F^- inside the cell (due to its neutral pH) and inhibits bacterial growth (Suzuki et al., 1999).

In heap leaching operations, temperatures inside the heap can reach values that support moderate thermophilic growth and this has a positive impact on both the leaching rate and metal extraction (Cruz et al., 2010). Assessing temperature effects on secondary sulphide bioleaching, mineralogical studies revealed the presence of fluorite (CaF_2) on the ore. Its dissolution in acid solutions produced total fluoride concentrations in some cases, higher than 100mg/L, which had an important negative impact on iron oxidation and solution potential. Therefore, this paper was undertaken aiming to assess fluoride ion impact on ferrous iron oxidation and how its detrimental effect could be overcome.

2. Materials and Methods

Sulfobacillus thermosulfidooxidans (strain DSMZ 9293) was grown in a medium containing: 0.4g/L $(NH_4)_2SO_4$, 0.8g/L $MgSO_4.7H_2O$, 0.4g/L K_2HPO_4, 2.5g/L ferrous iron ($FeSO_4.7H_2O$) and 0.1g/L yeast extract, at pH 1.5. The cells were maintained throughout the experiments in an orbital shaker (New Brunswick Scientific), at 50°C, and 200min^{-1} and provided the inocula for the bio-oxidation experiments from a 48h grown culture.

The bio-oxidation experiments were carried out in batch mode in a bioreactor (New Brunswick Scientific - BioFlo 110) with 2L of suspension containing 10% (volume) of inoculum. To produce the latter, 200mL of the inoculum (not previously adapted to either fluoride or aluminum) were transferred to the bioreactor and growth medium was added to produce a final solution volume of 2L. Afterwards, the pH was set at 1.5 and kept at this value throughout the experiment. A pH meter (Hanna 2221) and glass membrane electrode calibrated against pH 4.0 and 7.0 buffers was used for pH measurement. The pH was controlled during the experiments by the addition of either concentrate sulfuric acid or sodium hydroxide. Both the temperature and the stirring rate were maintained at 50^oC and 300min^{-1}, respectively. This latter was defined as the value which produced the highest ferrous iron oxidation rate. The solution was aerated at a rate of 1L/min and 5mL samples were withdrawn regularly and analysed for ferrous iron concentration and cell number.

Both bacterial growth and ferrous iron oxidation were assessed in experiments where fluoride ions (NaF) were added. The fluoride concentration was varied from 0 to

0.50mol/L 0-10mg/L), in the presence and absence of aluminium ($Al(OH)_3$) so that the following Al:F molar ratios were achieved: 0.0:0.13; 0.0:0.25; 3.0:0.0; 1.0:0.50 and 3.0:1.0.

Cell counts were performed using a Neubauer chamber in a light contrast microscope (Leica). Ferrous iron concentration was determined by titration with standard potassium dichromate solution in the presence of a 1 H_2SO_4: 1 H_3PO_4 solution using an automatic titrator (Schott - Tritoline Alpha). All chemicals used in this study were analytical grade reagents (AR), unless otherwise stated and all solutions were prepared with distilled water.

Statistical analysis was carried out using the Origin^{TM} version 8.0 software to determine the specific growth rate, the Fe(II) oxidation rate as well as the yield values for a 95% confidence interval. The data points used to calculate such parameters were those that produced linear regression with correlation coefficients (r^2) higher than 0.94.

3. Results and discussion

Bacterial growth in batch mode follows four different phases: (i) lag, (ii) exponential, (iii) stationary and (iv) death. During the lag phase, virtually there is no bacterial growth as the microorganism is adapting to the new environmental conditions. In this work, the lag phase was taken as the period between the beginning of the experiment and the first point of the exponential phase. The duration of the lag phase depends on many factors such as medium composition, energy availability, osmotic pressure, inoculum age and presence of toxic elements (Waites et al., 2001). The negative effect of fluoride on *S.*

thermosulfidooxidans growth can be noticed in figure 1. As the anion concentration increases so does the lag phase, which varies from 5 hours in the absence of the anion to 33 hours, in the presence of 0.25mmol/L (5mg/L) fluoride. Higher fluoride concentrations inhibited bacterial growth completely. This behaviour can be interpreted by noticing that HF is a weak acid (pKa = 3.2, at 25^{o}C and I ↦ 0) and at pH 1.5, around 98% of the total fluoride species is present as HF, which can easily cross the bacterial cell membrane. Inside the cell, due to the its neutral pH, HF dissociates to H^{+} and F^{-}, decreasing internal pH and affecting growth, accordingly (Suzuki et al., 1999).

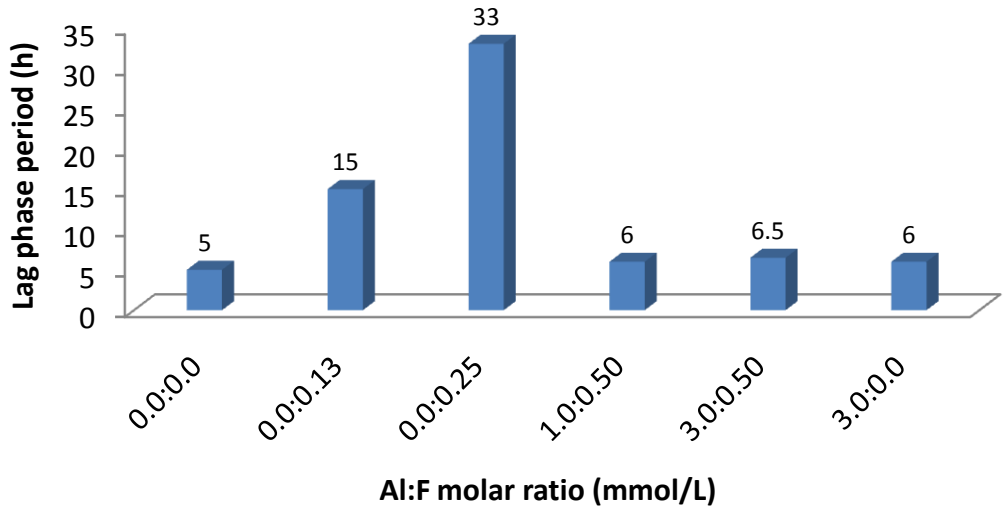

Figure 1: Effect of the NaF addition on the lag phase during Fe(II) oxidation by *S. thermosulfidooxidans.* Experimental conditions 2.5g/L Fe^{2+}; 0.1 g/L yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50°C.

With 0.50mmol/L total fluoride, no bacterial growth was observed within 60 hours and therefore this was taken as the reference in the experiments seeking to reduce its detrimental effects. This was accomplished with aluminium addition to the bioleaching reactor (Brierley and Kuhn, 2009). It can be observed (fig.1) that 1.0mmol/L Al^{3+} was

sufficient to overcome the negative impact caused by 0.50mmol/L NaF on the lag phase period, which as reduced from 60 hours to 6 hours. Aluminium forms strong complexes with fluoride, producing charged species (eq. 1-3), which cannot cross bacterial cell membranes.

$$Al^{3+} + F^{-} \leftrightarrows AlF^{2+} \qquad \log\beta_1 = 7.01 \quad (25^{o}C, I \mapsto 0) \qquad (1)$$

$$Al^{3+} + 2\ F^{-} \leftrightarrows AlF_2^{+} \qquad \log \beta_2 = 12.63 \quad (25^{o}C, I \mapsto 0) \qquad (2)$$

$$Al^{3+} + 4\ F^{-} \leftrightarrows AlF_4^{-} \qquad \log\beta_4 = 19.4 \quad (25^{o}C, I \mapsto 0) \qquad (3)$$

At pH 1.5, 1.0mmol/L total aluminium and 0.5mmol/L total fluoride, the predominant aluminium species on the system are Al^{3+} and AlF^{2+}, around 50% each, whereas the concentration of HF was reduced to values below 10^{-5}mmol/L. Therefore, fluoride complexation with aluminium significantly reduces the HF concentration and prevents any fluoride extensively entering the microbial cell. It is important to maintain a high Al:F ratio to avoid the formation of AlF_3(aq) (eq. 4) which can also potentially affects bacterial growth.

$$Al^{3+} + 3\ F^{-} \leftrightarrows AlF_{3(aq)} \qquad \log \beta_3 = 16.7 \quad (25^{o}C, I \mapsto 0) \qquad (4)$$

At the end of the lag phase, the bacteria are adapted to their environment and cell doubling starts (the exponential phase). If growth is not limited, doubling will continue so that a maximum growth rate $\left(\frac{dX}{dt}\right)$ is attained. In this situation, exponential growth is observed and cell doubling will continue at the so-called specific growth rate (μ).

$$\mu_i = \frac{1}{X_i}\frac{dX}{dt} \quad (5)$$

$$\left(\frac{dX}{dt}\right) = \mu.X \quad \text{or} \quad Ln\left(\frac{X}{X_0}\right) = \mu.t \quad (6)$$

Equation 6 applies to closed systems where growth is the only process affecting cell concentration (X) (Doran, 1995). A plot of LnX versus time gives a straight line with slope μ (figure 2).

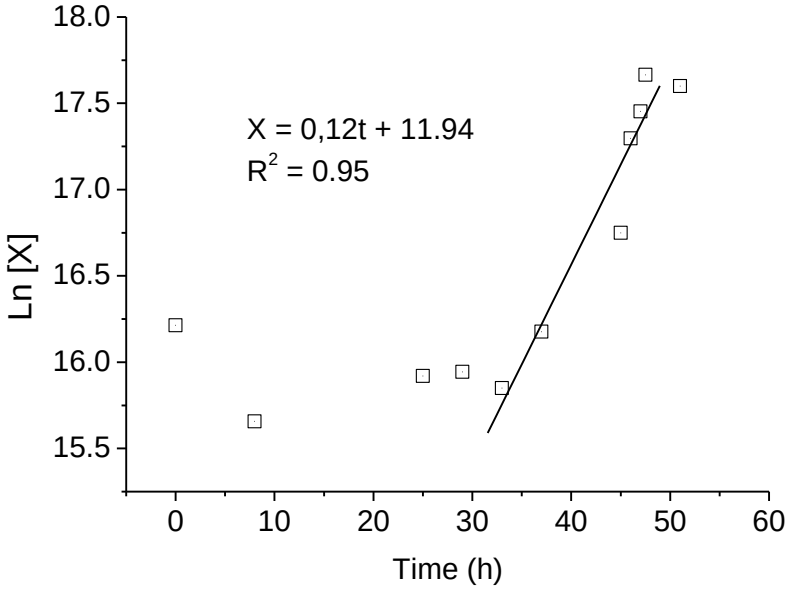

Figure 2: Bacterial counts as a function of time for the growth of *S. thermosulfooxidans* on Fe(II). $[Fe^{2+}]_0$ = 2.5g/L, 0.25mmol/L F^-, 50°C, 10% inoculum, pH 1.5, 300 min^{-1}.

Applying equation 6 to the bacterial counts determined in the experiments carried out in the presence of aluminium and fluoride ions, specific growth rate values were achieved and are depicted in figure 3. The specific growth rate (0.225±0.137h^{-1}), determined for the experiment carried out in the absence of both fluoride and aluminium ions (blank) is

consistent with previous studies on Fe(II) oxidation by *S. thermosulfooxidans* (0.197±0.012 h^{-1}) (Pina et al., 2010). Literature data on Fe(II) oxidation by moderate thermophiles and extreme thermophiles are scarce and sometimes contradictory. For instance, Nemati & Harrison (2000) reported specific growth rate of 0.043h^{-1} for *Acidianus brierleyi* in the presence of 1.8g/L Fe(II), while Konish et al. (1995) determined μ as 0.37h^{-1} for the same microorganism at 2.0g/L Fe(II).

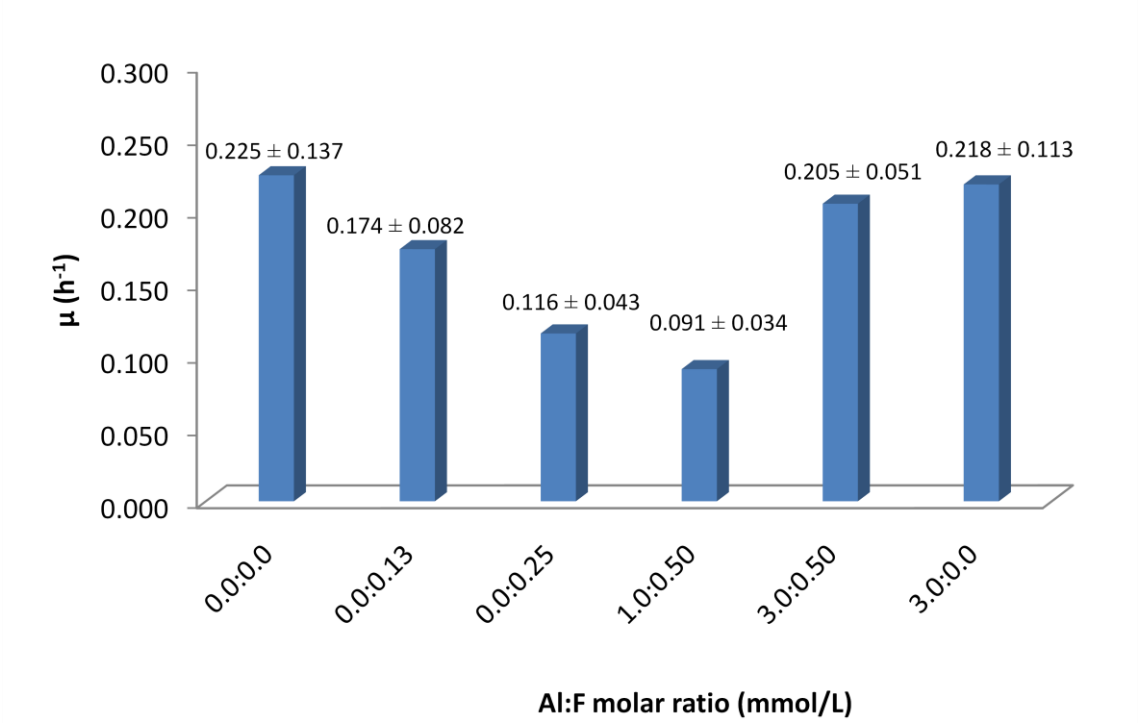

Figure 3: Effect of fluoride and aluminium addition on the specific growth rate (μ) during Fe(II) oxidation by *S. thermosulfidooxidans*. Experimental conditions 2.5g/L Fe^{2+}; 0.1 g/L yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50°C.

The presence of fluoride reduces the specific growth rate. With 0.25mmol/L total fluoride, μ is reduced to 0.116±0.043h^{-1}, i.e. half the value observed in the absence of the anion, reflecting the inhibitory effect caused by the toxic substance (Suzuki et al.,

1999). When aluminium was also added its detoxification effect becomes clear. As already stated, no growth was observed in the presence of 0.5mmol/L fluoride. When 1.0mmol/L Al was added to this fluoride concentration (Al:F = 2), growth could be observed and μ reached $0.091 \pm 0.034 h^{-1}$. Increasing the Al:F molar ratio to 6 (3.0mmol/L Al and 0.5.0mmol/L F) values similar to that produced in the absence of fluoride were achieved (figure 3).

Sulfobacillus thermosulfidooxidans utilizes Fe(II) as substrate for growth (Cruz et al., 2010). The maximum specific growth rate (μ_{max}) was determined for this bacterium growing in the presence of 2-20g/L Fe(II) and the value of $0.242 h^{-1}$ was observed (Pina et al., 2010). Fe(II) oxidation to Fe(III) has many technological applications including bioleaching, the removal of H_2S from gases, the desulfurization of coal and the treatment of acid mine drainage (AMD). Figure 4 presents the Fe(II) concentration behaviour in the experiments carried out in the presence of fluoride as well as aluminium. In the absence of aluminium (fig. 4a), increasing fluoride concentrations result in longer delays on the start of Fe(II) oxidation. This period is equivalent to the lag phase where growth is not expressive and therefore no substrate consumption is observed. As at 0.5mmol/L fluoride no bacterial growth was observed, Fe(II) oxidation was not detected either. In this latter concentration, aluminium additions enabled bacterial growth (figure 3) and substrate consumption (figure 4.b).

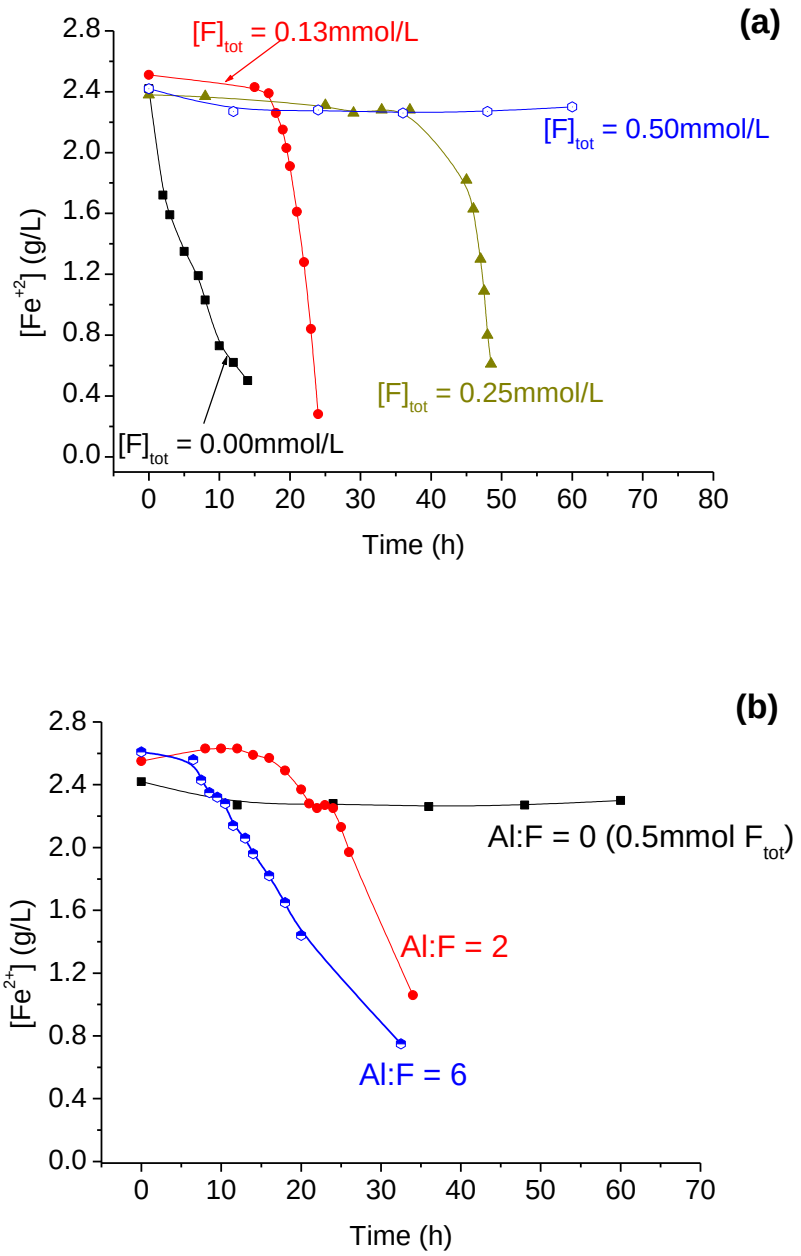

Figure 4: Effect of fluoride (a) and Al:F molar ratio (b) on Fe(II) oxidation by *S. thermosulfidooxidans.* Experimental conditions 2.5g/L Fe^{2+}; 0.1 g/L yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50°C. In figure b, fluoride concentration was set at 0.50mmol/L.

The ferrous iron consumption rate $\left(\frac{d[Fe^{2+}]}{dt}\right)$ is equivalent in absolute terms to the Fe(II) oxidation rate and was determined from the slope of the linear part of the profile of ferrous iron concentration versus time (figures 4a and 4b). The calculated values are shown in figure 5. Ferrous iron oxidation rate was determined as 0.114±0.014g/L.h in the absence of both aluminium and fluoride ions and is lower than the value observed by Pina et. al. (2010) determined as 0.292±0.034g/L.h in a similar experiment. However, this values is consistent with that reported for *A. ferrooxidans* (0.14 g/L.h) in the presence of 2.5g/L Fe(II) (Nemati and Harrison, 2000) and, as expected, higher than the value observed for *A. brierleyi* - 0.053 g/L.h - in 1.8g/L Fe(II).

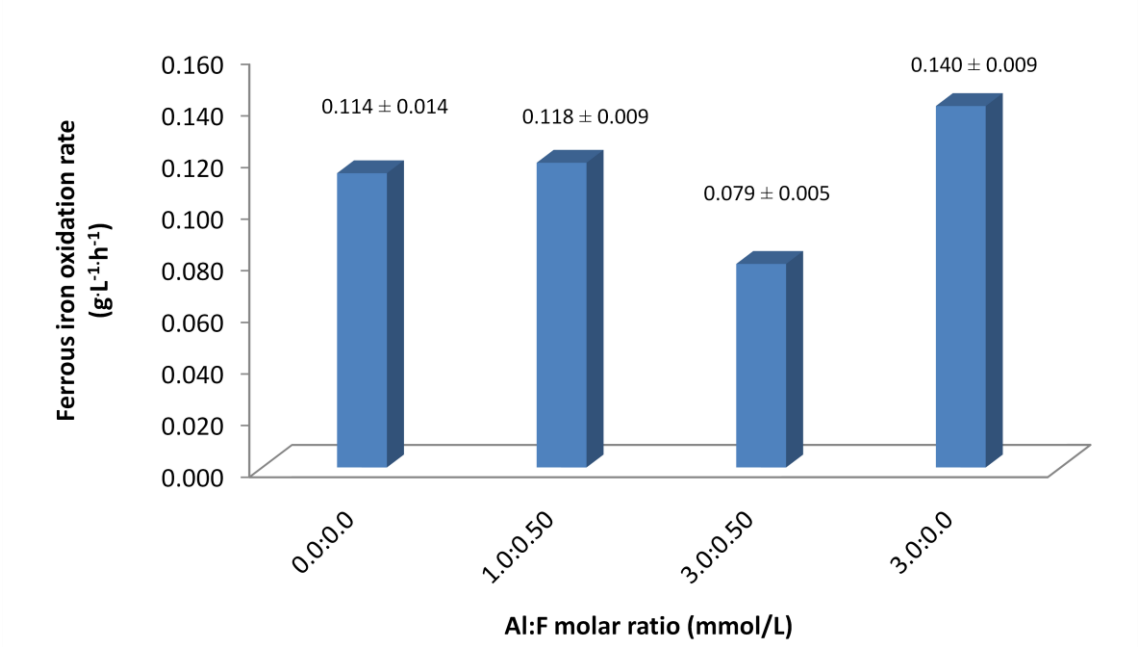

Figure 5: Effect of fluoride and aluminium addition on the Fe(II) bio-oxidation rate. Experimental conditions 2.5g/L Fe^{2+}; 0.1 g/L yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50°C.

Figure 5 shows that aluminium can overcoming the detrimental effect posed by fluoride ion during Fe(II) oxidation by *S. thermosulfidooxidans*, as already stated in the bacterial growth rate discussion (figure 3). The Fe(II) oxidation rate in the presence of the both ions (Al and F) is statistically similar to that observed in the blank experiment with a slightly lower value at 3.0mmol/L Al and 0.05mmol/L F. This is consistent with the results achieved by Ojumu et al. (2008) during Fe(II) oxidation by *L. ferriphilum*. The authors observed a deleterious effect on Fe(II) oxidation and bacterial growth only at high aluminium concentrations (10g/L Al).

Fluoride can also complex ferric iron, but the addition of aluminium ions seems to reduces the Fe(III)-fluoride complexes concentration as suggested by Brerley and Kuhn (2010). The authors observed the increase in solution potential when aluminium was added to the system containing Fe(III) and fluoride. This was due to the preferential formation of aluminium- over Fe(III)-fluoride complexes, which increased free Fe(III) concentration and, as result, the solution potential. However, this was not observed in this study due to the small fluoride concentration tested.

The results clearly show an increase in the lag phase period in the presence of fluoride species during ferrous iron oxidation. As the production of ferric iron is the main bioleaching mechanisms, the onset of metal extraction can became excessively longer than usually expected or even do not occur (Dopson et al., 2009). Unlike the Fe(II) oxidation experiments, bioleaching can be performed at much higher fluoride concentrations provided the ore contains elements such as aluminium which can complex free fluoride and reduce the HF concentration in the leaching liquor. If by any means the presence of fluoride- containing minerals is detected, extra care must be

taken during bioleaching studies specially when the leaching solution is not recirculated during the experiments (Brierley and Kuhn, 2009). Although fluoride toxicity can be reduced by the presence of aluminium, the build-up of both elements can lead to high ionic strength values which can also affect bioleaching. In this condition, solution bleeding would be required to reduce fluoride toxicity as well as solution ionic strength so that bioleaching can be performed properly.

4. Conclusions

Fluoride ions adversely affect S. *thermosulfidooxidans* growth, increasing the lag phase period from 5h to 33h, when 0.25mmol NaF was added to the system. During the exponential growth phase, the specific growth rate was reduced from $0.225\pm0.137h^{-1}$ to $0.116\pm0.043h^{-1}$ with the same concentration of fluoride. At 0.5mmol/L F, there is no bacterial growth in 60 hours. In acid solutions, HF is the main fluoride species which can cross the cell membrane, affecting bacterial growth. These effects can be overcome by the presence of aluminium (1mmol/L Al - 0.5mmol/L F) which can form complexes with the element, such as AlF^{2+}e AlF_2^+, reducing the HF concentration and fluoride toxicity, accordingly. This results in specific growth rates similar to those observed in the absence of both species. For those ores where fluoride-containing minerals are present, bioleaching can be performed provided aluminium sources are present or added to the bioleaching system. In heap bioleaching applications the build-up of aluminium and fluoride can lead to failures due to high ionic strength constraints and regular leaching solution bleedings may be required.

5. Acknowledgements

The financial support from the funding agencies FINEP, FAPEMIG, CNPq, CAPES as well as Vale is gratefully appreciated. The "Conselho Nacional de Pesquisas - CNPq scholarship to V. A. Leão is also acknowledged.

6. References

Booth, I.R., 1985. Regulation of cytoplasmic pH in bacteria. Microbiological reviews, 49(4): 359-378.

Brierley, J.A. and Kuhn, M.C. 2009. From laboratory to application heap bioleach or not. In: IBS 09 - International Biohydrometallurgy Symposium. E. Donati et al. (eds.) Bariloche, Trans Tech Publications. pp. 311-317.

Brierley, J.A. and Kuhn, M.C., 2010. Fluoride toxicity in a chalcocite bioleach heap process. Hydrometallurgy, 104(3-4): 410-413. DOI: 10.1016/j.hydromet.2010.01.013

Cruz, F.L.S., A.Oliveira, V., Guimarães, D., Souza, A.D. and Leão, V.A., 2010. High temperature bioleaching of nickel sulfides: Thermodynamic and kinetic implications. Hydrometallurgy, In Press. DOI: 10.1016/j.hydromet.2010.08.006

Dopson, M., Lövgren, L. and Boström, D., 2009. Silicate mineral dissolution in the presence of acidophilic microorganisms: Implications for heap bioleaching. Hydrometallurgy, 96(4): 288-293. DOI: 10.1016/j.hydromet.2008.11.004

Doran, P.M., 1995. Bioprocess Engineering Principles. Academic Press, San Diego, CA, USA, 439 pp.

Harahuc, L., Lizama, H.M. and Suzuki, I., 2000. Selective inhibition of the oxidation of ferrous iron or sulfur in *Thiobacillus ferroxidans*. Applied and Environmental Microbiology, 66(3): 1031-1037.

Konishi, Y., Shigeyuki, Y. and Asai, S., 1995. Bioleaching of pyrite by acidophilic termophile *Acidianus brierleyi*. Biotechnology and Bioengineering, 48: 592-600. DOI: 10.1002/bit.260480606

Nemati, M. and Harrison, S.T.L., 2000. A comparative study on thermophilic and mesophilic biooxidation of ferrous iron. Minerals Engineering, 13(1): 19-24. DOI: 10.1016/S0892-6875(99)00146-6

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

Petersen, J. and Dixon, D.G., 2007. Principles, mechanisms and dynamics of chalcocite heap bioleaching. In: E.R. Donati and W. Sand (Editors), Microbial Processing of Metal Sulfides. Springer, Dordrecht, The Netherlands, pp. 193-218.

Pina, P.S., Oliveira, V.A., Cruz, F.L.S. and Leão, V.A., 2010. Kinetics of ferrous iron oxidation by Sulfobacillus thermosulfidooxidans. Biochemical Engineering Journal, 51(3): 194-197. DOI: 10.1016/j.bej.2010.06.009

Rawlings, D.E. and Johnson, D.B., 2007. The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. Microbiology, 153(2): 315-324. DOI: 10.1099/mic.0.2006/001206-0

Suzuki, I., Lee, D., Mackay, B., Harahuc, L. and Oh, J.K., 1999. Effect of various ions, pH, and osmotic pressure on oxidation of elemental sulfur by Thiobacillus thiooxidans. Applied and Environmental Microbiology, 65(11): 5163-5168.

Waites, M.J., Morgan, N.L., Rockey, J.S. and Higton, G., 2001. Industrial Microbiology: An Introduction. Wiley-Blackwell, Oxford, 304 pp.