Hydrometallurgy 106 (2011) 19–25

Contents lists available at ScienceDirect

Hydrometallurgy

journal homepage: www.elsevier.com/locate/hydromet

The influence of two thermophilic consortia on troilite (FeS) dissolution

M.A. Giaveno [a,*], G. Pettinari [a], E. González Toril [b], A. Aguilera [b], M.S. Urbieta [c], E. Donati [c]

[a] Facultad de Ingeniería, Universidad Nacional del Comahue, IDEPA – CONICET, Buenos Aires 1400, (8400) Neuquén, Argentina
[b] Centro de Astrobiología, Instituto Nacional de Técnica Aeroespacial, Carretera de Ajalvir Km 4, (28850) Torrejón de Ardoz, Madrid, Spain
[c] CINDEFI (CONICET LA PLATA, UNLP), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, (1900) La Plata, Argentina

ARTICLE INFO

Article history:
Received 17 August 2010
Received in revised form 15 November 2010
Accepted 15 November 2010
Available online 1 December 2010

Keywords:
Thermophiles
Troilite
Dissolution
Consortia

ABSTRACT

Dissolution of a natural troilite by thermophilic consortia collected from two hot springs placed in Copahue geothermal region (Neuquen — Argentina) and later enriched in specific media for sulphur-oxidisers is reported in this paper. The enrichment was carried out at a temperature (65 °C) far away from those measured in the original hot springs (40.5 °C and 87 °C) in order to analyse the flexibility of the consortia to keep viability under other temperature conditions. Different microscopic techniques (SEM, TEM, fluorescence microscopy) allowed the partial characterisation of the cultures used as inocula in the bioleaching experiments. Results show that, as other metal sulphides, troilite dissolution can be strongly catalysed by sulphur (and iron) wild oxidising microorganisms present in the consortia from Copahue hot springs. According to our results, the addition of sulphur increased the bioleaching rate although the troilite dissolution is not limited by such addition because sulphur is in situ generated by chemical oxidation. Iron solubilised from troilite was partially precipitated mainly as jarosite. An additional and interesting result of our studies indicates that natural consortia can have a wide thermal flexibility and there are some strains among them – especially archaeas from *Sulfolobales* genus – that are able to survive at temperatures far away from the ones registered in the place where they were collected.

1. Introduction

Troilite is stoichiometric FeS, without vacancies in its crystalline–hexagonal-structure and consequently is non magnetic compared with magnetic-monoclinic or hexagonal-iron sulphides belonging to pyrrhotite group which are iron-deficient (Rochette et al., 2010). Moreover, troilite can be found as a native mineral in the Earth usually associated to other iron sulphides like pyrrhotite, pyrite, marcasite, arsenopyrite and mackinawite, whose oxidation is the main source for the production of acid mine drainage (AMD) (Sracek et al., 2004). AMD is surely the most serious environmental problem provoked by the metallic mining activity (Akcil and Koldas, 2006). In addition, dissolution of troilite and mackinawite has been associated to arsenic mobilisation (Jeong et al., 2010). Thus, studies about its solubilisation and that of other sulphides can be relevant in relation to problems of water and soil pollution.

During the production of AMD, the chemical oxidation of sulphides is highly enhanced by the activity of acidophilic microorganisms. According to the physicochemical conditions different species can play a predominant role; under mesophilic conditions, genus like *Acidithiobacillus* and *Leptospirillum* seem to be dominant (Johnson and Hallberg, 2005). Some species belonging to those genus are able to oxidise iron generating ferric iron which can dissolve sulphides and they are usually used in bioleaching processes (Falco et al., 2003). In addition all *Acidithiobacillus* species catalyse the oxidation of sulphur generating sulphuric acid which is enough to dissolve some of those sulphides (Rohwerder and Sand, 2007; Giaveno and Donati, 2001). Similar reactions occur in geothermal environments where pools, hot-springs and fumaroles are characterised by low pH values. These habitats are colonised by thermophilic microorganisms including many iron and sulphur oxidisers.

Although the anoxic dissolution of troilite is dependent on the proton concentration – indicating its capability of dissolving in acid medium – (Chiriţ and Descostes, 2006a), its aerobic oxidation also implies the oxidation of sulphur in the surface layers (Chiriţ and Descostes, 2006b). Even the presence of sulphur into the crystal structure has been mentioned (Thomas et al., 2003). Thus, sulphur oxidising microorganisms are perfectly able to dissolve troilite probably through direct oxidation of sulphur present into the structure or mainly through the production of sulphuric acid.

The geothermal Copahue–Caviahue (GCC) system is a volcanic area located at latitude 37°50′S and longitude 7 l°05′W mainly in the north-west of Neuquén Province in Argentina. This geothermal field is on the east side of Los Andes, in the ridge, which forms the watershed separating the river basins of the Pacific and Atlantic sides. The overall area comprises approximately 20 km^2 and rises to about 2000 m above sea level. In the GCC Field, there are five active geothermal manifestations, which mainly consist of fumaroles, hot springs and

* Corresponding author. Fax: +54 299 4490385.
E-mail address: agiaveno@hotmail.com (M.A. Giaveno).

0304-386X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.hydromet.2010.11.015

mud pots. Four of these manifestations are located in Argentina: Las Maquinas, Termas de Copahue, Las Maquinitas and El Anfiteatro, and the fifth on the Chilean side, Chancho Co (Mas et al., 1996). Many microorganisms have been isolated and characterised from these hot springs (Chiacchiarini et al., 2010); also some of them were utilised in bioleaching processes (Lavalle et al., 2008; Chiacchiarini et al., 2007). Two of the five active geothermal manifestations in GCC, Baño 9 (B9) belong to Termas de Copahue and Las Maquinitas (LM), were selected for the studies described in this paper.

Analysing the influence of those extremophilic microbial communities – not previously described – on sulphides like troilite allows getting significant information about the community behaviour; in addition it can be relevant for understanding AMD processes. That is why the aim of this study is to evaluate the dissolution of a natural troilite by thermophilic consortia collected from two hot springs placed into Copahue geothermal region and later enriched into specific media for sulphur-oxidisers. This enrichment was carried out at a temperature far away from those present in the hot springs in order to analyse the flexibility of the consortia to keep viability under other temperature conditions.

2. Materials and methods

2.1. Sample collection

Two sites in Copahue geotermal region, Neuquén, Argentina, were selected for water sample collection, Baño 9 (B9) and Las Maquinitas (LM). Samples were taken in December of 2009. Both sites are hydrothermal pools; which present some anthropogenic influence. Probably, it is higher in the first case where also some animals live there. Physicochemical parameters were measured in situ. Temperature and pH values for Baño 9 and Las Maquinitas were 40.5 °C and 2.7 and 87.0 °C and 2.0, respectively. Water samples were collected in one litre sterile plastic jars and kept on ice until further processing. As soon as possible samples were filtered through 0.22 μm Millipore membranes and stored at −20 °C until cellular DNA extraction.

2.2. Culture enrichment

Environmental samples were cultivated in M88 medium (Brock et al., 1972), recommended by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, German Collection of Microorganisms and Cell Cultures) for favouring thermophilic microorganisms growth. Medium was prepared as a 10× stock solution as follows (per litre): $(NH_4)_2SO_4$ 13.0 g, KH_2PO_4 2.8 g, $MgSO_4 \cdot 7H_2O$ 2.5 g, $CaCl_2 \cdot 2H_2O$ 0.7 g, $FeCl_3 \cdot 6H_2O$ 0.2 g, $MnCl_2 \cdot 4H_2O$ 18.0 mg, $Na_2B_4O_7 \cdot 10H_2O$ 45.0 mg, $ZnSO_4 \cdot 7H_2O$ 2.2 mg, $CuCl_2 \cdot 2H_2O$ 0.5 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.1 mg, $VOSO_4 \cdot 2H_2O$ 0.1 mg, $CoSO_4$ 0.1 mg, and yeast extract (Difco) 1.00 g. pH value was adjusted to 2 with H_2SO_4 10 N. All medium components, except yeast extract, were autoclaved for 20 min at 1 atmosphere overpressure. Yeast extract was prepared and autoclaved separately and added to the medium when dilution was made. Cultures were routinely re-cultivated in that medium supplemented with sulphur or sucrose for enhancing the growth of sulphur oxidisers and/or heterotrophic microorganisms – tolerant to those conditions – which could contribute to the stability of the consortia. Sulphur was added at a final concentration of 5 g/l (previously it was sterilised by steaming for 45 min three successive times). Sucrose was added at a final concentration of 1.0 g/l, prior autoclaving it for 20 min at 121 °C. The selected temperature condition was 65 °C, lower than that measured in Las Maquinitas and higher than that detected in Baño 9 in order not to be the optimal for the original consortia but intermediate between both. Even at such temperature, natural samples showed a significant growth in M88 medium supplemented with sulphur; however, when sucrose was supplemented growth was just detected in Baño 9 sample. After cultivating several times in the same medium it was assumed that stable consortia had been achieved. Such consortia were used in the bioleaching experiments (see below). Cells suspended in medium or attached to different surfaces were used to analyse morphological characteristics using a LEO EVO 40 XVP scanning electron microscope (SEM). Microorganisms ultra structure was studied by transmission electron microscopy (TEM) using a JEOL 100 CXII microscope.

2.3. Sample fixation, FISH and CARD FISH

Fluorescence in situ hybridisation (FISH) is a powerful technique to detect, quantify and identify microorganisms. Probes – belonging to a specific order, genus or species – marked with a fluorescent terminal dye recognise a specific sequence in the nucleic acids within cells giving colour which can be detected in an epifluorescent microscope. CARD-FISH is a modification of that technique which allows the amplification of FISH signal. 4′,6′-diamidino-2-phenylindole (DAPI) is non-specific DNA staining and it shows all cells in the sample. Through FISH (or CARD-FISH) plus DAPI analysis, it is possible to calculate the percentage of certain cells into a sample (Amann, 1995; Pernthaler et al., 2002).

Water samples were fixed for FISH during sampling collection. Approximately 500 μl of water sample were incubated with the corresponding volume of paraformaldehide to reach a 4% final concentration. Incubation times were between 1 and 12 h. Fixed samples were diluted in approximately 15 ml of sterile pH 2 water and filter through GTTP 025 Millipore filter (0.22 μm) using a filtration column. Filters were washed and neutralised with 20 ml of PBS buffer (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.2) and air dried. Fixed samples were stored at −20 °C until hybridisation reaction. Hybridisation and DAPI staining were performed as described previously (Amann, 1995). Cy-3 labelled EUB 338 and ARCH 915 probes for FISH were provided by Bonsai Technology (Barcelona, Spain). An Axioskop microscope (Zeiss, Germany) equipped with the proper filter set was used to visualise the FISH and CARD FISH hybridisations. Cell counting was carried out as described by Kepner and Pratt (1994). For CARD FISH hybridisation protocol reported by Pernthaler et al. (2002) was used with the following modifications: no overnight treatment with active diethyl pyrocarbonate was done, as the samples did not have high endogenous peroxidase activity. For further permeabilisation filters were treated with achromopeptidase (0.6 U/m final concentration; buffer contained 0.01 M NaCl, 0.01 M Tris–HCl. pH 8.0; incubation al 37º for 30 min) then washed for 1 min with ultrapure water. Peroxidases were inhibited by treating the filters with 20% methanol 0.015% H_2O_2 solution for 30 min at room temperature. Hibridisation was done at 46 °C for 2 h. EUB 338 and ARCH 915 probes were used at 35 and 20% of formamide respectively.

2.4. Mineral and solid residues characterization

The mineral troilite was milled up to a particle size less than 74 μm. Quantitative chemical composition was obtained by X-ray fluorescence spectrometry (XRF) using a Shimadzu energy dispersive X-ray fluorescence spectrometer EDX-800HS. Fluorescence X-ray chemical characterisation showed the following percentages for major and minor components: Fe_2O_3 (51.3%), SO_3 (48.2%), CuO (0.25%), ZnO (0.12%), MnO (0.10%), Cr_2O_3 (0.05%), Mn_2O_3 (0.04%), and NiO (0.04%). The analysis of these data shows Fe/S atomic relation of 1.067 close to that expected for troilite.

X-ray diffraction (XRD) analysis was performed using a Rigaku DII-Max, CuKα equipment with a Ni filter. Diagrams were run from 10° to 70°2θ, by steps $0.05° s^{-1}$. It was possible to identify troilite (FeS) as the main component and marcasite (FeS_2) as its main impurity. The electron scanning micrograph of milled mineral showed flat-sided crystals and aggregates of smaller size of cubic habit like troilite.

The solid residues from the bioleaching tests were recovered by centrifugation and washed with distilled water. After being air-dried, the samples were ground an agate mortar. X-ray diffraction (XRD) analyses of a top fill powder mounts were conducted using CuKα radiation. All specimens were scanned from 10° to 70°2θ, by steps 0.05° s^{-1}.

Selective dissolution of the most poorly crystalline ferric iron compounds formed during troilite degradation was accomplished by using acid ammonium oxalate (AO) solution (Schulze, 1994). In this procedure 0.5 g of residue was mixed with 20 ml of 0.2 M of AO solution (pH = 3) and shaken vigorously for 15 min in the dark at 22 °C. The solids were recovered by centrifugation, air-dried and analysed by XRD. A differential XRD (DXRD) pattern was then obtained by subtracting the post dissolution pattern from that of the original sample. The resulting DXRD pattern could then be used to characterise the material solubilised by AO solution. In a similar way, a more vigorous dissolution of the solid residues products was attempted by using 5 M HCl solution following the same procedure to obtain the XRD and the DXRD patterns (Bhatti et al., 1993). Air-dried bioleaching residues were observed under a Philips JEOL 515 scanning electron microscope.

2.5. Bioleaching experiments

Troilite was sterilised as described for sulphur and added at a final concentration of 4%. Different inocula from the consortia (growth on sulphur or on sucrose) were added to M88 medium (pH = 2) in shake flasks. Inocula description can be found in Table 1. The flasks were incubated at 150 rpm and 65 °C for 70 days. As it was indicated above, such temperature was chosen in order not to be the optimal for the original consortia but intermediate between both. During this period, pH and Eh were monitored using a platinum electrode against Ag/AgCl (4 M KCl) reference electrode. Total soluble iron was determined by atomic absorption spectrophotometry and the percentage of solubilisation was calculated regard to the amount of iron present in the initial troilite. An abiotic control (where inoculum was replaced by fresh medium) was used for evaluating chemical leaching. The experiments were carried out in duplicate.

3. Results and discussion

FISH analysis of Baño 9 original sample using general bacteria and archaea domain probes showed that a high percentage of the cells stained with DAPI were also hybridised with archaeal general probe ARCH 915. Practically no bacteria were detected when hybridising with bacteria domain probe EUB 338. For Las Maquinitas original sample it was impossible to use FISH stain due to the great amount of autofluorescent material present in the sample. To avoid this problem CARD-FISH stain was used. Results were very similar to those for B9, most of the prokaryotes in the sample were archaea while very few cells hybridised with EUB 338 probe. Even though CARD FISH helped to improve hybridisation resolution, DAPI stain images were very difficult to analyse because of the sample background complexity, and that made it very inaccurate to calculate hybridisation percentages.

Table 1
Inocula used in bioleaching experiments.

Culture Name	B9 + S	B9 + Su	LM + S
Sample source	Baño 9	Baño 9	Las Maquinitas
Growing medium	M88 + sulphur	M88 + sucrose	M88 + sulphur
Bacteria/mL	$2{,}5 \times 10^8$	$3{,}5 \times 10^8$	$1{,}9 \times 10^8$
Culture morphology in optic microscope	Mainly coccoid and lobed shaped cells	Varied: coccoid, different sizes rod chains, thin treads etc.	Varied: different sizes rod chains, thin treads, less coccoid, etc.

FISH analysis for LM samples using general probes for archaea (ARCH 915) and a specific probe for *Sulfolobales* order were carried out after the enrichment in M88 plus sulphur. These results indicate that the culture medium used during the enrichment steps favoured the development of *Sulfolobales* species. Similar results were obtained from B9 samples.

Morphological characteristics of the cells present into the enriched cultures can be seen in SEM micrographs (Fig. 1). Pictures A and B are from cultures coming from Baño 9 grown in M88 medium supplemented with sulphur (B9 + S) and sucrose (B9 + Su) respectively. Picture C belongs to a culture from Las Maquinitas grown in M88 medium with sulphur (LM + S). Micrography A is dominated by cocci and lobular cells; B shows a couple of cells representing most of the cells in the culture although it was possible to find other cells with different morphology. The same situation was found in the case of LM + S although it was not possible to get a good image of both types of cells together. Internal structure was studied by transmission electron microscopy and the results can be seen in Fig. 2. Pictures A, B, C and D are from the same enrichments cultures shown in Fig. 1 (B9 + S, B9 + Su and LM + S, respectively).

In all cases two different shapes can be distinguish; some of them show the typical irregular shape of archaea (it is clearer in A). In some of the pictures – especially in those from cultures with sulphur – it is possible to see some precipitates and also some of the grains seem to be inside the cell bodies.

According to the results of microscopic and preliminary molecular analysis, inocula coming from natural places with different temperatures were enriched in similar populations when they were cultivated at the same temperature (intermediate between the original ones). Deeper molecular studies (cloning and sequencing) should be carried out in order to confirm that hypothesis.

Troilite dissolution was firstly monitored through the amount of total soluble iron present in the solution (Fig. 3). Bioleaching experiments showed a first slow increase of iron in solution surely due to the acid dissolution showed by Eq. 1; after 10 days a significant dissolution in cultures with sulphur (higher for B9 + S culture) was observed. That suggested a possible microbial action on sulphur generating sulphuric acid (Eq. 2) which would allow the progress of the acid dissolution.

$$FeS + 2H^+ \rightarrow Fe^{2+} + H_2S \quad (1)$$

$$S^0 + 1.5O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-} \quad (2)$$

B9 + Su started to move away from the sterile control one week later indicating the possible presence of sulphur at that time probably produced by chemical oxidation (Eq. 3).

$$2FeS + O_2 + 4H^+ \rightarrow 2Fe^{2+} + 2S + 2H_2O \quad (3)$$

After 70 days, the percentages of iron solubilised were 60 ± 12%, 48 ± 9%, 32 ± 7%, and 15 ± 2% for B9 + S, LM + S, B9 + Su and abiotic control, respectively. Actually the dissolution of troilite occurred at a greater extent but iron was partially precipitated as different iron(III) compounds (see below). Since the precipitation process depends not only on the concentration of total soluble iron but also on the pH value of the solution, there is not a simple relationship between the extension of troilite dissolution and the soluble iron present into the solution. In order to analyse the extension of the dissolution, the most intensity peak from troilite in XRD pattern (2.05 A) was compared normalised to untreated troilite. In this way, the percentages of troilite dissolution were 90.5%, 85.7%, 81.0% and 52.4% for B9 + S, LM + S, B9 + Su and abiotic control, respectively. Although the tendency is the same, the last values are higher than those of iron solubilised because they also include iron re-precipitated.

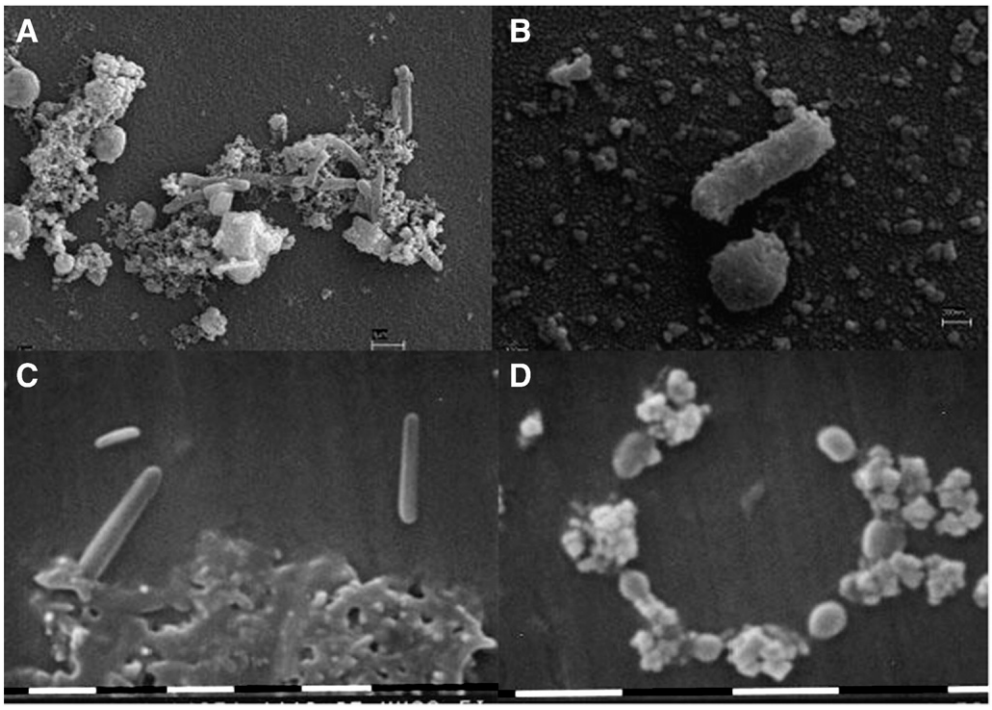

Fig. 1. Scanning electron micrographs for Baño 9 enrichment growing on sulphur (20140X, bar = 1 μm) (A) or sucrose 60,000×, bar = 300 nm) (B) and Las Maquinitas growing on sulphur (20,000×, bar = 1 μm) (C).

In addition, such abundant iron precipitation could cover part of the troilite surface stopping the attack of the sulphide. The higher solubilisations were reached in the cultures with sulphur (B9 consortium was more efficient than LM consortium) that also reached the lower pH values.

Eh and pH behaviours fitted to typical kinetics of iron dissolution. Eh and pH values during the bioleaching experiments are shown in Fig. 4. In the first five days, pH values increased due to the proton consumption (Eqs. 1 and 3) while Eh values decreased due to the iron (II) release (Eqs. 1 and 3). This behaviour is typical of troilite and other

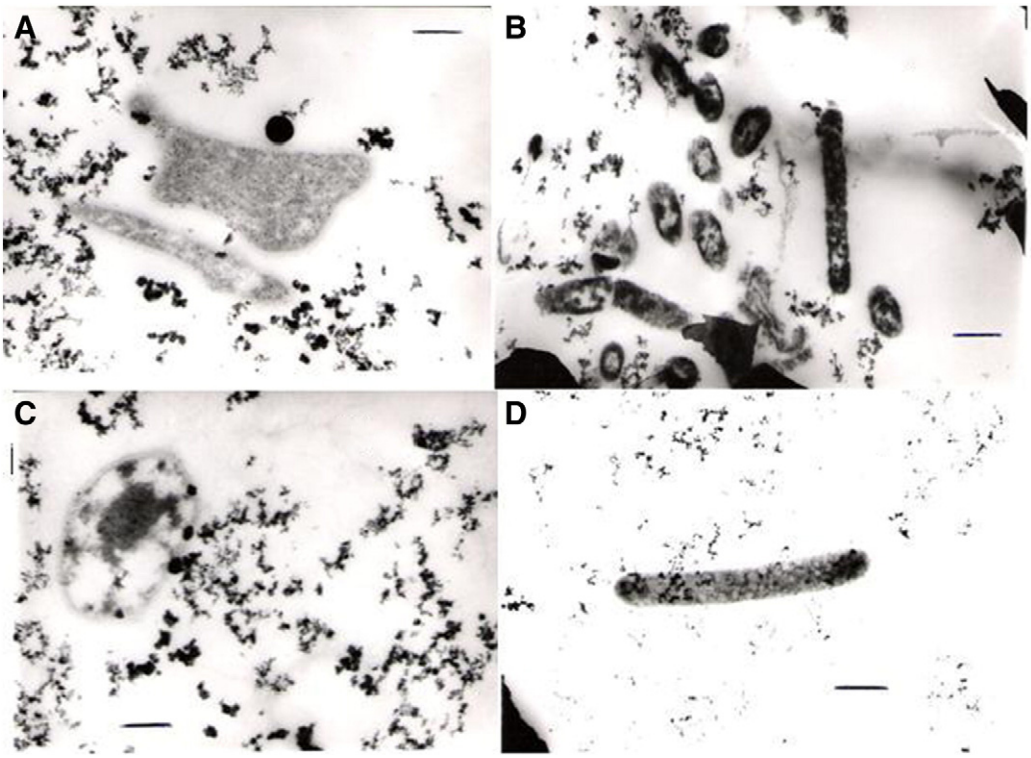

Fig. 2. Transmission electron micrographs for Baño 9 enrichment growing on sulphur (27,000×, bar = 0.31 μm) (A) or sucrose (14,000×, bar = 0.61 μm) (B), and Las Maquinitas growing on sulphur (27,000×, bar = 0.31 μm) (C) and (10,000×, bar = 0.85 μm) (D).

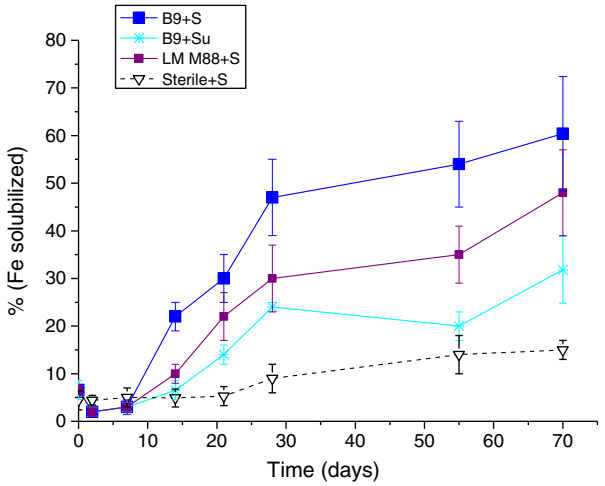

Fig. 3. Total iron evolution during chemical and biochemical leaching experiences.

iron sulphides acid dissolution at the oxidation initial stages (Bhatti et al., 1993).

Abiotic control maintained initial pH value through the whole experience. In the cultures pH decreased, mainly in those grown in M88 medium with sulphur where pH values around 1 were reached. As it was indicated above, this behaviour can be explained by sulphuric acid generation due to microbial sulphur oxidation (Eq. 2); sulphur was also present even in the cultures where sulphur was not supplemented due to the process indicated by Eq. 3. Redox potential significantly increased (up 600 mV) in those cultures inoculated with B9 + S. In the other systems as well as in the abiotic control the Eh values reached only 300 mV. These results would suggest that M88 allowed the development of iron-oxidisers in the culture. The capability of oxidising ferrous iron was probed for these consortia in independent experiments (data not shown) confirming the presence of iron-oxidising microorganisms within them. Ferrous iron oxidation can occur in abiotic medium (faster at high pH values) and that is strongly catalysed by iron oxidising microorganisms. Ferric iron hydrolysis also contributes with pH decrease. Through hydrolysis there can be generated different precipitates like: goethite (FeOOH), jarosite ($KFe_3(SO_4)_2(OH)_6$) and schwertmannite ($Fe_8O_8(OH)_6SO_4$).

Precisely, residues from bioleaching experiences visually showed a wide range of characteristic colours of the different precipitated oxides and remaining iron sulphides, ochre from jarosite, strong red from hematite, yellow from goethite and orange from schwertmannite. Most of them can be verified combining XRD analysis with a selective solubilisation. As it can be seen in Fig. 5, mineralogical species in the solid residues were sulphur (S), jarosite (J), hematite (H), goethite (G) and troilite (T). After selective solubilisation with acid ammonium oxalate (AO) the species detected were sulphur, jarosite, goethite, schwertmannite (Sw) and troilite. DXRD-AO allowed schwertmannite identification, while that it was not possible to do this from the XRD-total pattern because schwertmannite was masked by other minerals due to its low crystallinity. Schwertmannite is a poorly crystalline iron oxyhydroxide that has been reported as a product of pyrite and other iron sulphides bacterial oxidation and is one of the main precipitates found near coal and mineral mines that generate acid mine drainage (Bigham et al., 1994; Cornell and Schwertmann, 2003; Murad and Rojik, 2003; Caraballo et al., 2009; Pogliani and Donati, 2000).

Table 2 shows the main mineralogical species in all the solid residues. Solid residues from abiotic controls were similar to each other and the same species were detected in all the XRD patterns from all the treatment applied. In all solid residues from all assays a remnant of troilite was identified. This was confirmed by dispersion X-ray analysis (EDAX) of a black portion of precipitate from one of the systems assayed. EDAX determined atomic percentages of 48.5% for S, 51.5% for Fe and Fe/S ratio of 1.06. Electron scanning micrograph of the same residue portion showed the characteristic crystal of troilite (see Fig. 6). Marcasite was resistant to biological oxidation and it was detected in all solid residues.

The composition of iron(III) compounds formed during the precipitation strongly depend on the pH value of the solution: jarosite precipitates at pH<3, schwermannite does it between 2.5 and 5.5, hematite at pH>2.0, goethite between 4.5 and 6.5 and ferrihydrite precipitates at pH values higher than 6. In addition, shwermannite and ferrihydrite are metastable and can be transformed to goethite and hematite (Kumpulainen et al., 2008; Schwertmann and Murad, 1983). In the case of the residues from the abiotic control, mainly jarosite was detected in agreement with Murad and Rojik (2003). Jarosite was also the main iron(III) compound in the culture inoculated with B9 + S while goethite was the most abundant phase

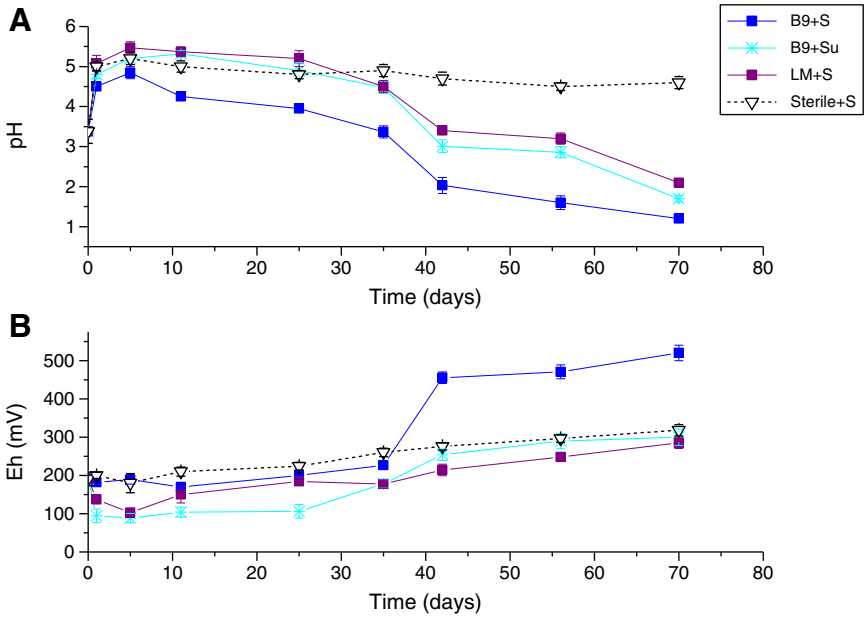

Fig. 4. Eh and pH evolution during chemical and biological leaching experiences.

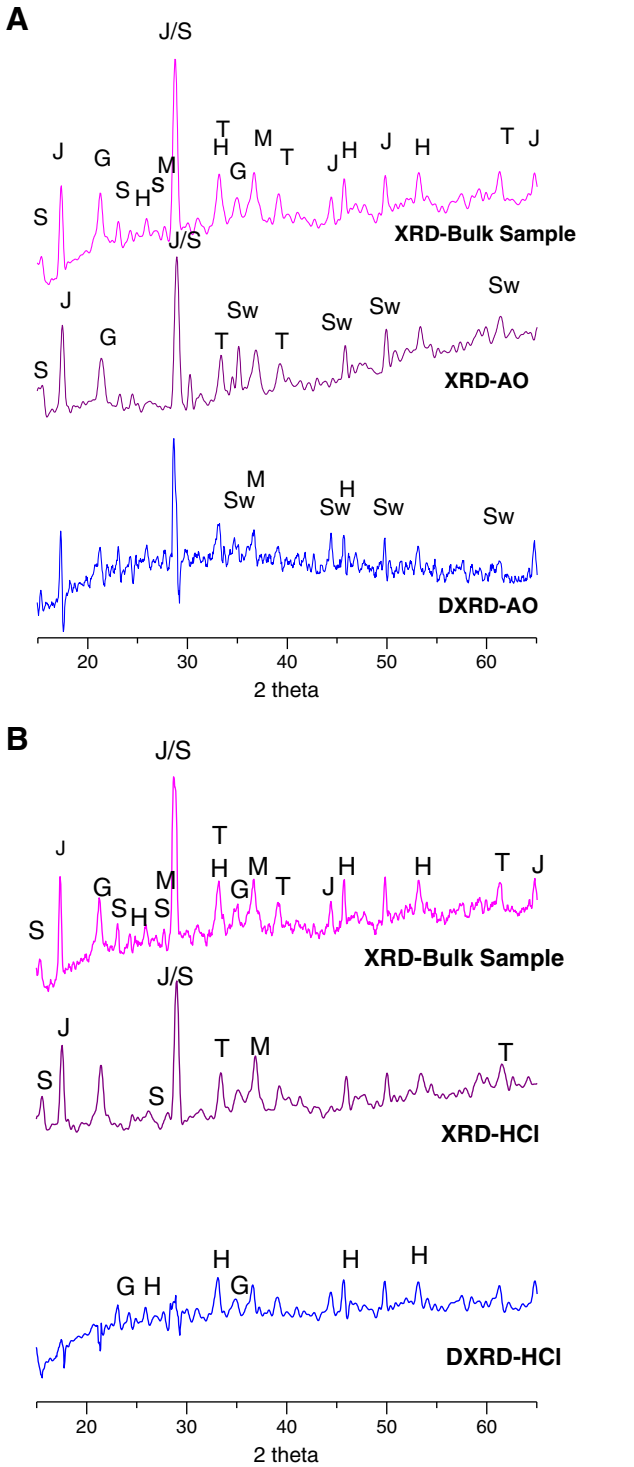

Fig. 5. Powder XRD patterns of troilite leach residues using different tests: A) with acid ammonium oxalate treatment B) with HCl treatment. Both figures include the resulting DRXD patterns and the Bulk sample diagrams without any additional treatment. Sulphur (S), jarosite (J), hematite (H), goethite (G), troilite (T), schwertmannite (Sw), marcasite (M).

in the culture with B9 + Su. Ferrihydrite was not detected in any of the systems assayed because pH never reached such high values.

Our results show that, as other metal sulphides, troilite dissolution can be strongly catalysed by sulphur (and iron) oxidising microorganisms present in two different consortia from hot springs located into the geothermal zone of Copahue reaching percentages of 80–90% (about 50% in the abiotic control). Abundant iron precipitation was

Table 2
Mineral identified in solid residues by XRD and DXRD techniques (see material and methods). Sulphur (S), Jarosite (J), Hematite (H), Goethite (G), Marcasite (M), Schewetmannite (Sw), Troilite (T). XRD-Total without any additional treatment; XRD-AO and XRD-HCl after treatment with ammonium oxalate pH = 3 and HCl 5 M respectively; DXRD differential XRD.

Assays	XRD-Total	XRD-AO	XRD-HCl	DXRD	DRXD
		pH = 3	5 M	Total-AO	Total-HCl
Raw mineral[a]	T–M				
B9 + S	S–J–T–M–G–H	S–J–T–G–Sw	S–J–T–M	M–H–Sw	G–H
B9 + Su	S–J–T–M–G–H	S–J–T–G–M–Sw	S–J–T–M	H–Sw	G–H
LM + S	S–J–T–M–G–H	S–J–T–G–Sw	S–J–T–M	M–H–Sw	G–H
Abiotic Control	S–J–T–M	S–J–T–M	S–J–T–M		

[a] Mineral before bioleaching process.

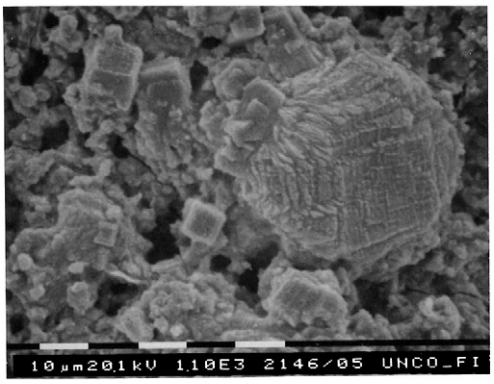

Fig. 6. Electron scanning micrograph of a black residue assumed to be trolite (bar = 10 µm).

detected in all the cultures (between 30 and 60%); it was even higher for the abiotic control. According to our results the addition of sulphur increased the bioleaching rate although the troilite dissolution is not limited by such addition because sulphur is in situ generated by chemical oxidation. An additional and interesting result of our studies indicates that natural consortia can have a wide thermal flexibility and they have some members – especially archaeas from Sulfolobales – which are able to keep their viability at temperatures far away from that measured in their natural habitat in the Geothermal Copahue Caviahue Field.

Acknowledgments

This work was partially supported by grants PICT25300, PICT339, PIP 5147 and by Universidad Nacional del Comahue.

References

Akcil, A., Koldas, S., 2006. Acid mine drainage (AMD): causes, treatment and case studies. J. Cleaner Prod. 14, 1139–1145.
Amann, R., 1995. In situ identification of microorganisms by whole cell hybridization with rRNA targeted nucleic acid probes. In: Akkermann, A.D.L., van Elsas, J.D., Brujin, F.J. (Eds.), Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1–15.
Bhatti, T., Bigham, J., Carlson, L., Tuovinen, O., 1993. Minerals products of pyrrhotite oxidation by *Thiobacillus ferrooxidans*. Appl. Environ. Microbiol. 56, 1984–1990.
Bigham, J.M., Carlson, L., Murad, E., 1994. Schwertmannite, a new iron oxyhydroxysulphate from Pyhasalmi, Finland, and other localities. Mineralog. Mag. 58, 641–648.
Brock, T., Brock, D., Belly, K.M., Weiss, R.T., 1972. *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch. Microbiol. 84, 54–68.
Caraballo, M.A., Rötting, T., Nieto, J.M., Ayora, C., 2009. Sequential extraction and DXRD applicability to poorly crystalline Fe- and Al-phase characterization from an acid mine water passive remediation system. Am. Mineralog. 94, 1029–1038.

Chiacchiarini, P., Giaveno, A., Lavalle, L., Donati, E., 2007. Zinc recovery in an air-lift bioreactor by isolated *Leptospirillum ferrooxidans*. Hydrometallurgy 89, 117–126.

Chiacchiarini, P., Lavalle, L., Giaveno, A., Donati, E., 2010. First assessment of acidophilic microorganisms from geothermal Copahue–Caviahue system. Hydrometallurgy 104, 334–341.

Chiriţă, P., Descostes, M., 2006a. Anoxic dissolution of troilite in acidic media. J. Colloid Interface Sci. 294, 376–384.

Chiriţă, P., Descostes, M., 2006b. Troilite oxidation by hydrogen peroxide. J. Colloid Interface Sci. 299, 260–269.

Cornell, R.M., Schwertmann, U., 2003. The Iron Oxides: Structure, Properties, Reactions, Occurrences, and Uses. Wiley-VCH, Weinheim, Germany. 664 p.

Falco, L., Pogliani, C., Curutchet, G., Donati, E., 2003. A comparison of bioleaching of covellite using pure cultures of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* or a mixed culture of *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans*. Hydrometallurgy 71, 31–36.

Giaveno, A., Donati, E., 2001. Bioleaching of heazelwoodite by means *Thiobacillus* actions. Process Biochem. 36, 955–962.

Jeong, H.Y., Han, Y.-S., Park, S.W., Hayes, K.F., 2010. Aerobic oxidation of mackinawite (FeS) and its environmental implication for arsenic mobilization. Geochim. Cosmochim. Acta 74, 3182–3198.

Johnson, D.B., Hallberg, K.B., 2005. Acid mine drainage remediation options: a review. Sci. Total Environ. 338, 3–14.

Kepner, R.L.J., Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev. 58, 603–615.

Kumpulainen, S., Räisänen, M.L., Von der Kammer, F., Hofmann, T., 2008. Ageing of synthetic and natural schwertmannites at pH 2–8. Clay Miner. 43, 437–448.

Lavalle, L., Giaveno, A., Pogliani, C., Donati, E., 2008. Bioleaching of a polymetallic sulfide mineral by native strains of *Leptospirillum ferrooxidans* from Patagonia Argentina. Process Biochem. 43, 445–450.

Mas, G.R., Mas, L.C., Bengochea, L., 1996. Hydrothermal surface alteration in Copahue Geothermal field (Argentina). Proceedings of Twenty-First Workshop on Geothermal Reservoir Engineering. Stanford Geothermal Program Workshop Report SGP-TR-151, pp. 241–246.

Murad, E., Rojik, P., 2003. Iron-rich precipitates in a mine drainage environment: influence of pH on mineralogy. Am. Mineral. 88, 1915–1918.

Pernthaler, A., Pernthaler, J., Amann, R., 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. 68, 3094–3101.

Pogliani, C., Donati, E., 2000. Immobilisation of *Thiobacillus ferrooxidans*: importance of jarosite precipitation. Process Biochem. 35, 997–1004.

Rochette, P., Gattacceca, J., Ivanov, A.V., Nazarov, M.A., Bezaeva, N.S., 2010. Magnetic properties of lunar materials: Meteorites, Luna and Apollo returned samples, Earth and Planet. Sci. Lett. 292, 383–391.

Rohwerder, T., Sand, W., 2007. Oxidation of inorganic sulfur compounds in acidophilic prokaryotes. Eng. Life Sci. 7, 301–309.

Schulze, D.G., 1994. Differential X-ray diffraction analysis of soil minerals. SSSAJ 677, 412–428.

Schwertmann, U., Murad, E., 1983. Effect of pH on the formation of goethite and hematite from ferrihydrite. Clays Clay Miner. 31, 277–284.

Sracek, O., Choquette, M., Gelinas, P., Lefebvre, R., Nicholson, R.V., 2004. Geochemical characterization of acid mine drainage from a waste rock pile, Mine Doyon, Quebec, Canada. J. Contam. Hydrol. 69, 45–71.

Thomas, J.E., Skinner, W.M., Smart, R.S.T.C., 2003. A comparison of the dissolution behavior of troilite with other iron(II) sulfides; implications of structure. Geochim. Cosmochim. Acta 67, 831–843.