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BIOREMEDIATION OF AN INDUSTRIAL ACID MINE WATER BY METAL-TOLERANT SULPHATE-REDUCING BACTERIA

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ABSTRACT

The microbiological diversity associated with mining environments is a very well proven fact. One of the communities appearing in these environments is that formed by anaerobic sulphate-reducing bacteria (SRB) which can be used for the decontamination of acid mine drainage waters. In this work, the potential of a mixed population of SRB, isolated from the bottom of a pyritic tailing pond situated in the Spanish pyritic Belt, has been investigated with the main objective of treating the effluent generated in the same disposal site. The efficiency of the system is based on the presence of an important amount of reducing agents contained in the acid mine drainage received in the pond. Results showed that this option is effective for the precipitation of the dissolved metals (copper and iron), for the reduction and removal of sulphates and even for the alkalising of the waters. SRB were able to remove up to 9,000 ppm of sulphate ion efficiently, to grow in the presence of up to 100 ppm of copper and 30 ppm of iron, and alkalise the medium, provided that this was not extremely acidic (pH>4). Finally, according to the results obtained, the possibility of applying this method to the treatment of a real effluent is discussed.

Keywords
Acid rock drainage; sulphide ores; tailing disposal; bacteria; environmental

INTRODUCTION

One of the most important problems affecting mining companies around the world is the treatment of acid mine drainage (AMD) also known as acid rock drainage (ARD). AMD is characterised by its high acidity, high concentration of metals (for instance, Cu, Fe, Zn, Al, Pb, As, Cd, etc.) and high concentration of dissolved sulphates. Typically, the pH is lower than 3 and the sulphate concentration is higher than 3,000 ppm (Lyew *et al.*, 1994). Therefore, the treatment of mining effluents is aimed at water neutralisation and removal of the dissolved metals and sulphates.

The techniques traditionally used for the treatment of residual acid mine waters have been based on chemical methods of neutralisation and precipitation. These techniques, even though quick and effective, present several disadvantages, such as the need for building additional treatment plants, the high cost of the chemical reagents used and the generation of an important volume of sludges which need to be relocated.

A possible alternative to the chemical treatment of these effluents is bioremediation using anaerobic sulphate-reducing bacteria (SRB), taking advantage of the fact that these microorganisms grow in mining environments. In fact, several SRB have been isolated from the bottom of mining tailing ponds (Dugan, 1975; Babij *et al.*, 1980). Likewise it has been proven that aquatic ecosystems contaminated by acidic waters have been capable of naturally recovering the initial situation thanks to the presence of species with the capacity to buffer the medium. The anaerobic bacterial processes, as in denitrification and biological reduction, are implied in these phenomena (Gyure *et al.*, 1990).

SRB are useful to abate AMD due to two fundamental reasons. Firstly because of their capacity to reduce sulphate to sulphide. These sulphides react additionally with certain metals dissolved in the contaminated waters, such as copper, iron and zinc, forming insoluble precipitates. On the other hand, the system acidity is reduced by their own action of sulphate reduction and by the carbon metabolism of the bacteria.

Nevertheless bacteria have certain specific environmental requirements that must be fulfilled: an anaerobic environment with a redox potential lower than -100 mV and a pH higher than 5.5. Both factors can limit the AMD treatment when the medium conditions have to be fixed. In any case, in contrast to this fact, biological activity in wetlands and lake sediments receiving AMD has been observed, due to the presence of active SRB populations even at pH around 3 (Lyew *et al.*, 1994).

Another questionable aspect is whether these bacteria can grow in the presence of high concentrations of metals, especially in the presence of copper which is often present in AMD, and moreover which is toxic to them.

In addition, the possible application of a mixed culture of SRB, isolated from the system studied, to treat a real AMD was considered. For this, different variables were analysed: pH range in which the bacteria remain active, growth potential in the simultaneous presence of up to 200 ppm of copper and 30 ppm of iron, and efficiency of reducing up to 9,000 ppm of sulphate from AMD.

MATERIALS AND METHODS

Sampling procedure

The biological material used for the different tests was formed by a mixed culture of SRB isolated from the sludges deposited at the bottom of a pyritic tailing pond, belonging to a mine of polymetallic sulphides, situated in the Southwest of Spain.

For the physical, chemical and microbiological characterisation of the system, samples were taken at 12 m depth with the help of a sludge sampler (Ballcheck KB) which is especially used for this objective because it avoids the mixture of water layers with the sample. All samples were maintained in sterile flasks, totally full to avoid the influx of dissolved oxygen in the solution, until their transfer to the analysis laboratory. Samples of superficial waters of the pond were also taken in sterile flasks. The samples for the identification of SRB were handled in anaerobic conditions.

Isolation and enumeration of SRB

Several enrichment cultures both in Postgate's C medium (Postgate, 1984) at pH 7 and 30 °C for sludge samples and in Postgate's C medium, double concentrated, for superficial waters were prepared. In both cases 10% (v/v) inocula were used. The growth of SRB was detected by the formation of black precipitates at the bottom of the flasks (in the solid–liquid interface) which also appeared on the flask walls. In addition the smell provoked by the hydrogen sulphide was obvious. Even so, to confirm that SRB activity and to strengthen that hydrogen sulphide formation, and the consequent precipitation of sulphides, was due to the

sole presence of these anaerobic bacteria and not to any possible chemical reaction, the same inocula in Postgate's C medium with 0.01 M sodium molybdate were carried out. This last anion is an analogous of sulphate which interferes in the first enzymatic step of this ion and more concretely in the enzyme sulfurilase (Taylor and Oremland, 1979). The appearance of solid sulphides in the flasks, where the cultures were grown in the presence of molybdate, will discard to the bacteria as the sole responsible of reduction. In addition, SRB was checked by means of their growth in the presence of oxygen and in a medium without sulphate ion. The bacteria were counted in Postgate's C medium using the Most Probable Number (MPN) method (Pochon and Tardiex, 1974).

Isolation and enumeration of acidophilic lithoautotrophic bacteria and total heterotrophic bacteria

The superficial samples of water taken from the pond, in accordance with their chemical characteristics, were grown at 35 °C in 9K medium (Silverman and Lundgren, 1959) to identify acidophilic lithoautotrophic bacteria and in A and I media at 30 °C to identify total number of heterotrophic bacteria. Again the bacteria were counted using the MPN procedure.

Culture media

Postgate's C medium

0.5 $g.L^{-1}$ KH_2PO_4, 1.0 $g.L^{-1}$ NH_4Cl, 0.06 $g.L^{-1}$ $MgSO_4.7H_2O$, 3.5 $g.L^{-1}$ sodium lactate (70%), 1.0 $g.L^{-1}$ yeast extract, 1.0 $g.L^{-1}$ $CaSO_4$, 0.01 $g.L^{-1}$ $FeSO_4.7H_2O$, 4.5 $g.L^{-1}$ Na_2SO_4, 0.06 $g.L^{-1}$ $CaCl_2.6H_2O$, 0.3 $g.L^{-1}$ sodium citrate, pH was adjusted to 7 with NaOH (10% w/v).

Nutrient agar medium

Oxoid CM3 (1.0 $g.L^{-1}$ "Lab-Lemco" powder, 2.0 $g.L^{-1}$ yeast extract, 5.0 $g.L^{-1}$ peptone, 5,0 $g.L^{-1}$ sodium chloride and 15 $g.L^{-1}$ agar.

9K medium without iron

3 $g.L^{-1}$ $(NH_4)_2SO_4$, 0.1 $g.L^{-1}$ KCl, 0.618 $g.L^{-1}$ $K_2HPO_4.3H_2O$, 0.5 $g.L^{-1}$ $MgSO_4$, 0.013 $g.L^{-1}$ $Ca(NO_3)_2.4H_2O$. In addition, 9K medium was used containing one of the following elements or compounds as energy source: iron, tetrathionate, thiosulphate, or elemental sulphur. pH was adjusted to 2, 3 or 6 with the addition of sulphuric acid.

A medium

9K medium without iron at pH 3 with 10 $g.L^{-1}$ glucose, 5 $g.L^{-1}$ yeast extract and 15 $g.L^{-1}$ agar.

I medium

9K medium without iron at pH 3 with 0.5 $g.L^{-1}$ bactotryptone, 1 $g.L^{-1}$ malt extract, 10 $g.L^{-1}$ glucose and 15 $g.L^{-1}$ agar.

Adaptation of SRB

To prove the capacity of the bacteria to grow in acid media, different tests in Postgate's C medium at different pH (4, 5 and 7) were carried out. The medium pH was adjusted with the addition of dilute sulphuric acid. During four weeks and every 7 days 1 mL samples were taken for pH and Eh analysis (versus an Ag/AgCl electrode).

Biological precipitation of metals

For the bioprecipitation tests, Postgate's C medium with different concentrations of copper (25, 50, 100, 150 and 200 ppm, added as cupric sulphate pentahydrate) was used. The medium pH was adjusted to 5.

Parallel to the copper concentration, the iron concentration was followed. Fe was added to the initial solution through the Postgate's C medium as $FeSO_4.7H_2O$, with an initial concentration of 30 ppm. Metal concentrations in solution were determined by atomic absorption spectrophotometry. Samples for metal analysis were acidified immediately after collection.

Batch tests were carried out with two different cultures: a culture (Di) previously adapted at pH 5 by repeated subculturing in the presence of copper (varying concentrations) and 30 ppm of iron; and a culture (Ei) grown at pH 7.0.

Metal precipitation as an indirect consequence of biological activity was also checked by means of X-ray diffraction analysis of the obtained precipitates. For this a Phillips X'Pert d-MPD diffractometer was used.

Biological reduction of sulphates

The transformation of sulphate ion to sulphide ion was evaluated by following the variation of the sulphate concentration in the experiments prepared with Postgate's C medium with an initial concentration in sulphates of 9,000 ppm. This ion was analysed by a turbidimetric method (AWWA-APHA-WEF, 1998). The method was tuned in accordance with the alkaline characteristics of the samples. For this, it was necessary to acidify the sample with a solution of NaCl/HCl before the corresponding measurement. Turbidity was produced, provoking the reaction between 1 mL of sample and 1 mL of 0.1 M $BaCl_2$. Once taken, the samples were centrifuged for 10 minutes at 8,000 rpm, and always immediately before performing the analysis, because this sulphate determination is especially sensitive to the presence of solids in suspension.

Mineralogical and chemical analysis

X-ray diffraction and granulometric analysis were used to determine the mineralogical composition of the solids contained in the pulp samples. The chemical composition of AMD and sludge were carried out by atomic absorption spectrophotometry. The sulphur was analysed using an automatic analyser from Leco, and the sulphite was measured by volumetric analysis using iodine. The sulphate was analysed as described above.

RESULTS AND DISCUSSION

System description

The system studied was a decantation pond of pyritic tailings which belongs to a mining company in the Southwest of Spain. These tailings were produced from a flotation plant processing a complex metallic sulphide for the production of three different concentrates of copper, lead and zinc. The composition of the tailing generated in the plant corresponded to a pyrite pulp with 30% of solids. The most representative chemical characteristics of the aqueous phase poured to the pond is shown in Table 1 (drainage initial).

The pulp reached the pond by gravity through a channel of approximately 2.3 km which was open to the atmosphere. In the pond, the liquid–solid separation and several chemical and microbiological transformations took place. The final result was the generation of both a solid residue (sludge) deposited at the bottom of the pond and the AMD final whose chemical composition is also shown in Table 1. The composition of the initial and final drainages were very different because the pH of the solution decreased appreciably and led to an important increase in metal and sulphate concentration in solution because of a decrease in the sulphite concentration.

TABLE 1 Chemical analysis of the initial and final drainages

	Initial drainage	Final drainage (AMD)
pH	9 - 10	2.5 - 3.5
Eh (mV, versus Ag/AgCl)	(-20) - (+10)	220 - 350
Sulphate (ppm)	500 - 650	1,800 - 2,000
Sulphite (ppm)	88 - 120	2 - 3
Copper (ppm)	0.1 - 0.2	0.4 - 0.8
Iron (ppm)	0.4 - 2.0	50 - 55
Zinc (ppm)	0.1 - 2.0	30 - 50
Lead (ppm)	0.1 - 0.2	5 - 6
Calcium (ppm)	400 - 450	400 - 450
Magnesium (ppm)	15 - 20	50 - 55

Tailing characterisation

One of the main objectives of this work was to evaluate the possibility of using a mixed SRB culture isolated from the pond for the treatment of the AMD produced by the same system. The culture was isolated from the system studied because when the tailings are submerged under water, to avoid their oxidation, this kind of bacteria grows naturally. If under these conditions there is, in addition, organic matter, as it happens in the majority of industrial ponds, the natural biological reduction of sulphates takes place with the consequent formation of hydrogen sulphide and the bioprecipitation and stabilization of metals from the solution (Brierley and Brierley, 1997).

As described later, the water studied (AMD final in Table 1) did not have the usual characteristics of the classical AMD; although it was acid with an important amount of metals and sulphate dissolved, it did not show the extreme values of a conventional AMD. The differential factor with regard to the drainage of other mines was the presence of important concentrations of reducing agents (sulphite ion and other thiosalts) that conditioned its chemical characteristics. First, the intermediate species of sulphur were oxidised to sulphate with the consequent generation of additional acidity (García *et al.*, 1996), and second these reducing conditions rendered the conventional treatment methods difficult making the use of stronger oxidizers for the total elimination of the reducing compounds from the drainage necessary.

With this situation, although most of the mineral was under water, a practice that has been traditionally used to avoid generation of AMD (East *et al.*, 1994), the acidity of the water pond continued to increase, as a result of oxidation of the different intermediate sulphur species with oxygen dissolved in the water.

The final disposition of the tailings and the segregation of environments after disposal of the pulp in the pond produced decantation of solids at the bottom and two zones of liquid (an anaerobic zone at the bottom and an aerobic zone at the surface) as a function of oxygen gradient. The chemical compositions of the two zones appearing in the pond water are shown in Table 2.

Parallel to this chemical diversity a microbiological diversity was detected in the sludge whose characteristics are shown in Table 3. In the superficial water a great number of iron- and sulphur-oxidizing aerobic lithoautotrophic bacteria were detected. These bacteria did not prevail in the deepest samples. From the sludge of the pond bottom SRB were isolated. In this place, the bacteria found the optimum strict anaerobic conditions that they need for growth, in such a way that the sulphate-reducing activity was limited to the solid sediment zone of the pond.

TABLE 2 Chemical composition of the pond waters

	Superficial AMD	Sludge from the pond bottom
pH	2.5 - 3.5	6 - 7
Eh (mV, versus Ag/AgCl)	220 - 350	(-45) - (-3)
Sulphate (ppm)	1,800 - 2,000	400 - 500
Copper (ppm)	0.4 - 0.8	0.3 - 0.6
Iron (ppm)	50 - 55	3.3 - 5.0
Zinc (ppm)	30 - 50	0.1 - 0.3

TABLE 3 Microbiological diversity in the pond (MPN.g^{-1})

Location	SRB	Lithoautotrophic bacteria	Heterotrophic bacteria
Superficial AMD	0	$1.1 \times 10^2 - 7.0 \times 10^3$	1.0×10^3
Sludge from the bottom	$1.7 \times 10^2 - 2.0 \times 10^3$	$2.5 \times 10^1 - 9.5 \times 10^2$	6.0×10^1

As was mentioned before, the microorganisms were counted using the MPN method. In this procedure the possible error produced should be taken into account because when a synthetic growth medium is used the number of viable SRB is underestimated (Vester and Ingvorsen, 1998).

The chemical composition of the sludge (w/w) was follows: S, 39.3%; Fe, 39.0%; Pb, 1.70%; Cu, 0.45%; Mg, 0.23%; Mn, 0.12%; Zn, 0.10%; Ca, 0.01. The main mineral was pyrite (63.4%, w/w) with a particle size of 30 μm. Other components were carbohydrates and silicates, 34.0%; chalcopyrite, 1.44%; sphalerite, 0.70%; and galena, 0.46%.

Alkalising of acid mine drainage

The possibility of treating AMD waters from the pond was evaluated, taking advantage of the metabolic activity of SRB isolated from the system, by carrying out several tests of growth with mixed cultures at different pH values. As was stated previously, the pH values tested were 4, 5 and 7.

As a consequence of the bacterial metabolism an increase in the pH of the culture medium was observed, reaching values close to 8.5 (Figure 1). The reaction responsible for this increase was the following:

$$2\,H^+ + SO_4^{2-} + 2\,C_{org} \rightarrow H_2S + 2\,CO_2\,(g) \qquad (1)$$

The initial pH values appearing in Figure 1 at zero time do not coincide exactly with 4, 5, and 7. These last values were the pH values at which the respective culture media were prepared but the inoculum used increased the initial pH.

The redox potential however, was negative provided that the bacteria grew appropriately (Figure 2). The bacteria generated an appreciable negative potential, even starting from the potential of an oxidising medium.

Therefore, the chemical and microbiological data of the water indicated that the SRB were active. In this sense, the redox potential was decreasing, black precipitates appeared in the medium and the presence of hydrogen sulphide was detected in the aqueous phase, with the classical strong smell (Christensen *et al.*, 1996).

Although the optimum pH of growth for this type of microorganisms is between 7.0 and 7.5, the mixed cultures tested were adapted at pH 5 without problems. However bacteria grew with some difficulty on a moderately acidic medium (pH 4) and although a tendency for a decrease in the Eh and an increase in the pH was observed, which are indications of SRB activity, this acid medium was excessively acidic for the bacteria. This growth difficulty in acid medium has been mentioned often in the literature and it is a controversial aspect in the possible treatment of AMD using SRB (Tuttle *et al.*, 1969; Arnesen and Iversen, 1991). However, these low pH values do not only inhibit the bacteria activity (Dvorak *et al.*, 1992) but also contribute to increasing the solubility of the metallic sulphides formed, through the classical reaction of solubilization in the presence of oxidants and acids.

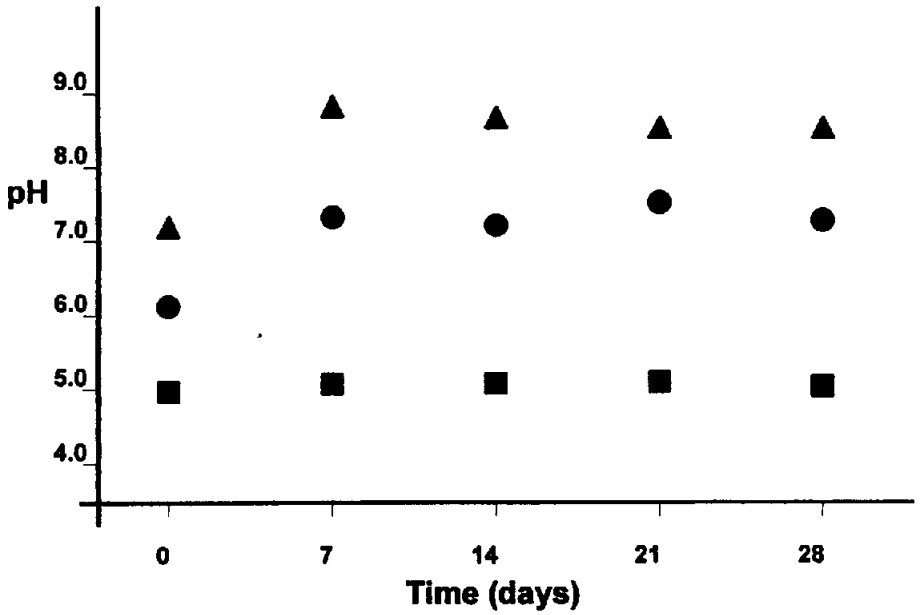

Fig.1 pH variation during SRB growth at different initial pH (■ pH 4, ● pH 5, π pH 7).

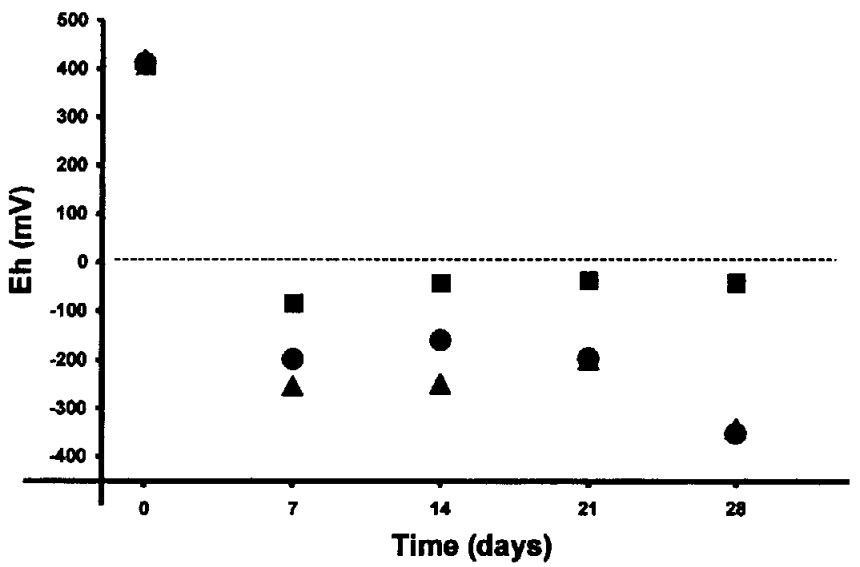

Fig.2 Eh variation during SRB growth at different initial pH (Eh versus Ag/AgCl electrode) (■ pH 4, ● pH 5, π pH 7).

In fact, to maintain SRB active in an ecosystem with extreme acidity, for instance in the presence of acid mine drainage, it is necessary either to adapt previously the bacterial populations to these aggressive conditions or to include in the AMD treatment process a previous soft neutralization step which is less expensive than the conventional chemical treatment and where the volume of sludges generated is also smaller (Béchard *et al.*, 1990).

Bioprecipitation of metals

One of the consequences of the SRB activity is the precipitation of the metals dissolved in the mine waters after their reaction with the hydrogen sulphide produced in the sulphate ion reduction (Kuyucak and St-Germain, 1994) according to reactions [1] and [2]:

$$H_2S + Me^{2+} \rightarrow MeS\downarrow + 2\,H^+ \qquad (2)$$

where Me represents Cu, Fe, Zn, Ni, Cd, Pb, etc., ions usually present in AMD waters.

If the biological reduction is totally effective for the metal ion removal, 1 mole of sulphate, after its reduction, would eliminate 1 mole of metal (reactions [1] and [2]). However the elimination of 1 mole of ferric iron would really require 1.5 moles of sulphate. Ferric iron, nevertheless, can also be precipitated as hydroxide as well, although its elimination probably occurs through the reduction to ferrous iron and the consequent later precipitation of the divalent metallic sulphide (Eger, 1994):

$$4\,Fe^{3+} + CH_2O + H_2O \rightarrow 4\,Fe^{2+} + CO_2 + 4\,H^+ \qquad (3)$$

In the bioprecipitation tests, SRB (Ei, culture not adapted) grew well in the whole range of chosen concentrations in such a way that after 21 days a total removal of copper from solution was obtained. However, iron was also efficiently removed (Table 4). With Di culture adapted to pH 5 good results were also obtained (Figure 3). In this figure the initial concentrations at time zero do not coincide exactly with the values indicated in the Figure legend (25, 50 and 100 ppm). The explanation is that, although the culture medium was prepared taking into account these concentrations, when the inoculum was added the pH increased and at the same time a certain metallic precipitation took place.

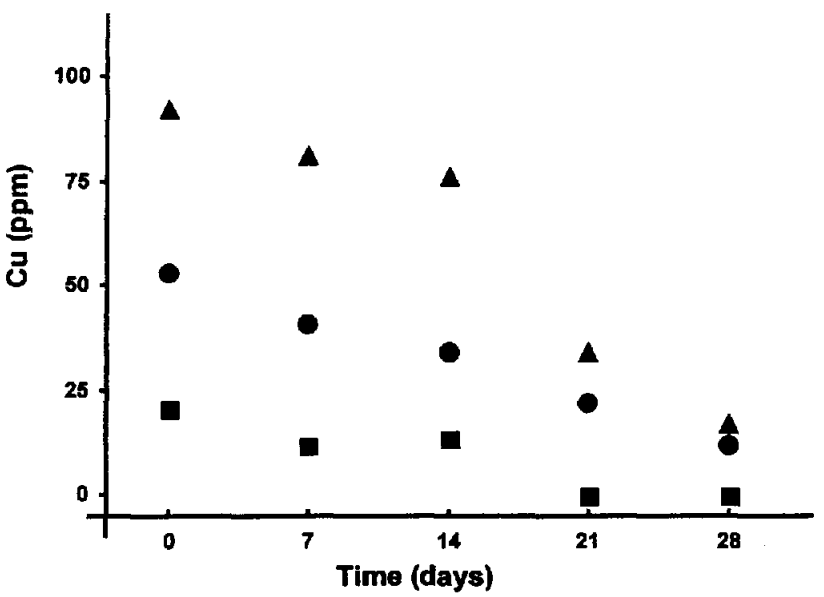

Fig.3 Biological removal of copper. The inocula was previously adapted at pH 5 (■ 25 ppm Cu, ● 50 ppm Cu, π 100 ppm Cu).

TABLE 4 Efficiency (%) for the biological removal of copper and iron after 21 days

Culture	25 ppm Cu/ 30 ppm Fe	50 ppm Cu/ 30 ppm Fe	100 ppm Cu/ 30 ppm Fe
% Fe removed (not adapted, pH 7, Ei)	93	93	97
% Cu removed (not adapted, pH 7, Ei)	99	99	99
% Cu removed (adapted, pH 5, Di)	99	58	63

Therefore the presence of copper and iron in the culture medium did not inhibit the growth of SRB which adapted well up to concentrations of 100 ppm copper and 30 ppm iron. Although an increase of copper concentration inhibits bacterial growth (as is reflected by both the time that the cultures took to reach negative potentials and the residual copper remaining in solution after similar periods of time), in all the cases (25, 50 and 100 ppm) bacteria were active which did not happen with the cultures grown in the presence of 200 ppm of copper.

When the cultures were adapted to increasing concentrations of copper, 200 ppm was the upper limit of concentration at which there was no bacteria growth. The concentration of 150 ppm did not prevent the growth shown by the formation of black precipitates and hydrogen sulphide. However growth kinetics were extremely slow.

The bioremoval of metals was also demonstrated by means of X-ray diffraction analysis of the precipitates obtained. Although iron can be precipitated by complexation with either sulphide or hydroxyl ion (Lyew and Sheppard, 1997), the most stable precipitate was FeS_2, pyrite, whereas copper precipitated as Cu_7S_4. It is important to stress that a high concentration of free hydrogen sulphide in water, without the capacity to react with dissolved metals could inhibit bacterial growth. Stucki *et al.* (1993) point out that 40 ppm of free hydrogen sulphide can negatively affect sulphate reduction.

Biological reduction of sulphates

The biological reduction of sulphates was also effective as well and in agreement with the preceding results the bacteria were active, from the point of view of their metabolism, both at pH 7 and at pH 5. The sulphate concentration decreased by about 85% in 27 days at pH 7 whereas at pH 5 a similar result was obtained in 9 days (from 9,000 ppm to 1,350 ppm). As it was predictable pH increased to alkaline values (pH 8.2) and Eh decreased to negative values (-250 mV) during the tests. In all the tests the SRB were capable of inducing their own environmental conditions (pH, Eh, etc.) for an optimal growth because they can control their microenvironment even when the bulk solution pH is below 5 (Eger, 1994).

Application of SRB to the bioremediation of a real AMD

In order to confirm the experimental results, an industrial AMD obtained from the studied pond and supplemented with copper (25 ppm) was treated using adapted cultures of SRB isolated from the pond. For the bioremediation three different effects were considered: metals precipitation, sulphates reduction and medium alkalising.

In principle, the use of SRB is sensible because at these levels of concentration the bacteria showed optimum behaviour. In fact, with copper concentrations of around 25 ppm and pH close to 5 the bacteria grew very favourably which was clearly proved through both the increase in pH values and the decrease in the redox potential of the medium (Figure 4). After 17 days, the 25 ppm of copper contained in the solution had been precipitated (Figure 5).

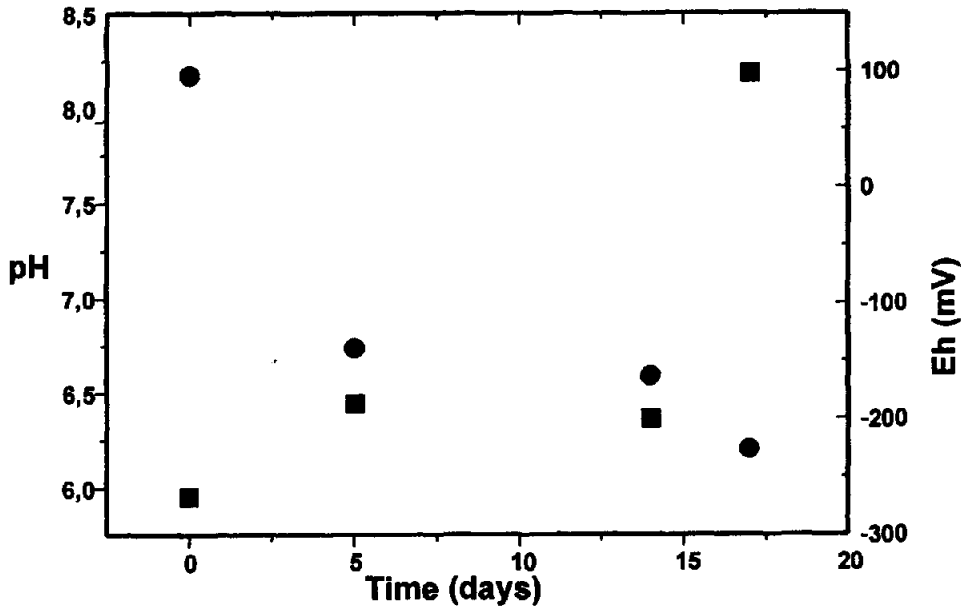

Fig. 4 Alkalising of AMD in the presence of SRB at pH 5 (■ pH, ● Eh).

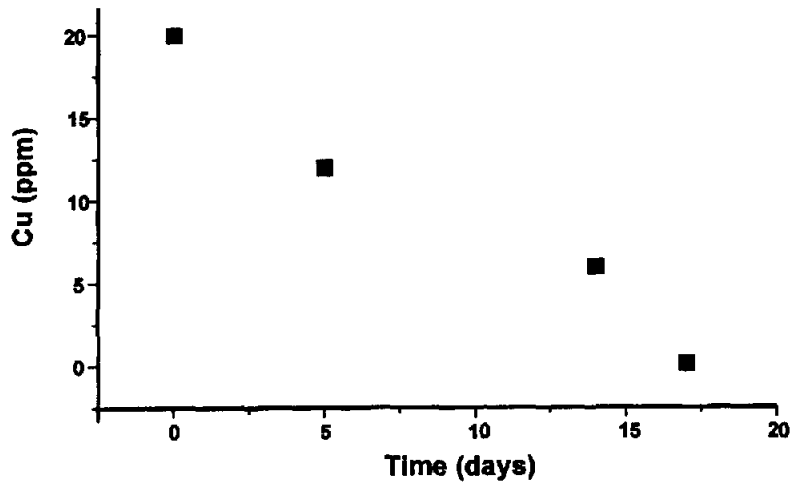

Fig. 5 Biological removal of copper in AMD by SRB at pH 5.

From a general point of view, there are two possible limiting factors for bacterial growth in the water: the sulphate concentration and the carbon source.

Concerning the first factor, the concentration of sulphate contained in the water pond was always smaller than the sulphate concentration tested in previous experiments in which concentration depends on the sulphate content in the nutrient medium used. Nevertheless, it is important not to forget two additional aspects: a residual sulphate concentration of around 2,000 ppm always existed in the pond, and the bacteria used in these tests come from this natural medium, the pond, where they were clearly active. Therefore this factor must not be limiting for the treatment of the pond waters.

Additionally, the other limiting factor can be the carbon source necessary for the SRB growth because in this type of aquatic systems the flux of organic matter to the sediments deposited at the bottom can control the rate of sulphate reduction (Gyure *et al.*, 1990). Similarly it is logical to consider that in the natural medium of the pond there exists some source of organic compounds providing the necessary carbon for the

evolution of the cultures. Among these media, the following could be considered: different products excreted by other microorganisms, organic matter blown away by the wind and deposited in the pond or even chemical reagents of organic nature from the flotation processes which are not lethal for this kind of bacteria.

In any case, the most significant limiting factor for the treatment of the pond waters using SRB could be the low level of pH reached (3.0–3.5) when the water remains still. These acid pH levels inhibit SRB growth. However, if the behaviour of the tailing is analysed in the system studied, it can be concluded that the pulp is discharged to the pond with a pH value of around 5, and only later the pH decreases. Therefore, the biological reduction of sulphate, the consequent metal precipitation and the alkalising of the medium might take place in the zone where the pulp is discharged to the pond, when the conditions are not yet extreme.

CONCLUSIONS

Results showed that mixed cultures of sulphate-reducing bacteria isolated from mine drainage water and adapted at pH 5, can increase the pH of the medium provided that the initial pH was higher than 4. Bacteria removed copper and iron simultaneously. In 21 days copper was removed either totally, when the initial concentration was 25 ppm, or partially (63%), when the initial concentration was 100 ppm. On the other hand, 30 ppm of iron were eliminated without special difficulties. In addition, these metals were recovered as stable sulphides: Cu_7S_4 and FeS_2, respectively. With reference to sulphates, about 85% of 9,000 ppm were reduced in 9 days. These results highlight the viability of applying anaerobic degradation as a treatment method for AMD.

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