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The effects of fluoride and aluminum ions on ferrous-iron oxidation and copper sulfide bioleaching with *Sulfobacillus thermosulfidooxidans*

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ABSTRACT

Microorganisms that grow at high temperatures can improve Fe(II) bio-oxidation and thereby its technological applications, such as bioleaching and H_2S removal. Conversely, elements present in industrial growth media, such as fluoride, can inhibit bacterial growth and iron bio-oxidation. In this work, the influence of fluoride on the kinetics of ferrous-iron bio-oxidation with *Sulfobacillus thermosulfidooxidans* was investigated. The effects of fluoride concentrations (0–0.5 mmol L^{-1}) on both iron oxidation and bacterial growth rates were assessed. In addition, the effect of the addition of aluminum, which was intended to complex free fluoride and reduce the concentration of HF through the formation of aluminum–fluoride complexes, was also investigated. The results show that 0.5 mmol L^{-1} NaF completely inhibited bacterial growth within 60 h. Nevertheless, fluoride toxicity to *S. thermosulfidooxidans* was minimized by control of the aluminum–fluoride ratio in the system because, at a 2:1 aluminum–fluoride molar ratio, bacterial growth was similar to that observed in the absence of fluoride ions. Despite a slower bacterial growth rate, fluoride concentrations less than the inhibitory concentration increased the Fe(II) oxidation rate. Successful copper bioleaching (80–100%) from fluoride-containing sulfide ores (1% total fluoride) was demonstrated in the presence of aluminum.

1. Introduction

Ferrous-iron bio-oxidation has many technological applications, including bioleaching [1], where ferric iron accounts for sulfide oxidation. Most Fe(II) bio-oxidation studies have been performed with mesophilic microorganisms such as *Acidithiobacillus ferrooxidans* [2] and *Leptospirillum ferriphilum* [3], although moderate thermophiles and extreme thermophiles can positively impact bioleaching because of faster sulfide oxidation at high temperatures. The electron pathway from ferrous-iron to oxygen has been proposed to include rusticyanin in *A. ferrooxidans* [4] and RuBP carboxylases in *Sulfobacillus* [5].

The growth medium can strongly influence the Fe(II) oxidation rate because industrial solutions may contain a plethora of dissolved elements. During heap bioleaching, for example, the gangue minerals can enrich the leach solution with elements that are harmful to the bacteria, thus impairing ferrous-iron oxidation kinetics [6]. Ojumu et al. [7] have shown the effect of increased ionic strength on mesophilic bacterial growth. In addition to reducing dissolved oxygen concentrations, a higher ionic strength reduces free water concentration, and the bacteria therefore lose water because of osmotic effects [8]. Anions affect Fe(II) and sulfur oxidation differently, but nitrate and chloride are the most important inhibitors of mesophilic bacterial growth [9]. Such species reduce the transmembrane potential and enable H^+ crossing of the cell membrane, thereby lowering the internal cell pH and impairing growth [10]. Another inhibition mechanism has been proposed for species such as HF, which are electrically neutral at the pH value where bioleaching occurs. A failure of a heap bioleaching operation due to the presence of fluoride ions on the ore has recently been reported [11], and this phenomenon was explained by the fluoride chemistry. At the low pH levels of bioleaching operations, fluoride ions are converted into HF, which, unlike F^-, can penetrate the cell membrane and dissociate into H^+ and F^-. The internal cell pH, which is neutral, is then lowered by H^+, whereas fluoride combines with some enzymes. The overall result is the inhibition of bacterial growth [8], and, therefore, the inhibition of ferrous-iron oxidation, irrespective of the bacterial strain (mesophiles, moderate thermophiles or extreme thermophiles) [12,13].

In bioleaching operations, inorganic weak acids such as HF may be present in the leaching liquor depending on the ore mineralogy. Because the processing of ores and concentrates that contain high contents of impurities are becoming commonplace, the industry is

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facing the challenge of dealing with such species, which are sometimes present in high concentrations [14]. Meanwhile, bioleaching by moderate thermophiles has been studied because sulfide oxidation is faster and because these microorganisms are found in heap leaching operations, where the heap temperature is high.

The effects of fluoride ions on the growth rate of bacteria relevant to bioleaching have not been extensively addressed. Fluoride effects are known to be overcome by the presence of aluminum. However, the effects of fluoride and aluminum on both bacterial growth and Fe(II) oxidation by *Sulfobacillus thermosulfidooxidans* have not yet been quantified. In addition, no consensus has been reached regarding the main aluminum–fluoride complexes formed during bioleaching of fluoride-containing ores. Whereas Dopson et al. [12] and Sundkvist et al. [13] have suggested AlF_2^+ as the main complex, Brierley and Kuhn [11] have proposed AlF^{2+} as the predominant species. Therefore, this paper was undertaken to assess the impact of fluoride ions on ferrous-iron oxidation by moderate thermophiles.

2. Materials and methods

2.1. Ferrous-iron bio-oxidation experiments

S. thermosulfidooxidans (strain DSMZ 9293) was grown in a medium composed of 0.4 g L^{-1} $(NH_4)_2SO_4$, 0.8 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.4 g L^{-1} K_2HPO_4, 2.5 g L^{-1} ferrous-iron ($FeSO_4{\cdot}7H_2O$) and 0.1 g L^{-1} yeast extract, at pH 1.5. The cells were maintained throughout the experiments in an orbital shaker (New Brunswick Scientific), at 50 °C and 200 min^{-1} and were used as the inocula for the bio-oxidation experiments when the potential reached the 580–600 mV (Ag/AgCl) range.

The bio-oxidation experiments (duplicate) were performed in a baffled bioreactor (New Brunswick Scientific, BioFlo 110) with 2 L of suspension that contained 10% (volume) of the inoculum. To produce the suspension, 200 mL of the inoculum (not previously adapted to either fluoride or aluminum) was transferred from the shaker to the bioreactor, and growth medium (supplemented with yeast extract) was added to produce a final solution volume of 2 L that contained between 5×10^6 and 5×10^7 cells mL^{-1}. The pH was manually adjusted to 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 buffer solutions was used for the pH measurements. The pH was controlled during the experiments by the addition of either concentrated sulfuric acid or sodium hydroxide solution. The temperature and the stirring rate were maintained at 50 °C and 300 min^{-1} (dual Rushton impeller, 5 cm diameter), respectively. This stirring rate was defined as the value that produced the highest ferrous-iron oxidation rate [15]. Aeration was provided by oil-free compressors at a rate of 1 L min^{-1}, and 5 mL samples were regularly withdrawn and analyzed for ferrous-iron concentration and cell counts. No additional CO_2 was added so that the yeast extract was the main carbon source.

In the bioreactor experiments, both bacterial growth and ferrous-iron oxidation were assessed in experiments where fluoride ions (NaF) were added. Fluoride concentrations were varied from 0 to 0.50 mol L^{-1} (0–10 mg L^{-1}), in the presence and absence of aluminum ($Al(OH)_3$) so that the following Al:F molar ratios were achieved: 0.0:0.13; 0.0:0.25; 1.0:0.50; 2.0:0.5 and 3.0:0.5, 3.0:0.0.

2.2. Bioleaching experiments

Copper sulfide bioleaching experiments were performed with two secondary ores. The first sample contained 0.99% copper (high-grade ore), and the second contained 0.73% copper (low-grade ore). Mineralogical analysis performed by optical microscopy and SEM–EDS indicated that the high-copper ore sample contained biotite (42.3%), magnetite (21.5%) and silicates, especially amphibole (18.9%) and garnet (6.9%). In addition, the low-copper ore contained approximately the same amount of biotite (34.9%) and amphibole (25.2%), less magnetite (9.5%) and more garnet (16.7%). The copper-containing minerals comprised bornite (36%) as well as chalcocite (64%) in the high-copper ore, whereas the low-copper ore contained 39% bornite, 55% chalcocite and 6% chalcopyrite. Both ores also contained 0.58–0.73% chloride and 0.53–0.75% fluoride as either fluorite (CaF_2) or fluoride-containing silicates. The iron and aluminum compositions were 27.8% Fe and 5.0% Al in the low-copper ore and 33.7% Fe and 3.9% Al in the high-copper sample.

The bioleaching potential of both ores was assessed in 250 mL Erlenmeyer flasks. A volume of 50 mL of the growth medium (supplemented with yeast extract) was adjusted to the required pH and transferred to the flasks. The amount of required Fe(II) was added as an acid solution that contained 50 g L^{-1} Fe(II) (as $FeSO_4{\cdot}7H_2O$). Afterwards, 5 g of the ore (corresponding to 5% (w/v) pulp density) were added, and the flasks were inoculated with a 10 mL aliquot of the bacteria that contained 1×10^7 cells mL^{-1}. Finally, sufficient distilled water was added to dilute the final slurry to a volume of 100 mL. The pH was subsequently adjusted to the required value (1.65), and the flask weight was recorded. Unless otherwise stated 350 mg L^{-1} and 200 mg L^{-1} Al (as aluminum sulfate) were added to the bioleaching tests with the high-and low-grade ores, respectively. A temperature-controlled orbital shaker (New Brunswick) provided mixing (at 200 min^{-1}). Each flask was sampled by the removal of a 2 mL aliquot of the leach solution, which was then used for elemental analysis. The redox potential (*Digimed*) (vs. Ag/AgCl reference) was recorded. Evaporation losses were compensated by the addition of the growth medium to the recorded weight. Sterile controls were also run in the presence of 0.015% (v/v) methylparaben–0.01% (v/v) propylparaben solutions as a bactericide.

2.3. Analysis

Cell counts were performed using a Neubauer chamber in a light-contrast microscope (Leica). Aluminum and fluoride were analyzed by ICP–OES and ion chromatography, respectively. Ferrous ion was titrated against a 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 performed using the Origin™ version 8.0 software program to determine the specific growth rate, the Fe(II) oxidation rate and 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) greater than 0.95.

3. Results and discussion

3.1. Ferrous-iron bio-oxidation

Both organic and inorganic weak-acid contaminants are well known to industrial microbiologists because they induce metabolic alterations that can result in complete inhibition of the microbial activity [16]. In the following paragraphs, the effects of fluoride ions on the bio-oxidation of Fe(II) by *S. thermosulfidooxidans* will be quantified. In this work, this inhibition was monitored through cell growth as well as through substrate consumption (Fe(II) bio-oxidation). Cell growth in batch conditions comprises at least four different phases: (i) lag, (ii) growth, (iii) stationary and (iv) death

[17]. In the lag-phase, growth is essentially zero because cells are adapting to the new environment, and new enzymes and structural components are being produced. After the lag-phase, the bacterial population starts to increase (growth phase); eventually, as nutrients become depleted or inhibitory products accumulate, the stationary phase is attained [17]. Fluoride ions have been shown to impair the growth of different bacterial strains [18], including mesophilic bioleaching microorganisms [19]. This result was also verified in the present work for the growth of the moderate thermophile *S. thermosulfidooxidans* in ferrous-iron. No bacterial growth was observed within 60 h during Fe(II) bio-oxidation experiments performed with 0.50 mmol L^{-1} (10 mg L^{-1}) total fluoride. This detrimental effect was observed because HF is a weak acid (pK_a = 3.2, at 25 °C and infinite dilution) that exists primarily as HF (98% of the fluoride-containing species) at the pH utilized in this study (1.5). HF is a highly permeant solute, with a permeability through lipid bilayer membranes that is seven orders of magnitude greater than that of F^- [20]. In acidophiles, although the external solution is acidic, the cytoplasmic pH is neutral because the cytoplasmic membrane, despite allowing the passage of ions and molecules to support metabolism, hinders protons from entering the cell. The entry of protons is reduced by an inverted transmembrane potential ($\Delta\Psi$), which contributes to the neutral cytoplasmic pH [21]. Small, uncharged molecules such as HF can cross the cell membrane. After entering the cell, HF dissociates into H^+ and F^-, which decreases the internal pH and affects microbial growth accordingly [8]. Growth is also impaired because fluoride itself can inhibit the activity of many enzymes [18].

The detrimental effect posed by fluoride on bacterial growth can be overcome by the addition of aluminum to the system [19], as depicted in Fig. 1. Fig. 1 shows no lag-phase in the experiment performed without either element (blank). However, growth was somewhat affected in the presence of 0.5 mmol L^{-1} fluoride and 2 mmol L^{-1} aluminum (Al/F = 4) and a lag-phase was observed. When the aluminum concentration was increased to 3 mmol L^{-1} at the same fluoride concentration (Al/F = 6), this lag-phase disappeared, which points to the detoxification effect of aluminum on fluoride toxicity [11,12].

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 at a constant growth rate, which characterizes the exponential growth phase, in which cell doubling will continue at the so-called specific growth rate (μ). This growth phase applies to closed systems where growth is the only process that affects cell concentration (X) [17]. A plot of $\ln X$ versus time gives a straight line with slope (μ) (Fig. 1).

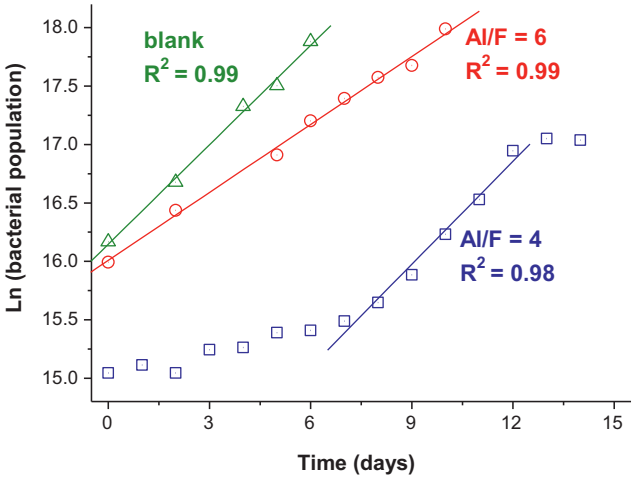

Fig. 1. Bacterial counts as a function of time for the growth of *S. thermosulfooxidans* on Fe(II) at different Al/F molar ratios. $[Fe^{2+}]_0$ = 2.5 g L^{-1}, 50 °C, 10% inoculum, pH 1.5, 300 min^{-1}, $[F]_{total}$ = 0.5 mmol L^{-1}. Blank experiment: $[Al]_t = [F]_t = 0$.

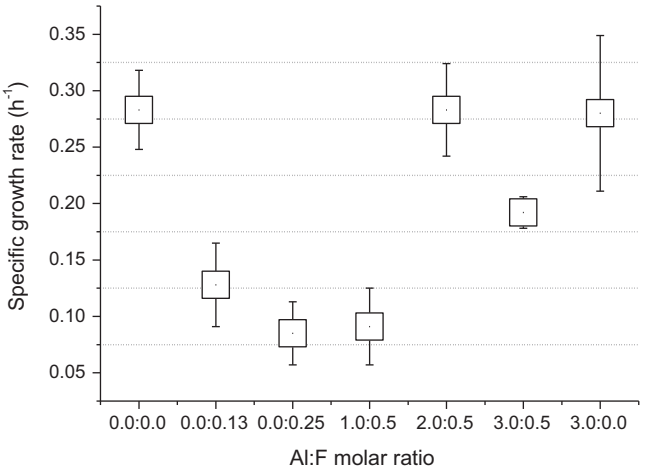

Fig. 2. Effect of fluoride and aluminum addition on the specific growth rate (μ) during Fe(II) oxidation by *S. thermosulfidooxidans*. Experimental conditions 2.5 g L^{-1} Fe^{2+}; 0.1 g L^{-1} yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50 °C.

The specific growth rate (μ) was determined from bacterial counts performed in the bioreactor (Fig. 2). The specific growth rate ($0.283 \pm 0.035\ h^{-1}$) calculated for the experiment performed in the absence of both fluoride and aluminum (blank) is consistent with previous studies on Fe(II) oxidation by *S. thermosulfidooxidans* ($0.220 \pm 0.025\ h^{-1}$) [15]. The maximum specific growth rate (μ_{max}) was determined for this bacterium grown in the presence of 2–20 g L^{-1} Fe(II) and the value of 0.242 h^{-1} was observed [15].

As shown in Fig. 2, the presence of fluoride ions reduced the specific growth rate. At a total fluoride concentration of 0.125 mmol L^{-1}, μ was reduced to $0.128 \pm 0.037\ h^{-1}$, i.e., less than half the value observed in the absence of the anion. The specific growth rate was further decreased to $0.085 \pm 0.028\ h^{-1}$ for higher fluoride concentrations (0.25 mmol L^{-1}), which reflects the inhibitory effect of HF on bacterial growth [8]. When aluminum was also added to the bioreactor, its detoxification effect became evident. As already stated, no growth was observed in the presence of 0.5 mmol L^{-1} (10 mg L^{-1}) total fluoride. However, when 1.0 mmol L^{-1} Al was added to this fluoride concentration (Al/F = 2), growth was detected, and a specific growth-rate value of $0.091 \pm 0.034\ h^{-1}$ was measured. Fluoride inhibition was not completely overcome at this aluminum concentration because the specific growth rate was statistically similar to those achieved in the absence of aluminum and at lower fluoride concentrations (0.125 mmol L^{-1}–0.25 mmol L^{-1}). A value similar to that produced in the absence of fluoride was achieved when the Al:F molar ratio was increased to 4 (2.0 mmol L^{-1} Al – 0.50 mmol L^{-1} F) (Fig. 2). A further increase in the Al/F molar ratio to 6 (3.0 mmol L^{-1} Al – 0.50 mmol L^{-1} F) resulted in a smaller growth rate than when only 3.0 mmol L^{-1} aluminum was present. This parameter is again statistically similar to that produced when none of the elements were present. The anomalous growth rate observed at Al/F = 6 may be due to the predominance of uncharged aluminum fluoride species (AlF_3), but this result requires further investigation.

The positive effect of aluminum on bacterial growth in the presence of fluoride can be explained by aluminum–fluoride complex formation Eq. (1–4), which produces species that cannot cross the bacterial cell membranes.

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

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

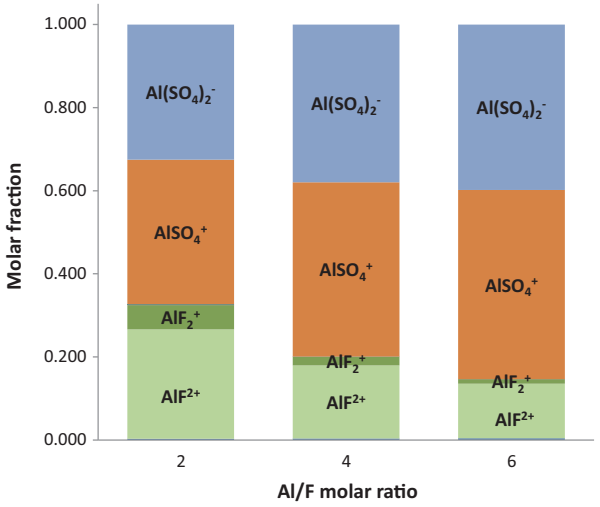

Fig. 3. Estimated aluminum speciation (molar fraction) in the presence of both sulfate and fluoride ions. Conditions: 25 °C, pH 1.50, infinite dilution (I → 0). Total concentrations: 0.5×10^{-3} mol L^{-1} (fluoride); 76.5×10^{-3} mol L^{-1} (sulfate); 44.8×10^{-3} mol L^{-1} (ferrous-iron). Al^{3+}, AlF_3 and AlF_4 together represents less than 0.5% of the aluminum species and therefore do not appear in the diagram.

$$Al^{3+} + 3\,F^- \leftrightarrows AlF_{3(aq)} \quad \log \beta_3 = 16.7 \quad (25\,^\circ C, I \rightarrow 0) \tag{3}$$

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

The composition of the bio-oxidation solution (aluminum, iron, fluoride and sulfate) was used in a thermodynamic study performed to estimate the distribution of soluble aluminum–fluoride species in the system. Fig. 3 presents the distribution of aluminum complexes for the three different Al/F molar ratios studied in the present work (at pH 1.50 and with 2.5 g L^{-1} Fe(II)) at 25 °C and infinite dilution, with data obtained from both the NIST database [22] and the results of Gimeno Serrano et al. [23]. Values of stability constants for the temperature and ionic strength of the moderate thermophile leaching could not be found. Therefore, actual values are somewhat different, but it is believed, however, that the main findings can be applied to the experimental conditions studied here. This analysis covers the beginning of the experiments when Fe(II) was the main iron species, i.e., ferric iron concentrations were too low to affect aluminum–fluoride speciation or to form jarosite. For all the Al/F molar ratios studied, the calculations indicate AlF^{2+} as the predominant Al/F complex, followed by $AlF_2{}^+$ (Fig. 3); these results are consistent with the work of Brierley and Kuhn [11], who also indicated AlF^{2+} as the main aluminum–fluoride complex during mesophilic bioleaching of secondary copper ores. Nevertheless, AlF^{2+} and $AlF_2{}^+$ represent more than 97% of the aluminum–fluoride complexes, and both species likely predominate at 50 °C.

Because sulfate was also present in the reactor, Fig. 3 also suggests that the complexes $AlSO_4{}^+$ and $Al(SO_4)^-$, which represent between 67% (Al/F = 2) and 85% (Al/F = 6) of the aluminum species, are also important. This result is consistent with previous findings [13]. Furthermore, the HF concentration was decreased to less than 6×10^{-5} mol L^{-1}, which represents only 12% of the fluoride-containing species (unlike the 98% observed in the absence of aluminum). The decreased HF concentration positively affected the bacterial growth. In summary, fluoride complexation with aluminum reduces the HF concentration and prevents fluoride from extensively entering the microbial cell.

S. thermosulfidooxidans utilizes Fe(II) as a substrate for growth [24]. Fig. 4 presents the Fe(II) profile in the experiments performed in the presence of fluoride and aluminum. In the absence of aluminum (Fig. 4a), increased fluoride concentrations resulted in longer delays for the start of Fe(II) oxidation. This time span

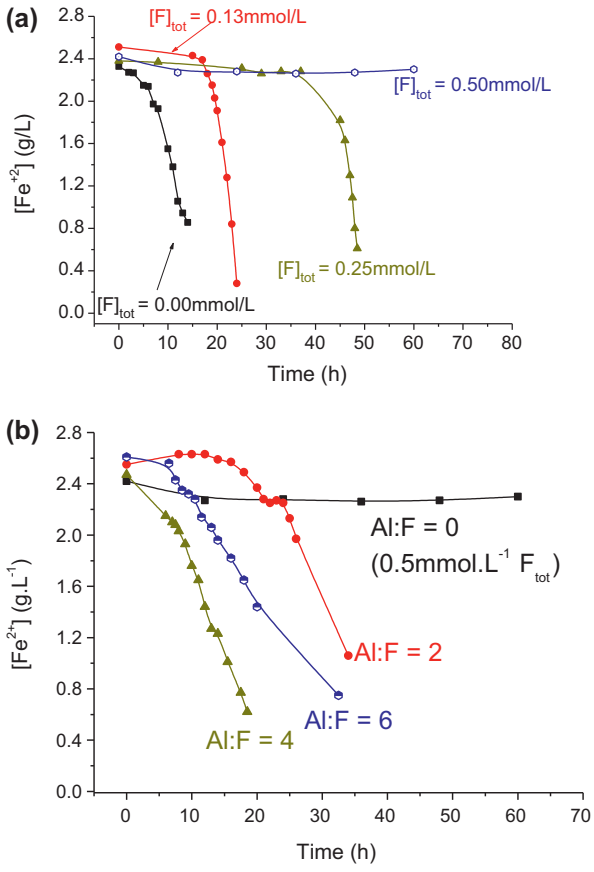

Fig. 4. Effect of fluoride (a) and Al:F molar ratio (b) on Fe(II) oxidation by *S. thermosulfidooxidans*. Experimental conditions 2.5 g L^{-1} Fe^{2+}; 0.1 g L^{-1} yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50 °C. In (b), fluoride concentration was set at 0.50 mmol L^{-1}.

matches the lag-phase period (data not shown), during which growth was not expressive and therefore no substrate consumption was observed. At 0.5 mmol L^{-1} fluoride, no bacterial growth was observed nor was Fe(II) oxidation detected. As previously discussed, at a fluoride concentration of 0.5 mmol L^{-1}, the addition of aluminum enabled bacterial growth (Fig. 1) and substrate consumption (Fig. 4b); the Fe(II) concentration was consequently reduced with time.

The yield (Y) can be determined from the change in biomass concentration (X) and substrate consumption (S) [25] and the results are shown Table 1. Higher yield values imply better substrate utilization by the bacteria. In all the experiments, the yield was within the same order of magnitude (10^{10} cells g^{-1}-Fe(II)), irrespective of the Al:F molar ratio. Nevertheless, the yield was lower when only fluoride was present, which implies that enhanced metabolic activity is required to sustain growth [16]. For *Saccharomyces cerevisiae*, the energy required to activate the plasma-membrane ATPases,

Table 1
Effects aluminum and fluoride additions on the yield coefficient (Y) during Fe(II) oxidation by *S. thermosulfidooxidans*. Experimental conditions 2.5 g L^{-1} Fe^{2+}; 0.1 g L^{-1} yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50 °C.

Aluminum (mmol L^{-1})	Fluoride (mmol L^{-1})	Yield (10^{10} cells g^{-1} Fe(II))
0.0	0.00	5.30 ± 1.00
0.0	0.13	2.83 ± 0.12
0.0	0.25	3.06 ± 0.74
1.0	0.50	–
2.0	0.50	3.62 ± 0.75
3.0	0.50	5.03 ± 0.65
3.0	0.00	4.57 ± 1.10

Table 2
Effects aluminum and fluoride additions on the Fe(II) bio-oxidation rate. Experimental conditions 2.5 g L^{-1} Fe^{2+}; 0.1 g L^{-1} yeast extract; Norris growth medium, pH 1.5; 300 min^{-1} and 50 °C.

Aluminum (mmol L^{-1})	Fluoride (mmol L^{-1})	Fe^{2+} oxidation rate (g L^{-1} h^{-1})
0.0	0.00	0.179 ± 0.019
0.0	0.13	0.323 ± 0.047
0.0	0.25	0.426 ± 0.042
1.0	0.50	0.118 ± 0.009
2.0	0.50	0.128 ± 0.006
3.0	0.50	0.146 ± 0.014
3.0	0.00	0.140 ± 0.009

which pump protons out of the cell, has been shown to result in an increase in the respiration rate with a decrease in cell growth and thus cell yield [26]. Slightly higher yield values were observed in the experiments with aluminum—a consequence of its positive effect on bacterial growth (Fig. 2). These results are consistent with those observed during ferrous-iron oxidation with *A. brierley* in two different studies. Konishi et al. [27] determined an yield value of 2.05×10^{10} cell g^{-1} in the presence of 2.0 g L^{-1} Fe(II), whereas Nemati and Harrison [28] achieved 5.38×10^{10} cell g^{-1} with 1.8 g L^{-1} Fe(II).

The ferrous-iron consumption rate (d[Fe(II)]/dt) is equivalent in absolute terms to the Fe(II) oxidation rate, and the latter was determined from the slope of the linear part of the ferrous-iron concentration profile shown in Fig. 4a and b. This approach was selected because the first-order kinetics model proposed by Franzmann [29] did not produce good fits to the experimental data. The calculated values are shown in Table 2. The ferrous-iron oxidation rate was determined as 0.179 ± 0.019 g L^{-1} h^{-1} in the absence of both aluminum and fluoride ions (blank); this value is lower than that observed by Pina et al. [15], who determined an oxidation rate of 0.292 ± 0.034 g L^{-1} h^{-1} in a similar experiment. However, this latter value is consistent with that reported by Watling et al. [30], who investigated growth in 10 g L^{-1} Fe(II) (~0.12 g L^{-1} h^{-1}). It is also consistent with the growth of *A. ferrooxidans* (0.14 g L^{-1} h^{-1}) in the presence of 2.5 g L^{-1} Fe(II) [28], and, as expected, higher than the value observed for *A. brierleyi* (−0.053 g L^{-1} h^{-1}) in 1.8 g L^{-1} Fe(II) [28].

Two additional important outcomes can be discerned in Table 2. First, aluminum can overcome the detrimental effect posed by fluoride ions during Fe(II) oxidation by *S. thermosulfidooxidans*, as already stated in the discussion that covered the bacterial growth rate. However, in the experiments where the cation is present, Fe(II) oxidation rates are slightly lower than those observed in the absence of both elements (blank). Similar findings have not been observed for this strain, and inhibitory effects have been reported only for higher aluminum concentrations and other microorganisms. Blight and Ralph [31] have observed a reduction in cell numbers and duplication time during ferrous-iron bio-oxidation at aluminum concentrations greater than 2.7 g L^{-1} for an unidentified mesophilic culture, whereas Ojumu et al. [7] observed, during Fe(II) oxidation by *L. ferriphilum*, deleterious effects on Fe(II) oxidation and bacterial growth only at high aluminum concentrations (10 g L^{-1}).

Among the results in Table 2, the effect of low fluoride concentrations on the Fe(II) oxidation rates are also noteworthy. Although the presence of fluoride induced a longer lag-phase during bacterial growth (Fig. 1), iron oxidation was faster in the presence of fluoride (Al:F ratios of 0.0:0.13 and 0.0:0.25) as soon as the exponential phase began. Iron oxidation reached a rate of 0.426 ± 0.042 g L^{-1} h^{-1} in the presence of 0.25 mmol L^{-1} fluoride, whereas the rate in the blank experiment was 0.179 ± 0.019 g L^{-1} h^{-1}). A possible explanation for this behavior is the need to activate cell metabolism as a resistance mechanism to the presence of low concentrations of fluoride [16], for which the growth rate is decreased and the length of the lag-phase is extended. It is therefore proposed that the decrease in cell internal pH (caused by HF diffusion) forces the system to pump protons out (increased ATPase activity) to balance the diffusion of the HF molecules into the cell. Overall, the energy requirements are increased, which result in an increased substrate consumption rate without an increase in biomass yield, as observed in other studies [32].

Because fluoride toxicity can be overcome by the presence of aluminum during Fe(II) bio-oxidation by *S. thermosulfidooxidans*, bioleaching experiments were performed with two copper ores that contained fluoride in their gangue minerals [14].

3.2. Bioleaching experiments

Two different secondary copper sulfide ores that comprise mainly chalcocite, bornite and chalcopyrite as a minor phase were bioleached with *S. thermosulfidooxidans* at 50 °C in the presence of 1 g L^{-1} Fe(II) to ensure a fast increase in solution potential. An external ferrous-iron addition was later found not to be required because iron dissolution from the ores provided enough substrate for bacterial growth [14]. Both samples contained more than 90% cyanide-soluble copper [14], i.e., copper that is easily amenable to bioleaching [33]. Because of the observed rapid ferrous-iron oxidation by the bacterium (Fig. 4), a sharp increase in the solution potential was expected during these experiments. Nevertheless, although there was significant copper extraction in both the biotic and abiotic systems, ferric- and ferrous-iron concentrations were similar to those achieved in the control experiment (data not shown), and pulp potential values were never higher than 450 mV (Ag/AgCl), as shown in Fig. 5. Similar results have been reported during bioleaching of a chalcopyrite ore that contained fluoride [6]. These results should be compared with, for example, those observed during nickel sulfide bioleaching with the same strain, where potentials as high as 600 mV (Ag/AgCl) were observed [24]. Therefore, some harmful substance might have been impairing bioleaching. Fig. 5 also shows different copper extractions from the high-grade ore for both the biotic (100%) and abiotic (75%) experiments. This behavior was also observed with the low-grade ore, but with slightly lower yields (80% and 60% for the biotic and abiotic experiments, respectively), which might be due to the presence of chalcopyrite in the low-grade ore [14].

Both copper sulfides contained between 0.53% and 1% fluoride, part of which was fluorite. The gangue is also believed to contain silicate minerals such as chlorite [34] that contains fluoride because the total fluorite content in both ore samples was 1.0% maximum. These mineral phases have some solubility in acid media [34], which resulted in concentrations as high as 270 mg L^{-1} (high-grade ore) and 152 mg L^{-1} F^- (low-grade ore) in the bioleaching medium [14]. Similarly, aluminum dissolution from the high-grade ore produced concentrations of approximately 210 mg L^{-1}, whereas its concentration in solution was in the range 280–310 mg L^{-1} during bioleaching of the low-grade ore. Therefore, based on analyses of the Fe(II) bio-oxidation experiments (Section 1), aluminum dissolution from the ore is inferred as not being sufficient to overcome the detrimental effects of fluoride on *S. thermosulfidooxidans* growth. It is hypothesized that fluoride released by the ore slowed (but did not inhibit) ferrous-iron bio-oxidation because copper extraction was higher in the inoculated experiment relative to that in the control (Fig. 5a and b). Under these conditions, any ferric iron produced would be quickly reduced by the copper sulfides; consequently, no increase in the solution potential would be observed. This observation is supported by the findings of Brierley and Kuhn [11], who showed that copper leaching from chalcocite was less than 50% in absence of bacterial activity.

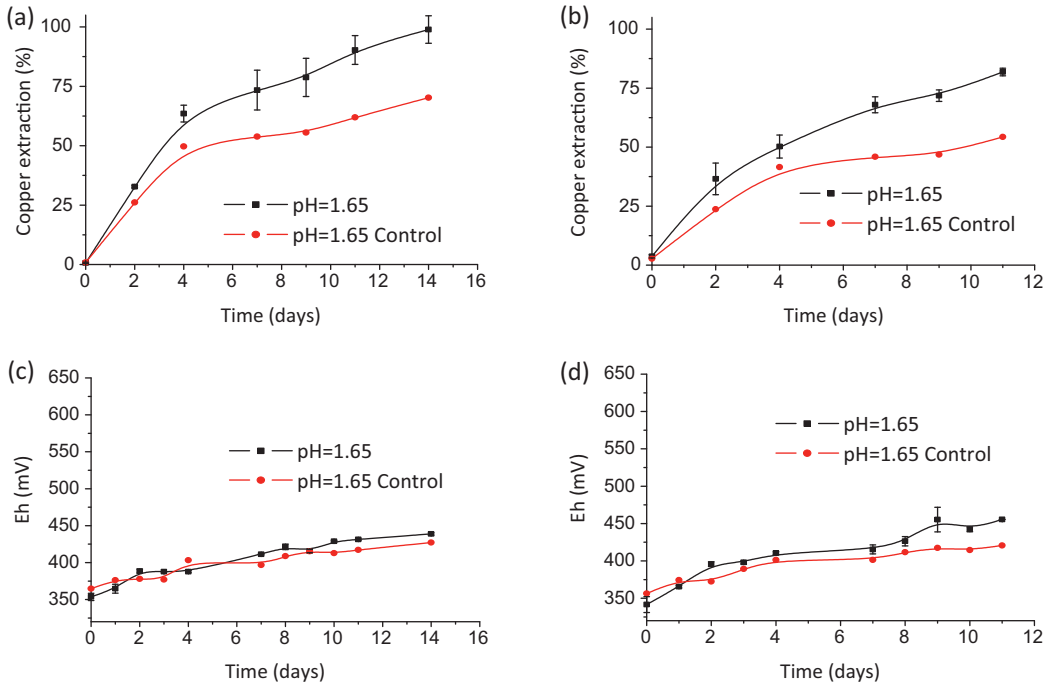

Fig. 5. Copper extraction (a) and (b) and solution potential (Ag/AgCl) (c) and (d) during the experiments with the high-grade (a) and (c) and low-grade (b) and (d) copper ores in the abscence of added aluminum ions. Experimental conditions: 5% solids; 75–53 μm; pH 1.65; 1 g L^{-1} Fe^{2+}; 200 min^{-1}, 10% Norris (v/v), 0.1 g L^{-1} yeat extract and 50 °C.

As previously shown, aluminum can overcome the detrimental effects of fluoride on Fe(II) oxidation, although the amount of aluminum dissolved from the ore did not ensure suitable conditions for bacterial growth. Therefore, a new series of experiments were performed in the presence of an external source of aluminum, i.e., 350 mg L^{-1} and 200 mg L^{-1} Al were added to the bioleaching tests with the high- and low-grade ores, respectively. This external source of Al ensured an Al/F molar ratio of at least 1.5 during bioleaching, which was shown to enable bacterial growth on ferrous-iron (Section 1) and to increase the solution potential to

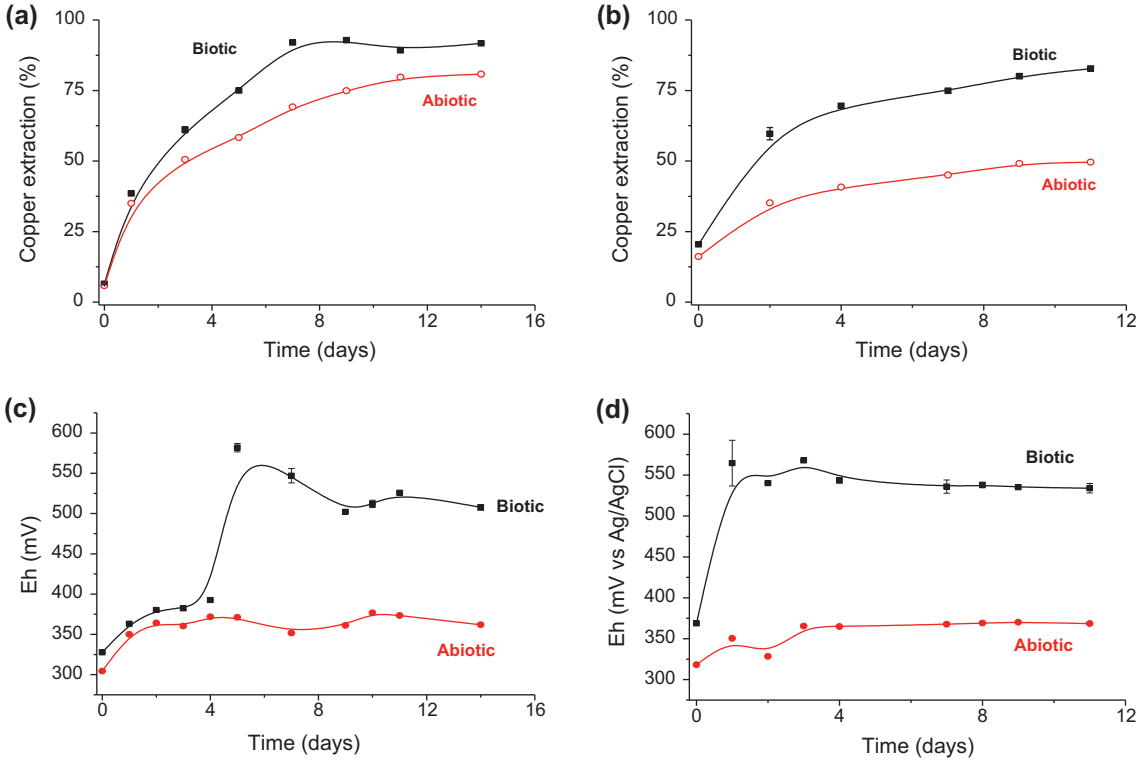

Fig. 6. Copper extraction (a and b) and solution potential (vs. Ag/AgCl) (c and d) during the experiments with the high-grade (a and c) and low-grade (b and d) copper ores in the presence of added aluminium ions. Experimental conditions: 1% solids; 75–53 μm; pH 1.65; 1 g L^{-1} Fe^{2+}; 200 mg L^{-1} Al^{3+} (low-grade ore) or 350 mg L^{-1} (high-grade ore) de Al^{3+}; 200 min^{-1} and 50 °C.

600 mV [14]. Fig. 6 depicts the values achieved for copper extractions and the solution potentials for both ores. In the presence of aluminum, the potential levels out in the 500–550 mV (Ag/AgCl) range within five days. These potentials are approximately 150 mV higher than the value observed in the control experiments (Fig. 6c and d), which confirms the predictions of Sundkvist et al. [13]. Therefore, bacterial activity was confirmed. Although final copper extractions were similar in the experiments without (Fig. 5) and with (Fig. 6) the external addition of aluminum, copper extraction was faster in the latter case. For example, for the low-grade ore, copper extractions were 70% and 50% at the fourth day of leaching in the presence and absence of aluminum, respectively.

The results show a clear increase in the lag-phase period when fluoride species are present during ferrous-iron oxidation by *S. thermosulfidooxidans*. Because the production of ferric iron is the main bioleaching mechanism, the onset of metal extraction became excessively longer than expected or even did not occur [11]. Notwithstanding, sub-lethal fluoride concentrations can double the ferrous-iron oxidation kinetics, which will result in faster sulfide oxidation. Unlike the Fe(II) oxidation, bioleaching can be performed at much higher fluoride concentrations if the ore contains elements such as aluminum that can complex free fluoride and reduce the HF concentration in the leaching liquor. If the presence of fluoride-containing minerals is detected, extra care must be taken during bioleaching, especially when the leaching solution is recirculated, such as in bio-heap-leaching operations [19]. Although fluoride toxicity can be reduced by the presence of aluminum, the ferrous-iron oxidation rate by *S. thermosulfidooxidans* is slightly decreased in the presence of the cation. Therefore, the build-up of both elements can lead to high ionic-strength values, which can also affect bioleaching. Under these conditions, solution bleeding would be required to reduce the fluoride toxicity as well as the ionic strength so that bioleaching can be performed properly.

4. Conclusions

At the pH levels typically found in bioleaching operations, fluoride ions can adversely affect the growth of *S. thermosulfidooxidans* because of the predominance of HF species in solution. The bacterial specific growth rate was decreased from $0.283 \pm 0.035\ h^{-1}$ in experiments without fluoride to $0.085 \pm 0.028\ h^{-1}$ when $0.25\ mmol\ L^{-1}$ total fluoride was present. Such detrimental effects can be overcome by the presence of aluminum ($1\ mmol\ L^{-1}$ Al – $0.5\ mmol\ L^{-1}$ F) which forms AlF^{2+} complexes that reduce the HF concentration and the fluoride toxicity accordingly. This reduction in toxicity results in specific growth-rate values similar to those observed in the absence of both aluminum and fluoride. Despite the increase in the lag-phase period, sub-lethal fluoride concentrations catalyze ferrous-iron oxidation, which reaches $0.426 \pm 0.042\ g\ L^{-1}\ h^{-1}$ with $0.25\ mmol\ L^{-1}$ total fluoride. The positive effect of aluminum on the bioleaching of copper sulfide ores that contain fluoride was demonstrated, and at least 80% copper extraction was achieved. Overall, for those ores that contain fluoride-containing minerals, bioleaching can be performed if aluminum sources are present or added to the bioleaching system. In heap bioleaching applications, the build-up of aluminum and fluoride can lead to failures due to high-ionic-strength constraints, and regular solution bleedings may be required.

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