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

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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

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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.