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Microbial activity in weathering columns

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Abstract

The aim of the present work was to evaluate the metabolic activity of the microbial population associated with a pyritic tailing after a column-weathering test. For this purpose, a column 150 cm high and 15 cm diameter was used. The solid was a tailing with 63.4% pyrite and with minor amounts of Cu, Pb and Zn sulfides (1.4, 0.5 and 0.8%, respectively). The column model was the habitual one for weathering tests: distilled water was added at the top of the column; the water flowed down through tailings and finally was collected at the bottom for chemical and microbiological analysis. Weathering was maintained for 36 weeks. The results showed a significant presence of microbial life that was distributed selectively over the column: sulfur- and iron-oxidizing aerobic bacteria were in the more oxygenated zone; anaerobic sulfur-reducing bacteria were isolated from the samples taken from the anoxic part of the column. Activity testing showed that (oxidizing and reducing) bacteria populations were active at the end of the weathering test. The quality of the water draining from the column was thus the final product of biological oxidation and reduction promoted by the bacteria consortia.

Keywords: Activity test; Weathering; Tailings; Microbiological oxidation and reduction; Column model

1. Introduction

As we know, the terms acid rock drainage (ARD) and acid mine drainage (AMD) refer to liquid effluents produced as a result of natural oxidation of sulfide minerals contained in rocks exposed to air and water. Such minerals are chemically (and biologically) stable in situations where both oxygen and water are excluded; however, when exposed to both moisture and air, sulfide-containing minerals will oxidize spontaneously, with either molecular oxygen or ferric ion acting as the oxidant. Metals such as cobalt, copper, iron, nickel, lead, zinc, cadmium, etc., are released when sulfides of these metals are exposed to the weathering conditions found in mines, tailing impoundments and waste rocks [1].

It has been proven that microbiology has an essential role in the AMD generation process. AMD communities are generally characterized by a very limited number of distinct species (AMD communities contain fewer prokaryotic lineages than many other environments), probably due to the small number of metabolically beneficial reactions available [2]. The oxidation of metals in AMD is limited by the availability of nutrients to the few microorganisms present in the medium [3]. Although chemolithotropic acidophilic bacteria are the main microbial species isolated in AMD, life in these waters is not restricted to iron-oxidizing bacteria but includes sulfur-oxidizing bacteria and archea, heterotrophic microorganisms and some lower eukaryotic life-forms [4]. Baker and Banfield [2] have also studied the particular microbiology associated with AMD environments and agree with this account, having reported that although eukaryotes (protists, fungi and yeasts) also live in AMD, prokaryotic components of these communities are the protagonists and autotrophic and heterotrophic archea and bacteria are involved in iron and sulfur oxidation and in sulfur reduction. The microbial communities associated to disposed sulfidic tailings are generally similar to those found in controlled bioleaching operations [5]. Whilst some indigenous microorganisms are responsible for accelerating sulfide mineral oxidation, thereby generating acidity and mobilizing metals, other types

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catalyse reductive processes that essentially reverse these reactions and thereby ameliorate polluted mine waters [3]. These types are sulfate and iron reducers. In this connection it is also important to note the presence of anaerobic sulfate-reducing bacteria (SRB) which tolerate acidic pH.

A tailing pond is a particular system (ecosystem) where waste fine-grain minerals are stored under water to reduce exposure to oxygen and the ensuing oxidative dissolution of acid-generating sulfidic minerals [6]. There are several unique aspects to these ecosystems. The first is the sharp gradation in dissolved oxygen, pH and concentration of sulfate and metals from the water table to the bottom: pH between 2 and 7 may be measured from the surface to a depth of only 1.5 m [6]. Matching this chemical gradation, a large variety of species has been found: moderately thermophilic acidophiles, iron- and sulfur-oxidizing acidophilic bacteria (including samples taken under water), aerobic heterotrophic acidophiles and sulfate-reducing bacteria [7].

Tailing degradation occurs simultaneously to a microbial succession. Initially, moderately acidophilic iron-oxidizing bacteria may prevail as the principal group, but heterotrophic acidophiles and other species emerge later [8]. Walsh and Mitchell [9] explained in a simple way how acid mine drainage occurs in natural environments. They maintained that AMD starts when chemical oxidation promotes a pH near 4.5. At this moment the bacteria *Metallogenium* may oxidize ferrous iron and reduce the pH of the media to around 3.5. And at that point *Acidithiobacillus ferrooxidans* can further reduce the pH to less than 2.5. When chemical conditions are near neutrality, heterotrophic species (bacteria, fungi, algae and yeasts) prevail [10]. As the chemistry changes, one population may push out another. *A. ferrooxidans* colonize and later acidify a circumneutral pH by first making pH dependent on bacterial succession [11]. *A. ferrooxidans* has been shown to colonise environments at neutral pH [12].

These chemically complex microbiologically induced reactions are at the origin of major environmental problems when mine tailings are abandoned. Nowadays environmental management is based on principles relating to prediction and prevention of these reactions rather than taking corrective action once the problem has arisen. Therefore, there is interest in reliable prediction tests for contamination and improvements in the prevention of acid rock drainage (ARD). In studies of the factors influencing the conversion of the tailings, columns and lysimeters have been used to reproduce particular systems [13]. Factors such as the availability of oxygen, amount of water in the tailing pores, pH of the natural leaching agents, ionic strength of solutions, type of ions in the medium, etc, have been found to be of major importance. Evolution of the bacterial population during the conversion of the tailings has also been studied [14]. Various researchers have published experimental designs in order to standardize the method, but methods must always necessarily be particularized because each system behaves in a unique way. Indeed, each system constitutes an ecosystem with its own particular chemical and microbiological characteristics.

Numerous papers have been published on the chemical process and the influence of the microorganisms in these kinds of systems [15–18]. Isolation of the bacteria associated with the tailings or the effluents from a weathering test is not enough to elucidate the real role of the microbiota in tailing conversion. It is necessary also to evaluate the activity, the kinetics and the particular role of the main microbial groups present (with their metabolism particularities).

The purpose of the present study was to reproduce the vertical heterogeneity found in a pyritic tailings pond using a column model. To verify the role of microbial groups during the conversion of pyritic tailings, we monitored not only the presence of bacteria but also the state of activity of the principal groups once the columns were dismantled. The role of the microbial groups in weathering of the tailings was evaluated by certifying their presence (through counting tests) and also by conducting activity tests.

2. Materials and methods

2.1. Site

The system studied using different columns (Fig. 1) was a pyritic tailing pond for storage of the waste produced during flotation of a complex sulfide ore. The pond belongs to a mine located in the Pyritic Belt of Southwest Spain. The waste collected in the pond was produced in a flotation plant where three different concentrates of zinc, copper and lead were obtained from the complex sulfide. The waste was sent to the pond for liquid-solid separation by way of a 3 km open-air channel taking advantage of gravity. The final disposal of the pulp in the pond caused the solids to settle at the bottom, giving rise to two different oxygen gradient zones: an anaerobic zone at the bottom of the pond (10 m), and an aerobic zone of surface solids and waters.

The tailing sampling point for the weathering column test was at the exit of the flotation plant, where the sample issued as a slurry. From there it was taken and transported to the laboratory in sterile 50 L bottles.

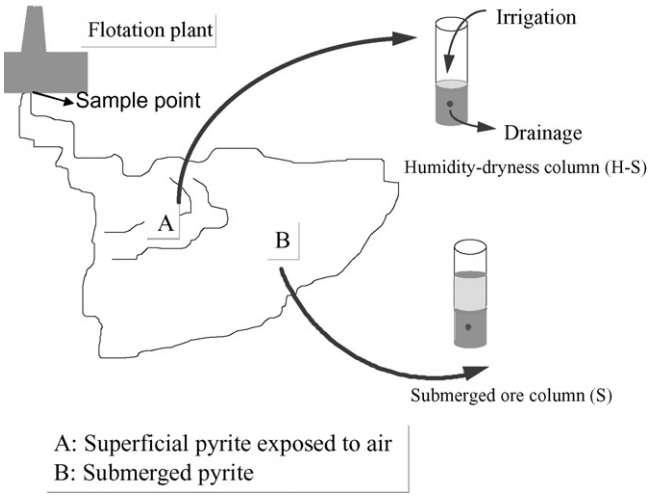

Fig. 1. The system and the model employed.

2.2. Weathering column tests

One large column (M column) and four small columns were used for weathering tests. Column designs are as follows.

2.2.1. M column

For weathering tests a 150 cm high, 15 cm diameter metacrylate column was used. The mineral was poured as a slurry on to a 20 cm bed of silica stones situated at the bottom of the column. The column was watered from the top and the effluents were collected using a pipe situated at the bottom [19–22]. The column was fed with 30 kg of pulp containing 30% solids (Fig. 2). The final bed height reached in the column once the solids settled was 65 cm. The column was heated at approximately 30 °C (between 28 and 33 °C) using a metal-box thermostatting system.

The first step in the test was to put the pulp in the column, leaving sufficient time for the liquid and solid phase distributes inside the system. The weathering of the residual solid in the column took place in two stages. In the first stage, which lasted 16 weeks, the column was watered continuously by means of a peristaltic pump whose flow was modified to suit the percolation conditions of the system. When the column was stabilized, it was observed that the optimum percolation flow was 2 mL h^{-1} (48 mL d^{-1}) using a watering flow of 45 mL d^{-1} distributed in additions of 15 mL every 8 h. In these conditions the solid was wet but no water accumulated on the pulp surface.

In the second stage of the experiment, the pulp was subjected to dryness and humidity cycles. Two litres of distilled water was used to water the column in each cycle. After watering, water was left in contact with the solid residue for 48 h; after which the columns were drained and left dry for the next 12 days. This second stage of the tests lasted 20 weeks.

The total time taken by the tests was 36 weeks.

2.2.2. Short weathering columns

Four glass columns (height 30 cm, diameter 6 cm) were prepared to monitor the solid weathering and to study the influence of different variables. Tailings (300 g) were placed in each column. The final bed height reached in the column in this case was 6 cm. The columns were periodically irrigated with 200 mL of distilled water [23–25]. The watering regimen also consisted of cycles of humidity and dryness. The methodology was as follows: the column was irrigated with 200 mL of distilled water, which remained in contact with the ore for 48 h (humidity period). The column was then drained and left to dry for 120 h. The models tested were: submerged ore (S) (Fig. 1), humidity–dryness (H–S) (Fig. 1), inoculated column (Li) and uninoculated column (L).

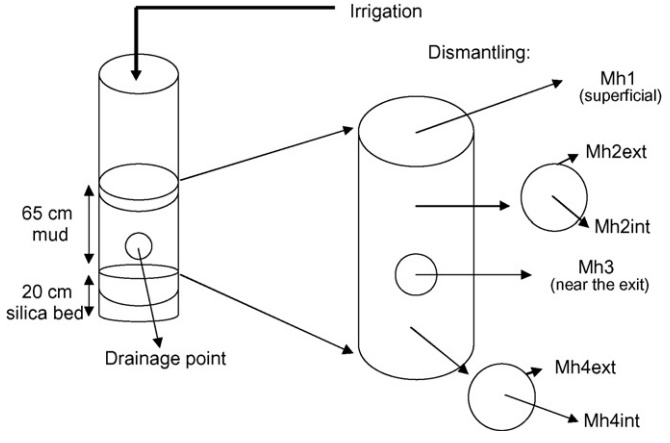

Fig. 2. Column M. Dismantling and sampling points.

2.3. Submerged ore and humidity–dryness columns

In the system under study (tailing pond), most of the ore is located under water. In order to reproduce the evolution of the waste in this zone and compare the results with the evolution of the ore exposed to air, two columns were designed. In the first, the ore (slurry) was totally submerged in water (S) (Fig. 1); and in the second, the ore (slurry without water; the water remaining with the mineral was eliminated by percolating) was subjected to humidity–dryness cycles (H–S) (Fig. 1). The tests took a total of 30 weeks.

2.4. Inoculated column

In this case two columns (L and Li) were assembled and loaded with 300 g of dry, finely dispersed tailings from the original sample. The first column (Li) was inoculated with 20 mL of a mixed culture containing iron- and sulfur-oxidizing bacteria obtained from an enrichment culture grown from the tailings themselves. For the mineral weathering, both columns were watered intermittently following the cycle system described before. In this case the tests took a total of 15 weeks. This column was compared with an uninoculated short column (L).

All characteristics of the columns are shown in Table 1.

2.4.1. Dismantling: sampling and residue characterization

Following completion of the tests, columns were dismantled as soon as the variables chosen to monitor the evolution of the system attained a given degree of stability. A final characterization of the solid tailings was performed and the conversion of the ore in the different layers was analysed. Each sample was characterized mineralogically and microbiologically as is described in the next Section 2.3. Samples were selected according the following criteria.

2.4.1.1. Column M. This column was sampled longitudinally and radially. As the height of the bed was 65 cm, samples were taken approximately every 20 cm. Double tests were performed at each height, one at the inner and the other at the outer location (see Fig. 2).

2.4.1.2. Small weathering columns. Only two samples were taken: one at the surface interface (X_s) and the other at the bottom (X_b).

Table 1
Column parameters

Column	Ø (cm)	H_{bed} (cm)	V_{bed} (cm^3)	Residue (g)	$V_{added\ water}$ (ml/cycle)	$V_{drained\ water}$ (ml/cycle)
M	15	65	11486	9000	2000	1800
S	6	13.5	382	300	200	–
H–S	6	13.5	382	300	200	150
L	6	13.5	382	300	200	150
Li	6	13.5	382	300	200	150

2.5. Characterization of samples

Various analyses were conducted on the samples taken at the pond (for system characterization) and from the weathering tests (column effluents). Light microscopy was also used to detect the possible presence of bacteria in the column effluents.

2.5.1. Solid samples

The solids contained in the pulp sample and in the samples taken from the column after dismantling were characterized by X-ray diffraction (Philips X'Pert-MPD, Germany) and granulometric analysis (Microtac FRA, USA). The chemical composition of the pulp (0.5 g) was treated with 5 mL HNO_3/HCl (3:1, v/v) for 20 min followed by heating on a hot-plate, diluted to 250 mL and the metals were determined by AAS (Perkin-Elmer 1100B; Germany). Sulfur content was determined using a Leco automatic analyser.

2.5.2. Liquid samples

pH was monitored by a Crison 2001 electrode. A photocolorimeter (Metrohm 662; Germany) was used to determine the turbidity of barium sulfate precipitates formed by reaction of sulfate ion with barium chloride for sulfate analysis [26]. Metals dissolved in the liquid samples taken from the pond in the successive column effluents were likewise monitored by AAS. Eh was measured with an Ag^0/AgCl electrode. For activity tests, Fe^{2+} was determined by photocolorimetry using *o*-phenanthroline as colorimetric reagent in acetic medium. A Metrohm 662 photocolorimeter was also used in this case [26,27]. Fe^{3+} was measured as the difference between total iron in solution and Fe^{2+}.

2.5.3. Microbiology

Samples taken at the flotation plant outlet and samples taken from the columns after dismantling were characterized microbiologically. Microbial were isolated in the following culture mediums: the aerobic chemolithotrophs, in the 9 K medium [28,29] containing ferrous iron (33.3 g/L) or an elemental sulfur spatula tip as energy source, for the iron and sulfur oxidizers, respectively. The pH of the 9 K medium was adjusted to 2 in the case of ferrous iron solution and to 3 in the other. The aerobic heterotrophs were isolated in the 9 K medium (adjusted to pH 3) containing 10 g/L glucose and 5 g/L yeast extract. The anaerobic were isolated in the Postgate's C medium, adjusted to pH 7 (ASTM D 4412-84 [30]). The same medium but with sodium molybdate was also used as control test.

Microorganisms were counted by the most probable number (MPN) method [26,29,31].

2.6. Activity tests

2.6.1. Iron-oxidizing activity

Specific growth tests were prepared to evaluate the activity of the bacteria associated with the ore at each point in the column. For aerobic iron-oxidizing activity, a 5 g sample was taken from each sampling point and inoculated in 250 mL sterile flasks containing 100 mL of 9 K medium with ferrous iron sulfate at pH 2. These flasks were kept at 30 °C with orbital agitation for 17 days. Samples (10 mL) were taken periodically for analytical control. Evaporation was compensated with sterile distilled water. pH and Fe^{2+} concentrations were measured in each test. In addition, the cultures were observed by light microscopy. Samples tested were: Mh1, Mh2ext, Mh2int, Mh3, Mh4ext and Mh4int (Fig. 2).

2.6.2. Anaerobic sulfate-reducing activity

To monitor the anaerobic sulfate-reducing activity, 20 g of each sample of the column was inoculated on 100 mL of Postgate's C medium at pH 7. The cultures were grown in hermetically sealed sterile flasks, which were kept at 30 °C. The test time was 40 days. Samples (10 mL) were taken periodically using a sterile pipette, at which time the flasks were opened. Nitrogen was bubbled through for 10 min to prevent oxygen influencing the cultures once the sample had been taken.

The variables chosen to monitor the bacterial activity in this case were pH and sulfate concentration. For sulfate analysis, the samples were first centrifuged for 10 min at 10,000 rpm to prevent turbidity influencing the measure.

The samples tested in this case were: Mh1, Mh2ext, Mh2int, Mh3, Mh4ext and Mh4int (Fig. 2).

3. Results and discussion

3.1. System description and sample characterization

The composition of the initial poured tailing met the specifications of a pyrite pulp with 30% (w/v) solids. This was the pulp used for the columns. The chemical, mineralogical and microbiological composition of the sludge is given in Tables 2–4. As the tables show, the composition clearly coincides with that of a pyrite; at the same time there is an appreciable presence of microorganisms with the potential to oxidize the tailing

Table 2
Chemical composition of the mud (%, w/w)

Cu	0.45
Pb	1.70
Zn	0.10
Fe	39.0
Mn	0.12
Ca	0.01
Mg	0.23
S	39.30

The rest until 100% corresponds to silica and other minor elements.

Table 3
Mineralogical composition of the mud

Mineral	% (w/w)
Chalcopyrite	1.4
Galena	0.5
Sphalerite	0.8
Pyrite	63.4
Silicates	33.0

Table 4
Initial number of microorganisms in the pulps (MPN g^{-1})

Pulp	
Fe-oxidizing	–
S-oxidizing	183.25×10^2
Heterotrophic	806.3×10
SRB	–

–: negative growth; SRB: sulfate-reducing bacteria.

associated with the pulp. The chemical composition of the aqueous phase of this pulp is shown in Table 5 (AMD initial).

As described earlier, the solid waste was sent to the pond through a 3 km open-air channel taking advantage of gravity. Once in the pond, solids and liquid from the pulp were separated, and different chemical and microbiological conversions took place [32]. The initial liquid residue (AMD initial) was therefore not the same as the final liquid residue (AMD final), whose composition is shown in Table 5. The main transformation was acidification of the liquids, which produced a considerable increase in metal and sulfate concentrations in solution, and a decrease in sulfite concentration. The disposition of the pulp in the pond produced a chemical gradient (Table 6): the surface water was acidic, with a high concentration of metals and sulfate, whereas the samples taken from the pond bottom had neutral pH and appreciably less metals and sulfate, with the exception of copper. As well as this chemical gradient, there was a microbiological gradient (Table 7). Aerobic iron- and sulfur-oxidizing bacteria were detected in the surface water. However, anaerobic sulfate-reducing bacteria were isolated in the sludge from the bottom of the pond.

Table 5
Chemical analysis of both the aqueous phase in the initial poured tailing (AMD initial) and the aqueous phase in the tailing pond (AMD final)

	AMD initial	AMD final
pH	9–10	2.5–3.5
Eh (mV)	−20 to +10	220–350
Sulfates (mg/L)	500–650	1800–2000
Sulfites (mg/L)	88–120	2–3
Copper (mg/L)	0.1–0.2	0.4–0.8
Iron (mg/L)	0.4–2	50–55
Zinc (mg/L)	0.1–2	30–50
Lead (mg/L)	0.1–0.2	5–6
Calcium (mg/L)	400–450	400–450
Magnesium (mg/L)	15–20	50–55

Table 6
Chemical gradient in the tailing pond

	Superficial waters	Samples of mud from the pond bottom
PH	2.5–3.5	6–7
Eh (mV)	220–350	−45 to M−3
Sulfate (mg/L)	1800–2000	400–500
Copper (mg/L)	0.4–0.8	0.3–0.6
Zinc (mg/L)	30–50	0.1–0.3
Iron (mg/L)	50–55	3.3–5

Table 7
Microbiological gradient in the tailing pond (MPN g^{-1})

Location	Chemolithotrophic aerobic bacteria	Anaerobic bacteria	Total heterotrophic
Superficial waters	1.1×10^2 to 7.0×10^3	0	1.0×10^3
Deep muds	2.5×10^1 to 9.5×10^2	1.7×10^2 to 2×10^3	6.0×10^1

3.2. Microbiological distribution in weathering columns

3.2.1. Column M

The results of the microorganism count in the various samples taken from column M are shown in Table 8. Clearly there was a considerable presence of microorganisms associated with the mineral throughout the column. The metabolisms underpinning these communities were two: autotrophic iron and sulfur oxidation and anaerobic sulfur reduction. As other researchers have found [2,14] the oxygen concentration was decisive in determining the distribution and selection of the various different species.

Comparison of these results with the initial microbiological characterization of the pulp (Table 4) showed that microbiological growth was enhanced by weathering of the mineral in two ways: on the one hand, the numbers of the species identified in the initial characterization of the pulp, such as

Table 8
Microbiological distribution in M column

Sample	Fe-oxidant bacteria (cells/g)	S-oxidant bacteria (cells/g)	SRB (cells/g)	Heterotrophic aerobic bacteria (cells/g)
Mhl	9.0×10^4	9×10^4	–	16×10^2
Mh2ext	27×10^2	12×10^2	–	4×10^3
Mh2int	12×10	12×10	10×10^1	8×10^2
Mh3	15×10^3	57×10^3	–	3.12×10^2
Mh4ext	9.0×10	18×10	9.0×10^4	4.0×10^3
Mh4int	5.7×10	6.6×10	10×10^4	3.0×10^3

sulfur-oxidizing bacteria (Table 4), increased; and on the other hand, iron-oxidizing and anaerobic bacteria that had not been identified in the initial microbiological characterization of the pulp now appeared or were selected at certain locations on the column, where specific microenvironments were established.

Heterotrophic acidophiles were likewise well represented and their numbers likewise grew. Although not directly connected with mineral conversion processes, bacteria of this kind play essential roles in maintaining the carbon cycle [2,33]. For example, an important symbiosis exits between heterotrophic and certain autotrophic species: autotrophs may depend on coexisting heterotrophs to remove organic compounds that are toxic to them, and heterotrophic acidophiles are able to utilize organic material produced by acidophilic autotrophs [2]. Heterotrophic acidophiles like *Acidiphilium* or *Acidiphilium acidophilum* have been isolated from acidic mine water and characterized. Interactions between members of microbial consortia are probably critical in optimization of AMD microbial community activity, since thanks to these symbiotic relationships the overall process is assisted by the coexistence of several species of microorganisms [34]. However, while certain heterotrophic acidophiles are able to utilize organic substances harmful to *A. ferrooxidans* as energy sources, there may also be some oxygen-consuming heterotrophs which excrete products that inhibit these *Acidithiobacilli* into the media [35].

As for the microbiological distribution throughout the column, sulfur-oxidizing bacteria were evenly spread in quantitative terms, although the most pronounced increase was detected in those associated with the surface mineral sample Mh1. There were two basic points of oxygenation in column M: the point corresponding to surface sample Mh1, with a considerable exposed free surface, and the nearby zone corresponding to sampling point h3, close to the column drainage point. In fact the most abundant distribution of iron- and sulfur-oxidizing bacteria was associated with these two points, and it was also at the same two points that sulfate-reducing anaerobic bacteria were found. As noted earlier, lithoautotrophic bacteria (which include sulfur-oxidizing species) are highly oxygen-dependent, which explains why it was precisely in sample Mh1, the most oxidized in the column, that preferential growth occurred. As Schippers et al. [36] have demonstrated, iron- and sulfur-oxidizing bacteria normally decline when a marked decrease in oxygen concentration occurs [36], although *A. ferrooxidans* [37] and *Sulfobacillus* spp. [38] have been shown to grow anaerobically.

These bacteria, which require anaerobic conditions to grow, were only detected in deep interior samples (Mh4ext, Mh4int and to a lesser extent Mh2int), in fact coinciding with the zones where growth of the lithoautotrophic bacteria was poorest.

Biological reduction of sulfate to sulfide by neutrophilic SRB has been observed and described in metal-rich environments, but there are very few reports of the isolation and characterization of acidophilic SRB [3]. However, anaerobic acid-tolerant bacteria have been isolated from different environments and even mixed cultures containing SRB were able to reduce sulfate in a bath culture at pH > 2.4. In a recent paper it is described sulfidogenesis at low pH media by a mixed population of acidophilic bacteria [39]. The anaerobic bacteria found in the column presented an

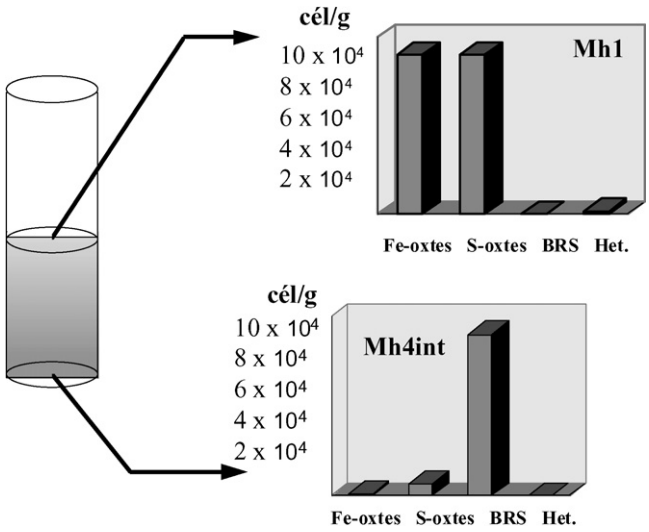

Fig. 3. Microbial selection in M column.

interesting tolerance of acidic conditions. This fact was verified with a series of experiments to characterize the behaviour of a mixed culture of SRB isolated from the tailing pond describe earlier. This interesting culture was able efficiently to remove 9000 mg/L of sulfate ion, to grow in the presence of up to 100 mg/L of copper and 30 mg/L of iron, and to alkalize the medium provided that the initial pH was higher than 4 [40]; also, it was successfully proven that this mixed culture was suitable for treating the acid mine drainage of the particular system studied [40]. The added advantage of the SRB is that not only are they able to generate net alkalinity, but they also effectively remove many toxic metals [3].

In column M, then, microbiological selection was established as a function of oxygen contribution (Fig. 3), so that there were two zones: a surface zone where lithoautotrophic bacteria grew preferentially, and an anoxic zone at the bottom, where mainly SRB grew.

3.2.2. H–S, S, L and Li columns

In dismantling the small columns, only two samples were taken from each one, of the surface mineral (X_S) and the bottom mineral (X_b). Bacteria counts were performed on these samples and on the final effluent from each column. The microbiological distribution in the columns is detailed in Tables 9 and 10.

At the conclusion of all the experiments, bacteria associated with the mineral were detected in all the small columns except for column S. Iron-oxidizing bacteria were found to be the most abundant in all columns, except column S as noted, where there was practically no growth of any kind. This microbiological finding was consistent with the chemical analyses conducted on the successive effluents in the experiments (see chemical analyses of the various effluents in [41]). There it had already been found that there was no pyrite weathering in column S.

Bacteria were associated with both surface and bottom samples, and there were no appreciable differences in the numbers of bacteria counted in samples from either point. The bed height of the columns was low (only 30 cm), and there was oxygenation

Table 9
Microbial distribution in the small weattienng columns

Column/specie	H–S_s	H–S_b	S_s	S_b	L_s	L_b	Li_s	Li_b
SRB	–	–	–	10×10	–	–	–	–
S-oxte (cells/g solid)	4.5×10^4	5×10^4	4.5×10	–	15×10^2	9.5×10^2	5×10^4	5×10^4
Fe-oxte (cells/solid)	$<0.1 \times 10$	$<0.1 \times 10$	–	–	0.08	0.08	0.18	0.18

–: negative growth.

via both the surface and the drainage tube; this means that there were no aeration-related factors that might induce selection of species.

The bacteria counts in the column effluents were likewise consistent with the counts of bacteria associated with the mineral in each column: bacteria were detected in all the effluents except those from column S.

A considerable proportion of iron-oxidizing bacteria were associated with the mineral, but the same was not true of sulfur-oxidizing bacteria, probably because these became more readily detached from the mineral through the successive washes. This was confirmed by the fact that both species were found in the effluents, with the S-oxidizing kind in the larger proportion.

All the results were consistent with the weathering assays [41,42]. For instance, the presence of bacteria associated with the mineral confirmed a bacterial role in the weathering of waste, as a result of which the degree of acidity generated was higher than when that activity was inhibited (column S) [42]. In fact, the absence of oxygen in the saturated column leads up to the acidic drainage as the result of the microbial activity did not occur. The pH value reached at column S was close to the initial value (close to 6). The concentration of metals in S column drainages was consistently to this pH value. The concentration of iron and zinc in the solutions from S column remained between 20 and 80 mg/L, and 25 and 80 mg/L, respectively. In column S, copper was not dissolved. In contrast to this result, the pH reached when bacterial was active (H–S column) and catalysing the oxidation process was 2 and the concentration of iron, copper and zinc reached oscillated, respectively, between 3000 and 5000, 200 and 400, and 1000 and 7000 mg/L [42].

This "natural" population which was associated with the mineral eventually created its own microenvironment, which enabled it to live in optimum growth conditions; this was borne out by the fact that columns H–S and L finally resembled column Li. In earlier experiments in an agitated flask [32], it was found that the degree of dissolution attained in inoculated experiments in optimum conditions was comparable to the degree attained in others where the pulp was allowed to evolve freely and where the bacteria eventually attained the same level of growth and activity. Thus, by the end of the experiments, the number of cells found in column H–S was similar to the number in the inoculated column (Li). Also, although values were lower in L, this was because experiment L/Li did not last as long as experiment H–S/S (15 weeks as opposed to 30). Given enough time, column L would eventually come to resemble column H–S, since they are identical. In consistence with this result, the pH evolution in columns L and Li drainages were identical, and the final value reached was close to 2 in the both weathering tests. Details on the chemicals are in the publications of García et al. [42].

The SRB count was negative in all cases (except column S). Since the columns were small, anoxic conditions did not develop because an exchange of oxygen took place both via the surface and through the drainage tube. This effect was further borne out by the fact that there were no differences between the numbers of aerobic microorganisms associated with surface and bottom samples in the same column.

Table 10
Microbial at the small column-weathering drainages

Specie/column	H–S	S	L	Li
SRB	–	–	–	–
Fe-oxidizing (cells/ml)	1.8×10^3	–	2.9×10^2	2.1×10^3
S-oxidizing (cells/ml)	1.8×10^5	–	6.5×10^2	1.8×10^4

3.3. Mineralogical analysis, column M

Fig. 4 shows diffractograms of three different samples. In the surface sample (Mh1), the intermediate sample (Mh2ext) and the bottom sample (Mh4), pyrite was the main component, with all its characteristic peaks clearly identifiable, indicating that the bulk of the mineral had not yet been transformed. This is consistent with the pH and the metal concentration measured in the successive effluents during weathering [41]. But even so, the waste did not evolve evenly throughout the column, so that in the more exposed surface zone the pyrite underwent some oxidation and hence the formation of sulfates. We specifically identified lead sulfate, a product of oxidation of lead sulfide, which was found as a minority component accompanying the pyrite in the initial sample (Tables 2 and 3). This behaviour is

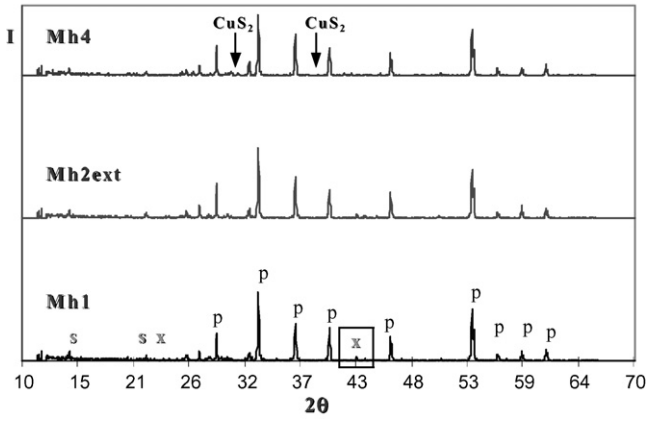

Fig. 4. Diffractograms corresponding M column dismantling (p, pyrite; *x*, lead sulfate; s, silicate).

to be expected of a sulfide exposed to oxygen and water as in surface sample Mh1. The lead sulfide present in minor quantities in the sample (Fig. 4) was oxidized preferentially with respect to the pyrite because it has less rest potential. As we know [43], any sulfide in contact with a given aqueous medium has a specific rest potential. When two or more mineral sulfides come into contact, the one with the highest rest potential acts as a cathode, while one with a lower rest potential may be the anode. It has been shown [43] that pyrite facilitates oxidation of mineral sulfides PbS, ZnS and $CuFeS_2$, while the same minerals retard pyrite dissolution. The rest potentials of pyrite, galena, sphalerite and calcopyrite respectively are 0.66, 0.40, 0.46 and 0.56 V for a medium at pH 4.

This lead sulfate is less well defined in the intermediate sample (Mh2ext) and does not appear in the bottom sample (Mh4), which is natural given the smaller degree of conversion.

The surface mineral behaved differently from the bottom sample: no lead sulfates were found associated with sample Mh4, while lead sulfides were identified there but not in sample Mh1. The explanation for this experimental finding is that certain lead sulfides, which might have accompanied the original mineral, along with a very small fraction of chalcopyrite, were attacked during the weathering process (watering) in the upper part of the column.

The dissolution of the sulfides further led to the appearance of dissolved lead and copper, which must have migrated to the bottom of the column during the successive waterings. These metals would encounter a reducing medium at the bottom promoted by the biological activity of the SRB, and the copper would precipitate again as a sulfide. This would explain why the characteristic peaks of CuS_2 appeared in the diffractogram for the bottom sample, Mh4.

The behaviour of sample Mh2ext was intermediate: on the one hand, it still had the products of pyrite oxidation associated with it; and on the other hand the effect of dissolution of the PbS and the $CuFeS_2$ was detectable in the low peak intensity.

The pyrite was also accompanied by traces of the silicates $Ca.5SiO_3{\cdot}5H_2O$ and $K_2Mg_5Si_{12}O_{30}$.

3.4. Activity assays

In the microbiological characterization of the samples from dismantling of the columns, it was important to take into account not only the number of bacteria present, but also the state of activity in which they were found. Since column M presented more variability and microbiological selection than the others (see Tables 8 and 9), it was decided to evaluate the oxidizing and reducing activity in the various layers of that column, and to this end two assays were prepared: one to monitor bacterial oxidation of the ferrous ion, and another to evaluate the biological reduction of the sulfate anions.

3.4.1. Iron-oxidizing activity

The bacteria associated with the two most oxygenated samples in the column (Mh1 and Mh3) were more active against oxidation of the ferrous ion than those from less aerated samples; no major differences in activity were observed at these locations (Fig. 5) even although they were not evenly distributed in quantitative terms (Table 8).

These findings can be explained in terms of the model chosen: because of the characteristics of the column (bed height, watering regimen, etc.), the chemical conditions throughout did not differ very much, since part of the oxygen was able to pass through the column by way of the preferential percolation channel that formed as weathering progressed.

3.4.2. Sulfate-reducing activity

Fig. 6 shows the results of the assays to evaluate sulfate-reducing activity. They indicate that the sulfate-reducing bacteria were highly sensitive to the chemical conditions prevailing at the various points, especially the presence of oxygen, so that these bacteria were only active in the samples lowest down the column (Mh4ext and Mh4int). There was no decrease in sulfate concentration or increase in the pH in the case of sample Mh2int, even although the counts had shown positive growth for that sample.

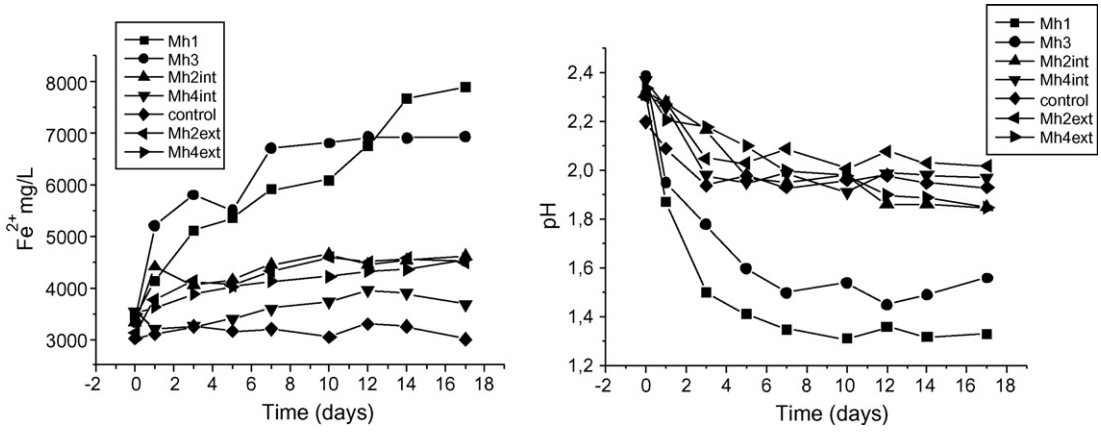

Fig. 5. Evolution of the Fe^{2+} concentration and pH at the activity tests.

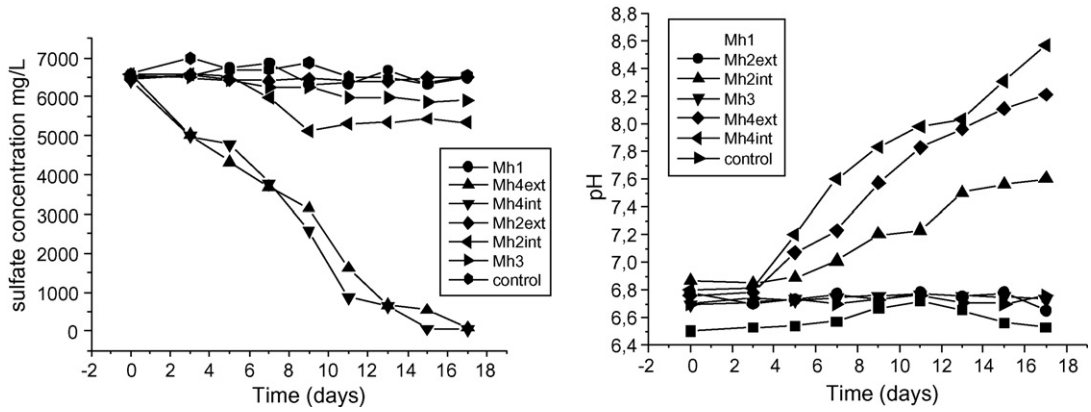

Fig. 6. Evolution of the SO_4^{2-} concentration and pH at the activity tests.

4. Conclusions

1. The ore itself had associated microflora which catalysed pyrite conversion. This "natural" population was associated with the mineral, eventually creating its own microenvironment at the various locations on the column, depending on the chemical conditions prevailing there (columns S, H–S and Li).
2. Weathering of the mineral generally enhances microbiological growth: with weathering in column M, iron-oxidizing species and anaerobic bacteria developed which had not been found in the initial characterization of the pulp. There was also an increase in the concentrations of sulfur-oxidizing bacteria and heterotrophic acidophiles.
3. Microbiological selection is established in a weathering column according to the oxygen contribution. There were two distinct zones in column M: a surface zone, where S- and Fe-oxidizing lithoautotrophic bacteria grew preferentially, and an anoxic zone at the bottom, where mainly SRB grew.
4. The quality of the water draining from a column is a product of the combined effect of microbiological oxidation and reduction processes. In our assays, the bacteria were not only present but active as well. The pH level and the metal and sulfate concentration measured in water draining column after weathering exhibited the habitual characteristic of acid mine drainage effluents. Obviously, water of this characteristics is inadequate to released to the environment without treatment (a biotreatment with sulfate-reducing bacteria may be a possibility [40]). The disposal of mineral under water is the possibility to prevent the acid mine generation. In this case, the quality of the water probably would let release the effluent to natural environment (with cautiously previous analysis).
5. The mineralogical data confirmed the results from the microbiological study: associated with the surface mineral was a higher proportion of aerobic bacteria capable of oxidizing the mineral, whose metabolic activity was reflected in the presence of sulfide oxidation products. The conditions predominating at the bottom of the column were absence of oxygen and metabolic activity of SRB, so that what was detected were not pyrite oxidation products but rather products of precipitation caused by a reducing environment containing H_2S.

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