Cooperative action of attached and planktonic cells during bioleaching of chalcopyrite with *Sulfolobus metallicus* at 70 °C

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The catalytic influence of *Sulfolobus metallicus* in the bioleaching of chalcopyrite at 70 °C and pH 1.5 was studied in shake flask experiments. Leaching was conducted in an iron-free basal medium with a −80 #+120 # chalcopyrite sample, and was characterized from monitoring in solution the pH, Eh, copper and iron concentration, and cell population. In order to evaluate separately the influence of planktonic and attached cells on the catalytic process, in some experiments the microorganisms were prevented from reaching the chalcopyrite surface by keeping them in a chamber separated by a 0.1 Millipore membrane. In a complementary experimental series, the concentration of different sulfur species dissolved in the solution was determined with HPLC at different conditions, under nitrogen or air, abiotic or inoculated with *S. metallicus*.

Maximum copper dissolution was reached when at least one fraction of the inoculated microorganisms were able to approach chalcopyrite surface. In this case, notably, the fraction of the population of microorganisms that were prevented from reaching chalcopyrite was still observed to increase. In experiments where all the inoculated microorganisms were prevented from reaching chalcopyrite, the cell population did not grow. In this case copper dissolution was only 50% of the amount reached when full bacterial contact was allowed, and was similar to the amount of copper dissolved at aerated–abiotic conditions. HPLC results on the analysis of dissolved sulfur species showed that when microorganisms were able to reach chalcopyrite there was accumulation in solution of thiosulfate $(S_2O_3)^{-2}$ and sulfite $(SO_3)^{-2}$, in addition to the formation of bisulfite $(HSO_3)^{-}$, bisulfate $(HSO_4)^{-}$ and sulfate $(SO_4)^{-2}$ which was also observed in aerobic–abiotic conditions. It could be concluded that in bioleaching of chalcopyrite in the presence of *S. metallicus* there is a cooperative action between attached cells which can oxidize sulfur-containing surface layers on chalcopyrite, forming thiosulfate, sulfite and bisulfite, and planktonic cells which further oxidize these intermediate compound to bisulfate and sulfate. Removal of surface passivating layers under the catalytic action of attached microorganisms is a key catalytic factor as it greatly enhances the oxidative action of ferric iron on chalcopyrite.

1. Introduction

Chalcopyrite can be dissolved at convenient rates in the presence of thermophilic microorganisms such as *Sulfolobus metallicus*, *Acidianus brierley* or *Metallosphera sedula* (Norris et al., 1986; Clark and Norris, 1996). The use of *S. metallicus*, a chemolitothrophic archaea that can grow autotrophically at temperatures between 65 and 80 °C, has been largely investigated in the bioleaching of chalcopyrite (d'Hugues et al., 2001; Blázquez et al., 1999; Muñoz et al., 1998; Rodríguez et al., 2003; Stott et al., 2003).

In the bioleaching of chalcopyrite with *S. metallicus* there is an efficient process of oxidation of residual sulfur compounds, i.e. sulfur and polysulfides, formed during the chemical dissolution of the sulfide (Escobar et al., 2003; Marsh et al., 1983; Shivvers and Brock, 1973). This oxidative process contributes to a dramatic increase in copper dissolution rate with respect to that obtained in chalcopyrite dissolution under simple indirect bacterial action (Jordan et al., 2006). However, SEM/EDS observations on chalcopyrite grains during its bioleaching with *S. metallicus* have shown that oxidation of sulfur compounds is not solely linked to the action of attached microorganisms. In fact, very efficient degree of oxidation of residual surface sulfur compounds has been observed even in situations where the degree of attachment of these microorganisms on the sulfide was very low (Davis, 2005).

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The aim of the present work is to study in more detail the catalytic role of *S. metallicus* during bioleaching of chalcopyrite in an iron-free basal medium, evaluating separately the contribution of attached and planktonic cells to the different oxidative reactions involved in the process.

2. Materials and methods

2.1. Mineral

Leaching experiments were conducted using a −80 #+120 # granulometric fraction (150 μm average particle diameter) of a sample of chalcopyrite (93% chalcopyrite, 7% pyrite) prepared from a chalcopyrite massive sample obtained from the Codelco/Andina mine, Chile. X-ray diffractogram of chalcopyrite present in this sample coincided well with tetragonal chalcopyrite (JCPDS: 37-0471).

2.2. Bacterial strain and growth conditions

The microorganism was a pure strain of *S. metallicus* grown on particulate chalcopyrite contained in Norris basal medium ($MgSO_4 \cdot 7H_2O$: 0.5 g/l, $(NH_4)_2SO_4$: 0.4 g/l, KH_2PO_4: 0.2 g/l) at pH 1.5 and 70 °C. The inoculum prepared for the leaching experiments was separated by filtration in a 0.1 μm Millipore and washed several times with 2 g/l sulfuric acid solution to secure optimum removal of remaining iron.

2.3. Experimental procedure

Leaching experiments were conducted in 250 ml Erlenmeyer flasks containing 100 ml of iron-free basal medium at pH=1.5 and 1 g of chalcopyrite, incubated on an orbital shaker at 125 rpm and 70 °C. Some shake flasks were modified by introducing a central cylindrical compartment with one end sealed with a 0.1 μm Millipore filter (see Fig. 1). This compartment, compartment A, contained 40 ml of solution and its Millipore filter end was immersed in compartment B which contained 120 ml of solution. Chalcopyrite particles were contained in compartment B and microorganisms inoculated in compartment A participated in the solution chemistry of the process, but were prevented from reaching chalcopyrite surface during the leaching process.

In one experimental series, series A, four experiments of chalcopyrite leaching in iron-free basal medium were conducted in duplicate in the following conditions: experiment (a) in aerated conditions, with *S. metallicus* inoculated both in compartment A (3×10^8 cells/ml) and in compartment B (9×10^7 cells/ml), where the chalcopyrite particles were present; experiment (b) in aerated conditions, with *S. metallicus* inoculated only in compartment A (1×10^8 cells/ml). In addition, two blank experiments were conducted in standard shake flasks under abiotic conditions: experiment (c) in abiotic–aerated conditions, and experiment (d) in abiotic conditions under nitrogen. During each experiment solution samples were taken from the flasks at different time intervals, pH was measured and then copper and iron concentrations were determined by atomic absorption spectrophotometry. Sulfate ion concentration was determined by precipitation with barium sulfate (ASTM D 516-82). The number of free cells in the solution in both compartments was determined by direct microscope counting using a Petroff-Hausser chamber.

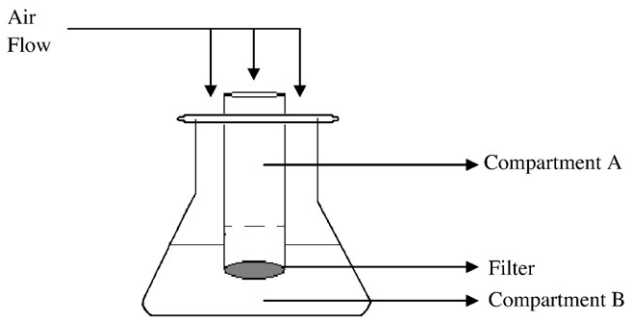

Fig. 1. Scheme of the modified shake flask used in inoculated experiments in series A. Chalcopyrite was in compartment B; microorganisms in compartment A could not contact chalcopyrite.

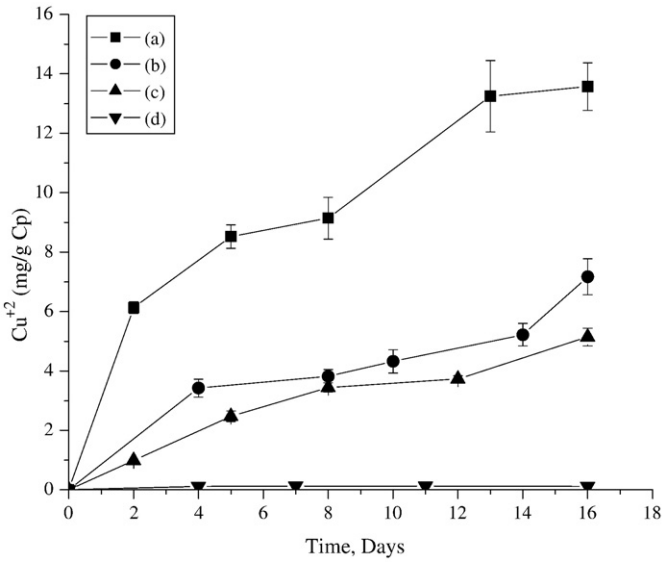

Fig. 2. Copper dissolution during chalcopyrite leaching. Series A: (a) microorganisms inoculated in compartments A and B; (b) microorganisms only inoculated in compartment A, (c) abiotic–aerated conditions; (d) abiotic conditions under nitrogen.

In a complementary experimental series, series B, three similar experiments of chalcopyrite leaching in iron-free basal medium were conducted in duplicate, using standard shake flasks: experiment (a) in aerated conditions with basal medium inoculated with *S. metallicus* (4×10^8 cells/ml); experiment (b) in abiotic conditions under air; experiment (c) in abiotic conditions under N_2 atmosphere. Experiments in this series, which reproduced experimental conditions used in series A, provided complementary information on Eh evolution and

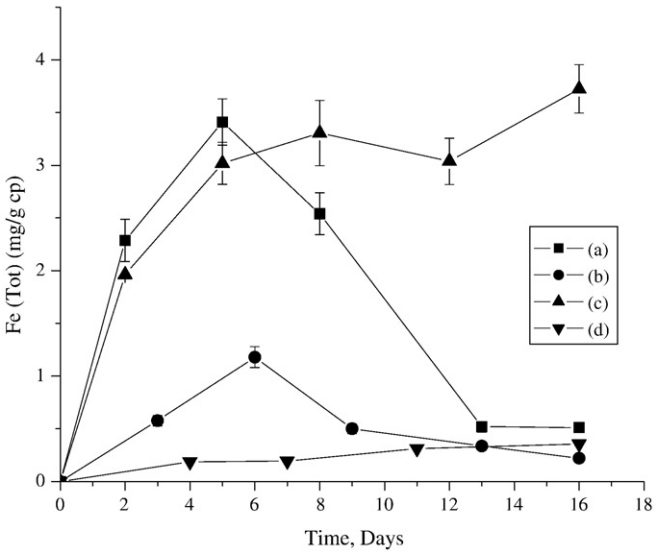

Fig. 3. Total iron dissolution during leaching of chalcopyrite. Series A: (a) microorganisms inoculated in both compartments; (b) microorganisms only inoculated in compartment A, (c) abiotic–aerated conditions; (d) abiotic conditions under nitrogen.

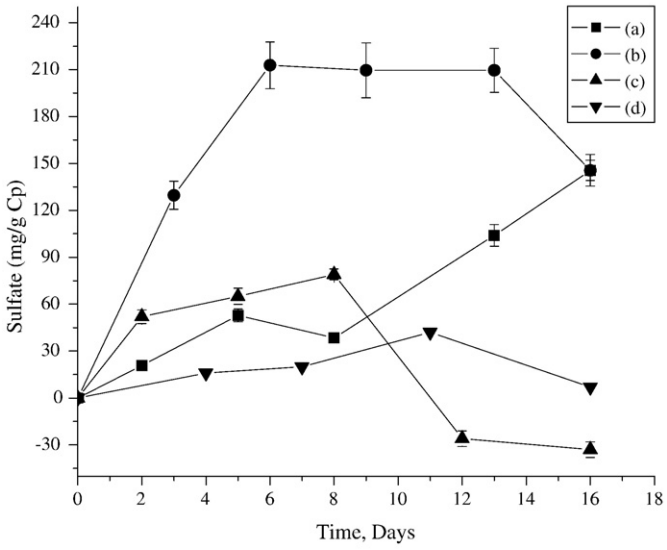

Fig. 4. Concentration of sulfate during chalcopyrite leaching. Series A: (a) microorganisms inoculated in both compartments; (b) microorganisms only inoculated in compartment A, (c) abiotic–aerated conditions; (d) abiotic conditions under nitrogen.

formation of intermediate sulfur soluble compounds during chalcopyrite leaching. Concentration in solution of soluble sulfur species formed by oxidation of residual sulfur compounds was determined with high-pressure liquid chromatography following the procedure described by Schippers et al. (1996), but using an ICSep ANSC Trangenomic column and a 4 mM $NaHCO_3$, 1.5 mM Na_2CO_3, pH=9.5 solution as eluent.

3. Results and discussion

3.1. Experimental series A

Results of experimental series A on copper dissolution, iron dissolution, sulfate and microorganisms population are shown in Figs. 2–5, respectively. Results in Fig. 2 show that maximum copper dissolution was reached in experiment (a) where at least one fraction of the inoculated microorganisms were able to approach chalcopyrite surface. In experiment (b), in which the microorganisms were inoculated solely in compartment A and could not reach chalcopyrite surface, copper dissolution was reduced to about 50% of that obtained in experiment (a). It is important to outline that total copper dissolved in experiment (b) was just slightly higher than the one obtained in experiment (c) conducted under abiotic–aerobic conditions. This implies that, as microorganisms were prevented from reaching chalcopyrite surface and the concentration of iron in the solution was very low, copper dissolution was still mainly the result of the chemical action of dissolved oxygen on chalcopyrite.

Results in Fig. 3 show that in chalcopyrite leaching under abiotic–aerobic conditions, experiment (c), iron dissolved at a similar rate as copper, consistent with their stoichiometric ratio in chalcopyrite. However, in experiments (a) and (b), both inoculated with *S. metallicus*, iron dissolution rate was well below their respective rate of copper dissolution. In addition, in these two experiments iron in the solution reached a maximum at about 5 days and then started to decay.

These results can be explained by the different iron oxidation states that predominated in abiotic and inoculated experiments, evidenced from monitoring solution Eh during chalcopyrite leaching. In abiotic conditions, either in aerated conditions or under nitrogen, Eh values stabilized in the range 600–620 mV/SHE, indicating that about 99.7% of dissolved iron was present as Fe^{+2}. On the other hand, in the presence of *S. metallicus*, Eh values rapidly stabilized in the range 780–800 mV/SHE, which indicated that about 74% of dissolved iron was present as Fe^{+3}. Therefore, in the abiotic situation, as ferrous ion predominated, all the dissolved iron remained in the solution and the 1:1 iron/copper dissolution ratio could be experimentally observed. However, in the presence of *S. metallicus* dissolved iron was rapidly converted to ferric ion which at the temperature of the experiment could readily precipitate as jarosites (Stott et al., 2001). In this situation, the expected 1:1 iron–copper dissolution ratio could not be observed as only one fraction of the iron dissolved from chalcopyrite remained in the solution. The maximum dissolved iron concentration reached in both inoculated experiments was the result of two competitive processes, iron dissolution and iron precipitation, which occurred simultaneously but with varying rates during the experiment. The time when this maximum was reached indicated the moment when the rate of iron precipitation became faster than the rate of iron removal from chalcopyrite. Naturally, the amount of dissolved iron reached at this maximum was larger in experiment (a), in which the rate of chalcopyrite dissolution was higher.

Fig. 4 shows sulfate formation during chalcopyrite leaching. Formation of sulfate in the presence of *S. metallicus* was much faster than in abiotic–aerobic experiment, even when the microorganisms

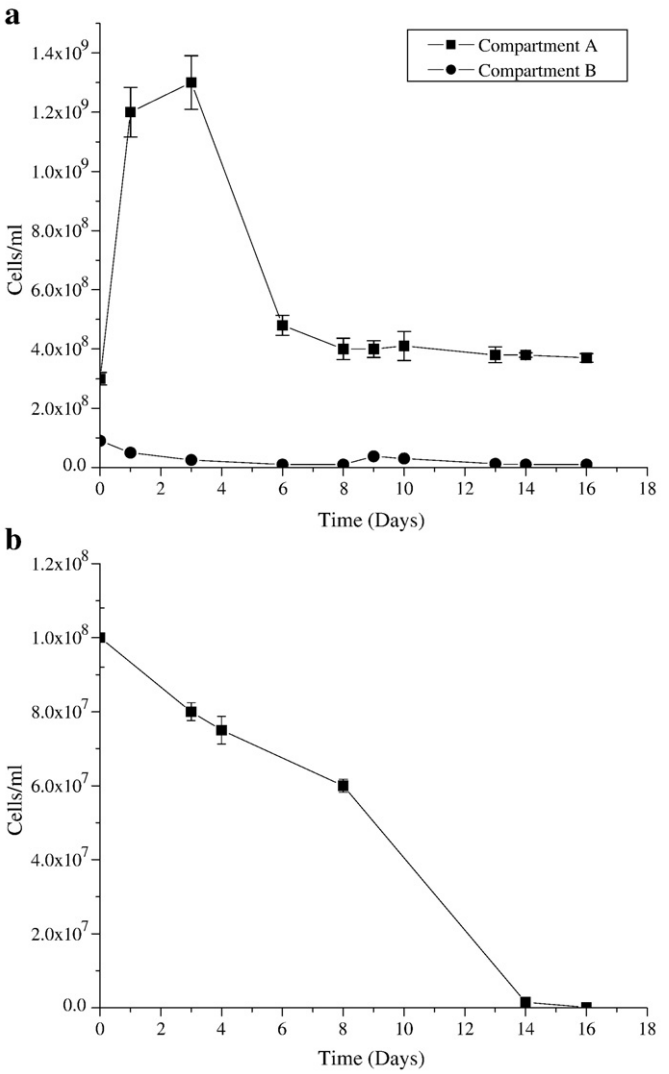

Fig. 5. Cell numbers of *Sulfolobus metallicus* during bioleaching of chalcopyrite. Series A: (5-a) microorganism inoculated in both compartments; (5-b) microorganisms only inoculated in compartment A.

were prevented from reaching chalcopyrite surface. Sulfate formation was paralleled by solution acidification evidenced in the decrease of the pH which lowered up to 1.35 (data no shown).

Fig. 5 shows results of monitoring the number of planktonic cells of *S. metallicus* during chalcopyrite leaching in both inoculated experiments. In experiment (a) (Fig. 5-a) the initial population of microorganisms in compartment B decreased since the start of leaching, indicating that most of the inoculated microorganisms (9×10^7 cells/ml) rapidly attached to chalcopyrite and remained on the sulfide during the whole experiment. Rapid attachment of *S. metallicus* on chalcopyrite has been also observed by other authors (Rodríguez et al., 2003; Escobar et al., 2003). On the contrary, the fraction of microorganisms maintained far from chalcopyrite in compartment A showed a well defined population increase during the first 4 days of leaching, and then rapidly decayed to a stable value close to the initial population. In experiment (b) (Fig. 5-b), in which none of the microorganisms present in the flask could reach chalcopyrite, the initial population of microorganisms inoculated in compartment A steadily decayed during the whole experiment.

Planktonic cell numbers determined in compartment A in experiment (a) reached up to 1.3×10^9 cells/ml during the first 4 days of leaching, concentration substantially higher than the concentration initially inoculated in that compartment, 3×10^8 cells/ml. The possibility that this increase in cell numbers is just the result of detachment of microorganisms growing in compartment B on chalcopyrite can be ruled out because, apart from the fact that both compartments were separated by a firmly placed 0.1 μm Millipore filter, planktonic population in compartment B remained very low during the whole experiment. Therefore, this increase is a clear evidence that the planktonic population of *S. metallicus* present in compartment A was growing out of the oxidation of some soluble species produced as intermediate compounds during chalcopyrite dissolution. These compounds, presumably dissolved ferrous iron and/or intermediate soluble sulfur compounds, were apparently produced in sufficient amount only when at least a fraction of the microorganisms could approach the sulfide surface.

The increase of planktonic cells observed in experiment (a) can be in principle analyzed in terms of the possible growth of these microorganisms out of the oxidation of dissolved ferrous ion produced during chalcopyrite dissolution. One can estimate the maximum population of microorganisms that could have grown assuming the extreme situation in which chalcopyrite is solely dissolved by ferric leaching and all the required ferric ion had been generated by the microorganisms. Assuming that ferric leaching of chalcopyrite occurred according to the stoichiometry: $4Fe^{+3}+CuFeS_2\rightarrow5Fe^{+2}+Cu^{+2}+2S^0$, the Fe^{+3} required to produce the amount of copper experimentally obtained, 14 mg (see curve a in Fig. 2), can be estimated as 49.3 mg. Using the value 1.13×10^6 cells/μg Fe^{+2} for the yield of the oxidation of ferrous ion with *S. metallicus* (Escobar and Vargas, unpublished results), the population of microorganisms grown out of the oxidation of 49.3 mg of Fe^{+2} can be estimated as 5.6×10^{10} cells. In the 160 ml of solution used in this experiment this means a concentration of 3.5×10^8 cell/ml. This result demonstrates that the oxidation of ferrous iron by *S. metallicus* cannot give account on its own for the population increase observed in compartment A, which reached 1.3×10^9 cells/ml. This implies that this population increase also involved the growth of planktonic cells out of the oxidation of intermediate soluble sulfur compounds.

3.2. Experimental series B

A complementary experimental series was conducted, series B, in which sulfur intermediate compounds were monitored during chalcopyrite leaching. Results of copper dissolution in these experiments (data not included) confirmed the trends observed in series A: maximum copper recovery was reached in experiment (a) where inoculated *S. metallicus* was in contact with chalcopyrite. However,

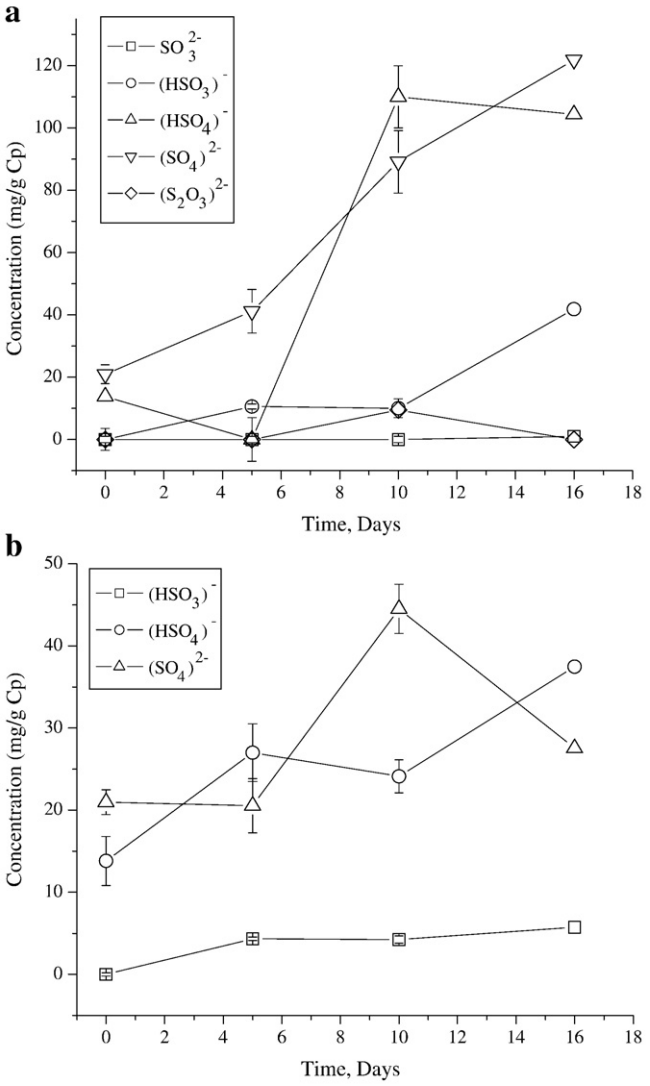

Fig. 6. Concentration of sulfur species in the solution: (6-a) aerated basal medium inoculated with *Sulfolobus metallicus*; (6-b) abiotic–aerated conditions.

copper dissolution was also important in the aerated abiotic case, experiment (b), where dissolved copper reached 40% of the inoculated case. Finally, without oxygen, experiment (c), copper dissolution reached only 4% of experiment (a).

A summary of the different intermediate soluble sulfur compounds detected under the three different experimental conditions is the following. When chalcopyrite was leached in abiotic conditions under nitrogen, experiment (c), only the species SO_4^{-2} and $(HSO_4)^-$ were present, in which sulfur is present as S^{+6}. These species were present in the initial basal medium, introduced by the addition of sulfuric acid, and their concentration did not increase during the process. When chalcopyrite was leached in aerated–abiotic conditions, experiment (b), the species $(HSO_3)^-$ was additionally detected, in which sulfur is present in the S^{+4} state. Finally, when chalcopyrite was leached in the presence of *S. metallicus*, apart from the previously mentioned species, the species $(SO_3)^{-2}$ and $(S_2O_3)^{-2}$ were additionally observed, in which sulfur is present as S^{+4} and S^{+2}, respectively.

Fig. 6 summarizes the results of the evolution of the concentration of the various sulfur species in experiments (a) and (b) in this series, under inoculated and abiotic–aerated conditions, respectively. It can be observed that in experiment (a) there was formation of 100 mg of SO_4^{-2} and 80 mg of $(HSO_4)^-$ while in experiment (b) there was formation of 10 mg SO_4^{-2} and

25 mg $(HSO_4)^-$. In other words, the presence of *S. metallicus* contributed to the formation of 180−35=145 mg of S^{+6} species, about 4 times the amount formed in the abiotic system. On the other hand, in experiment (a) there was an accumulation of 40 mg $(HSO_3)^-$ while in experiment (b) there was an accumulation of 5 mg $(HSO_3)^-$. Therefore, the presence of *S. metallicus* contributed to an accumulation of 40−5=35 mg of S^{+4} species, about 7 times their rate of accumulation in the abiotic system.

Following the scheme proposed by other authors for the oxidation of elemental sulfur by attached and planktonic *Acidithiobacillus ferrooxidans* (Shrihari et al., 1993), the oxidation of sulfur species in the bioleaching of chalcopyrite under the catalytic action of *S. metallicus* can be assumed to occur according to the following reaction scheme:

$$S^{-2}, S^{-1}, S^0(\text{in chalcopyrite surface}) + O_2 \rightarrow S^{+4}(\text{solution}) \quad (1)$$

$$S^{+4}(\text{solution}) + O_2 \rightarrow S^{+6}(\text{solution}) \quad (2)$$

Accordingly, the net rate of formation of the intermediate S^{+4} species,$(HSO_3)^-$, can be calculated adding the mass accumulated of this species (35 mg), to the amount consumed (145 mg), which corresponds to the formed mass of S^{+6} species, $SO_4^{=}/(HSO_4)^-$. Therefore, it can be concluded that in the presence of attached *S. metallicus* the rate of formation of intermediate S^{+4} species is 180 mg, about 24% faster than the rate of formation of S^{+6} species. This behaviour is presumably related to the fact that most of the inoculated microorganisms readily attached from the start to the sulfide and remained in close contact with the layer of reduced sulfur species formed on chalcopyrite surface, also previously observed by other authors (Rodríguez et al., 2003; Escobar et al., 2003).

The relatively faster rate of reaction (1) with respect to reaction (2) results in an accumulation of the intermediate S^{+2}/S^{+4} species in the solution, which enables the planktonic microorganisms to grow out of the oxidation of these species to sulfate even when they are prevented from approaching chalcopyrite. This mechanism could be proposed to explain the growth of planktonic microorganisms observed in compartment A in experiment (a) (see Fig. 5-a). However, the growth of these planktonic microorganisms was most significant at the start of the experiment. This indicates that initially most of the attached bacteria were catalyzing reaction (1) and the accumulation of S^{+2}/S^{+4} species is more significant. Later in the experiment, when the population of microorganisms in compartment B increased with leaching time, most of the produced S^{+2} and S^{+4} species could be also oxidized by microorganisms in the proximity of chalcopyrite, and the accumulation of these species in the solution was detained. This could explain why the growth of the microorganisms in compartment A eventually decayed with leaching time.

3.3. Role of attached and planktonic microorganisms in chalcopyrite dissolution

Data of copper dissolution in series A, experiments (a), (b) and (c), were analyzed according to the unreacted core kinetic model (Levenspiel, 1972) to determine the type of mechanism that controlled the kinetics of copper dissolution. For the three experiments the best fitting was found for the mechanism of diffusion control, the case in which copper conversion, X, is related to leaching time, t, by the equation $t/\tau = 1-3(1-X)^{2/3}+2(1-X)$ (see Fig. 7). τ is a parameter that represents the leaching time necessary to reach complete dissolution of copper contained in chalcopyrite. Results in Fig. 7 indicate that in the three experimental conditions the kinetics of copper dissolution is controlled by diffusion phenomena in the passivating layer formed during chalcopyrite leaching (Baur et al., 1974; Munoz et al., 1979; Jordan et al., 2006). With these antecedents it is now possible to assess in more detail the role of attached and planktonic microorganisms in the reported experiments.

In the leaching of chalcopyrite in iron-free basal medium at 70 °C under abiotic–aerated conditions, the presence of oxygen triggers dissolution of chalcopyrite, which shows as an increase in copper and iron concentration with respect to the one obtained under nitrogen (see Figs. 2 and 3). The presence of oxygen in these conditions also triggers the oxidation of sulfide ions present in chalcopyrite, which shows as a significant increase in sulfate concentration (Fig. 4) and the accumulation of $(HSO_3)^-$ (Fig. 6-b). However, at abiotic conditions the presence of oxygen is not sufficient to oxidize dissolved iron which accumulates in the solution as ferrous ion. One can then conclude that copper dissolution at these conditions is still mainly the result of chemical action of oxygen on chalcopyrite. Accordingly, results in Fig. 7 for this experiment can be interpreted in terms of copper dissolution kinetics controlled by oxygen diffusion through the formed passivating layer.

When *S. metallicus* was inoculated only in compartment A the main influence of their presence was that dissolved iron was oxidized to ferric ion, as shown from the sharp increase in the solution Eh. Therefore, the slight increase in the copper dissolution rate with respect to the aerated abiotic case (curve b, Fig. 2) can be mainly related to the shift from oxygen to ferric ion as the main leaching agent. The kinetics of copper dissolution still remains controlled by diffusion as the microorganisms cannot approach the sulfide to start dissolving the surface passivating layer. One can conclude saying that if none of the microorganisms can approach the sulfide and the passivating layer is not dissolved, ferric generation by the indirect action of *S. metallicus* does not contribute too efficiently to enhance chalcopyrite dissolution rate.

Finally, when at least one fraction of the inoculated *S. metallicus* could reach the chalcopyrite surface, a significant increase in copper dissolution rate was observed (curve a, Fig. 2). On the other hand, results in Fig. 7 for this experiment show that the rate of copper dissolution was still controlled by diffusion of ferric ion trough the passivating layer. However, the observed slope increase in Fig. 7 shows that the value of $1/\tau$ increases 7 times with respect to experiment (b), which is far more than the influence expected from the 3 fold increase in the maximum ferric iron concentration with respect to experiment (b). Therefore, the high increase in $1/\tau$ is indicating that the passivating layer formed on chalcopyrite is starting to be altered by the oxidative action of the microorganisms inoculated in compartment B, most of which remained attached to its surface during the whole experiment. Evidence of this catalytic action can be also seen from the increase of the rate of formation of sulfur intermediate compounds such as $(S_2O_3)^{-2}$ and $(HSO_3)^-/(SO_3)^{-2}$ in experiments with attached microorganisms (see Fig. 6-a). This result then confirms that

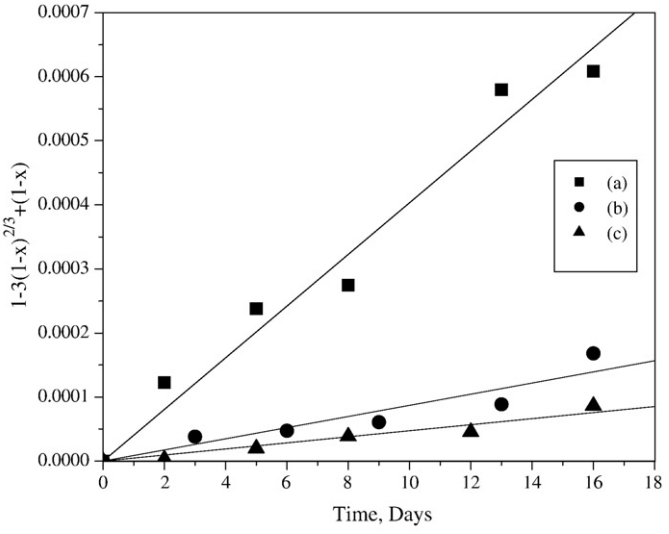

Fig. 7. Testing of copper dissolution data from experimental series A with the unreacted core model, case of control by diffusion in the passive layer. (a) microorganisms inoculated in compartments A and B (R^2: 0.97); (b) microorganisms only inoculated in compartment A (R^2:0.88), (c) abiotic–aerated conditions (R^2: 0.94).

when the presence of attached microorganisms triggered the dissolution of the passivating layer, ferric ion becomes a very active leaching agent for chalcopyrite dissolution (Jordan et al., 2006). Therefore, one can conclude saying that generation of ferric ion by the indirect action of *S. metallicus* becomes a very efficient mechanism for chalcopyrite dissolution only if a fraction of the inoculated microorganisms are allowed to approach the chalcopyrite surface.

4. Conclusions

- In bioleaching of chalcopyrite with *S. metallicus* at 70 °C in an iron-free basal medium a significant fraction of the copper and iron dissolved and the sulfate formed was the result of direct chemical action of dissolved oxygen.
- The presence of *S. metallicus* resulted in a significant increase in the rate of copper and iron dissolution and in the rate of sulfur solubilization with respect to the abiotic–aerated conditions only when a fraction of the microorganisms were attached on chalcopyrite.
- The population of *S. metallicus* attached to chalcopyrite catalyzed preferentially the oxidation of surface sulfur compounds to S^{+2}/S^{+4} soluble species (thiosulfite, bisulfite and sulfite). This oxidative activity is apparently linked to the dissolution of surface passivating layers on chalcopyrite and has a key catalytic influence on the rate of copper dissolution during chalcopyrite leaching.
- Planktonic population of *S. metallicus* which cannot approach chalcopyrite can grow out of the oxidation of S^{+2}/S^{+4} soluble species accumulated in the solution, which are produced by microorganisms attached to chalcopyrite, and ferrous ion released from the sulfide.

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