BIOREMEDIATION OF ACID MINE DRAINAGE USING ACIDIC SOIL AND ORGANIC WASTES FOR PROMOTING SULPHATE-REDUCING BACTERIA ACTIVITY ON A COLUMN REACTOR

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Abstract. Acid mine drainage (AMD) is a serious environmental problem resulting from extensive sulphide mining activities. The old copper mine of S. Domingos in Southeast Portugal is an example of such a situation. The abandoned open-pit from the mining operations resulted in the creation of a large pit lake with acidic water (pH$\sim$2) and high contents of sulphate and heavy metals. Sulphate-reducing biological processes have been studied as a remediation technology for this problem. A new application based on a simple and semi-continuous process for the treatment of S. Domingos AMD has been presented herein. Experiments using bench scale fixed-bed column bioreactors were carried out to evaluate the efficiency of the process. Sewage, anaerobic sludge and soil from the mining area were tested as solid matrices and/or inocula, as well as sources of complex organic substrates. The addition of lactose as a supplementary carbon source, easily available at zero cost or at negative cost in the effluents of the local cheese industries, was also tested. The data obtained indicate that it is possible to use the matrices tested for the production of sulphide by sulphate reduction, and that the regular addition of lactose is effective. Results showed that the process is efficient for the precipitation of the main dissolved metals, for the reduction in the sulphate content and, most importantly, for the neutralization of the AMD. Moreover, the use of soil as solid support also showed the possibility of using this process for the decontamination of both waters and soils.

Keywords: acid mine drainage, bioremediation, heavy metals, sulphate, sulphate-reducing bacteria

1. Introduction

Mining and metallurgical operations have been considered to be the major sources of heavy metal contamination in the environment. In Portugal mining operation is age-old, dating back to the Pre-Roman and Roman periods (Mateus *et al.*, 2000). These activities played an important role in the Portuguese economy of the 19th and 20th centuries, but in recent decades practically all the mining extraction has been suspended (about 80 mine sites were estimated to exist in the country) (Santos Oliveira *et al.*, 2000), and, as a consequence, several mine sites have been left untreated. The old copper mine of S. Domingos, located in the Southeast part of Portugal in the Iberian Pyrite Belt, is an example of such a situation. The abandoned

open-pit from the mining operations resulted in the creation of a large pit lake with acidic water containing high contents of sulphate and heavy metals. This acid mine drainage (AMD) water represents a harsh and extreme environment for biological activity, killing plants and animals and inhibiting the normal decay of the organic matter, thus allowing the permanent pollution of the surrounding ecosystem. Therefore, it should be contained to avoid groundwater contamination and it must be subjected to suitable treatment before being discharged. To minimize the environmental impacts caused, several studies within the country have already been conducted and reported (Santos Oliveira *et al.*, 1999, 2000; Alvarenga *et al.*, 1998, 2004.).

The AMD problem arises whenever sulphidic waste rock or mine tailings are exposed to oxygen and water occurring from the following reaction:

$$2FeS_2 + 7O_2 + 2H_2O \Leftrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \tag{1}$$

leading to solutions characterized by low pH (1.5 to 3.5) (Steed *et al.*, 2000) and large concentrations of sulphate (usually higher than 3 g/L) and dissolved heavy metals (for instance Fe, Cu and Zn, Al, Pb, As, Cd, etc.) (Garcia *et al.*, 2001), being the ions and their concentrations variable depending on the composition of the minerals lixiviated (Castro *et al.*, 1999). The oxidation of most sulphides can follow several routes depending on various factors, such as the pH and the availability of potential oxidants (e.g. ferric ion) (McConchie, 2003). Additional pathways occur when the oxidation reactions are microbially assisted (e.g. *Acidithiobacillus ferrooxidans*). Bacterial activity can contribute to a great increase in the rate of acid generation, as a result of sulphide mineral decomposition and iron oxidation (McConchie, 2003). Acid can also be generated when some metal ions (e.g. Fe^{3+} and Al^{3+}) react with water (Castro *et al.*, 1999), in addition to the direct release mechanism of acid.

Traditionally, AMD waters have been treated by chemical precipitation such as *in situ* addition of alkaline agents, usually lime, or other high alkalinity reagents (e.g. soda ash, calcium, or sodium hydroxide) (Burgess and Stuetz, 2002; Shokes and Moller, 1999; Tuppurainen *et al.*, 2002), to raise the pH and induce the formation and precipitation of metal hydroxides. Lime treatment is simple and has produced a satisfactory water quality during decades (McConchie, 2003). However, this classical method is expensive (Garcia *et al.*, 2001; Burgess and Stuetz, 2002) and generates voluminous amounts of residual mixed metal hydroxide sludge (Garcia *et al.*, 2001; Foucher *et al.*, 2000), which have to be dumped over large areas. Furthermore, it cannot meet new and more restrictive metal discharge standards, nor can it reduce the sulphate concentration below 1500 mg/L (De Vegt *et al.*, 1998). In fact, the evolution of environmental constraints has significantly contributed to the development of new decontamination technologies, and hence several alternative approaches, based on less costly and on green technologies, have been proposed to remediate AMD and other metal contaminated sites (effluents and solids such as soils, sludge, and sediments). Among those solutions, the

use of natural acid neutralizing minerals (e.g. the Bauxsol process) (McConchie, 2003), sorption materials, such as ion-exchange resins, membranes, or biomass-based methods (e.g. Algasorb) (Prasad *et al.*, 1999; Barnes, 1998; Lima *et al.*, 2001), are a few of the examples already commercially available. Another new technique, that is being examined and that could be effective in arid zones, involves the reaction of metal-rich AMD with highly concentrated saline brines, which can neutralize the acid and allow the controlled precipitation of marketable metal salts (McConchie, 2003). Other strategies, which can eventually be applied to some dam or pit lake waters, include evaporation processes (McConchie, 2003), the use of permeable reactive barriers, PRBs (Benner *et al.*, 1999; Gibert *et al.*, 2003) or electrochemical techniques (Shokes and Moller, 1999), the latter allowing the recovery of some metals (particularly copper) as a commercial resource. However, when used alone, this approach cannot produce water with a suitable composition for discharge, and the income from metal recovery would seldom compensate the cost of the treatment (McConchie, 2003). Although some of those new technologies can produce very clean water, most solutions involve substantial capital investment and ongoing costs, making the processes economically prohibitive. Hence, in spite of all these promising technologies, most water treatment facilities still rely on the use of lime or other alkaline agents to increase the pH and to reduce the metal content by precipitation, absorption and co-precipitation, followed by sludge removal and disposal.

Microbial treatment of AMD based on the use of sulphate-reducing bacteria (e.g. *Desulfovibrio desulfuricans* or *Clostridium desulfuricans*), has been considered a promising approach, possibly the only one able to remove sulphate economically (Barnes, 1998). Sulphate-reducing bacteria (SRB) use sulphate as the terminal electron acceptor in the metabolism of the organic matter, reducing it to sulphide in anaerobic conditions, according to the following equation:

$$2H^+ + SO_4^{2-} + 2C_{(org)} \Leftrightarrow H_2S + 2CO_2 \tag{2}$$
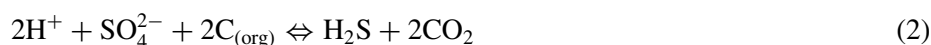
According to the literature (De Vegt *et al.*, 1998), the proposed 500-ppm sulphate standard and the, even more restrictive, 250-ppm sulphate drinking-water standard, can both be achieved. In fact, this natural technology has been identified as a potentially cost-effective way to overcome the limitations of traditional methods, since it can offer an efficient way to neutralize the acid in the AMD and to eliminate sulphate and metals simultaneously by converting them in very insoluble sulphides as represented by Equation 3:

$$M^{2+} + H_2S \Leftrightarrow MS(\downarrow) + 2H^+ \tag{3}$$

where M^{2+} represents the dissolved metal.

The metal sulphide sludge produced is more compact, faster settling and less subject to dissolution than the equivalent metal hydroxide sludge. Another advantage

of this method can be the possibility of the selective precipitation of the metals and several studies performed with that aim are reported (Foucher *et al.*, 2000; Luptakova *et al.*, 2003). As drawbacks were mentioned problems related with the maintenance of stable bacterial populations, and the fact that some metals (e.g. Al) do not form low soluble sulphides in water (McConchie, 2003).

Considering the promising features of microbial technologies involving SRB, this paper describes a laboratory scale investigation carried out to evaluate the feasibility of a new bioremediation process applied for the treatment of AMD from the S. Domingos mine.

2. Materials and Methods

2.1. AMD SAMPLES

AMD water samples from the mining area of S. Domingos were collected (November 2003) in two critical points of the mining area, indicated in Figure 1: one in the pit lake of S. Domingos (point A), and the other in the stream that comes from S. Domingos to Mosteirão River (point B). The water from the open pit was used as the columns influent.

2.2. EXPERIMENTAL SET-UP AND PROCEDURE

Glass columns of 5.5 cm diameter and 35 cm in length were constructed for the bioremediation experiments.

Three column experiments were performed.

Two of those columns (columns 1 and 2) were filled with three solid matrix layers, each with 200 g: solid sewage at the base of the column, anaerobic sludge in the middle and acidic soil (collected nearby the pit lake) on the top. Both solid sewage and sludge were from a waste water treatment plant, and along with the soil they were used as source of inocula and/or organic substrates.

Column 3 was filled using only two layers: 200 g of solid sewage in the base of the column and 200 g of anaerobic sludge above.

200 mL of a nutrient media for SRB (Postgate C), (Postgate, 1981), was also added to each column.

In column 1, the pH of the mixture of 200 g of the acidic soil and 200 mL of the nutrient medium was previously increased from about 1.4 to 6.5 by the addition of NaOH. In columns 2 and 3 no pH adjustment was made.

Thereafter, an acclimatization procedure was then started, lasting about 2 weeks, during which time the sulphate-reducing bacteria grow adapted to the matrix conditions. After that period, the addition of AMD from the S. Domingos pit lake started. Volumes varying from about 5 mL in the beginning, to about 50 mL in the end of the experiments, were generally added (and, a corresponding volume removed) to each column on a daily basis. All columns were gravity fed with the influent.
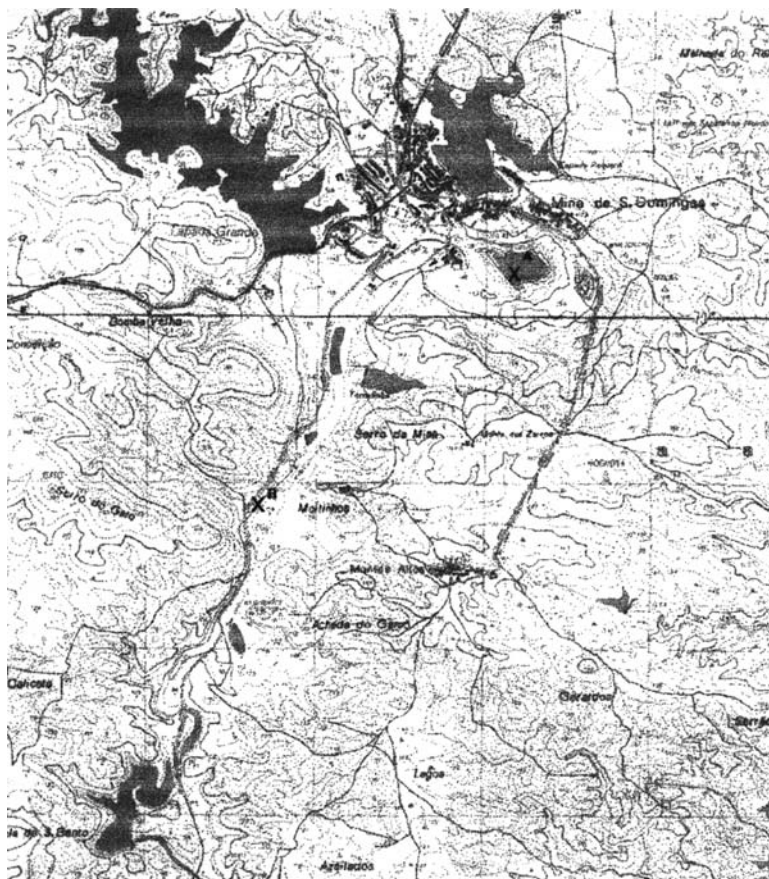
Figure 1. Location of the sampling points (X A and X B) in S. Domingos mining area. (Extract of chart n°559 of the Cartographic Service of Portuguese Army at scale 1/25000.)

After the biomass acclimatization, samples of the effluent were taken and analyzed every week. After pH monitoring, they were filtered and acidified (pH~2, by adding HNO_3) prior to sulphate and metals analysis.

Lactose was utilized as supplementary carbon source to revitalize "spent" matrices that were no longer efficiently removing sulphate. Thus, 1–2 g of lactose dissolved in the AMD was added to columns 1, 2 and 3, after 72, 123 and 110 days, respectively.

2.3. ANALYTICAL METHODS

Iron, zinc and copper concentrations in the AMD and in the effluents of the columns were determined by Atomic Absorption Spectroscopy (AAS) using a Shimadzu, AA-680 model spectrometer. For each sample, three aliquots were analysed and the

values were only accepted when a reasonable standard deviation was obtained. A multi-elemental analysis for the determination of additional metals (Ca, K, Mn, Co, Ni, As, Se, Sr, Cd, Pb, Cr) in the AMD and in an effluent of column 2, was carried out by Total Reflection X-ray Fluorescence Spectroscopy (TXRF), using an Atomika, model EXTRA-IIA, spectrometer. This is an analytical technique well known for both its high sensitivity and its suitability for environmental studies (Barreiros *et al.*, 2002). Water samples were analysed directly without pre-concentration using the internal standard method, adding a fixed volume of gallium standard solution. Aliquots were pipetted onto quartz sample carriers for TXRF measurements.

Standard Methods (APHA, 1998) procedures were used for analyzing COD (dichromate method) and sulphate (turbidimetric method with barium chloride). Sulphate measurements were performed in duplicate with a portable data-logging spectrometer from HACH, at 450 nm.

A solid sample taken from column 2 after the treatment was first examined using a stereo-microscope (Wild Heerbrugg M8), and then using a scanning electron microscope (SEM), coupled with energy dispersion system (EDS), from JEOL (JSM-35CF model). This sample was previously dried at room temperature under vacuum and then coated with gold. It was also semi-quantitatively analyzed by TXRF.

3. Results and Discussion

The composition of the AMD water from the pit lake of S. Domingos mine is presented in Table I. As a main consequence of pyrite oxidation, the pit lake water is very acidic and contains high amounts of heavy metals (especially iron, copper and zinc) and sulphate (Table I), thus representing a serious environmental problem among others associated with S. Domingos mine. The high content of these metals is due to the fact that in S. Domingos the base metal sulphides are generally limited to those containing Fe, Cu, Zn and Pb, while Mn, Cd, As, Bi, Hg and Sb are also present as geochemical minor but locally important elements (Report of the Mineo Project, 2000). Hence, in this investigation Fe, Zn and Cu were the metals chosen to monitor the efficiency of the bioremediation process under study.

Acid waters in S. Domingos are not confined to the pit lake. In fact, the mine is located in the hydrographic basin of Guadiana River, and the drainage of the mining area occurs along several kilometers as far as the Mosteirão River. The water samples collected in the stream that comes from S. Domingos mine (point B in Figure 1) have the chemical parameters and composition indicated in Table I. The aspect of the water in that stream presents different ferrous colours, due to the high amounts of iron. At present, as iron sulphide, always as a pyrite and/or pyrrhotite, is an ubiquitous component of all massive sulphide deposits, and because such pyrite was not entirely recovered during mineral processing, this sulphide constitutes the most

TABLE I

Chemical parameters and composition of AMD from the pit lake and from the stream of S. Domingos mine

Chemical parameters	Open pit	Stream
pH	2.4	2.3
E_h (mV)	497	489
COD (g/L)	0.25	–
SO_4^{2-} (g/L)	3.1	4.2
O_2 (%)	70	95
Metal composition: (mg/L)		
Fe	497	250
Cu	49	40
Zn	107	50
Ca	163	–
K	2	–
Mn	75	–
Co	3.7	–
Ni	<0.5	–
As	0.4	–
Se	<0.04	–
Sr	0.26	–
Cd	0.5	–
Cr	<0.2	–
Pb	0.3	–

environmentally important constituent of the massive sulphide wastes, which are particularly sensitive to weathering with the subsequent generation of AMD waters.

Comparing the water samples from the pit lake with the water samples from the stream, it can be concluded that both are very similar in pH, and that the water from the stream presents a higher sulphate content. According to previous studies (Matoso, 1998), the existence of old retaining dikes containing acidic water favours the infiltration of surface water, and the permeability of the dumps increases the infiltration of contaminated water and consequently the leaching of this material. An extensive monitoring study has been conducted in the mining area by COBA (2000), which also concluded about the existence of high contents of heavy metals in the waters from the streams, as well as in the sediments.

3.1. COLUMN EXPERIMENTS

This paper reports the first laboratory-scale investigation performed with the aim of developing a simple and low cost biochemical process for the treatment of

AMD waters from S. Domingos mine site. This site, typical of other AMD sites in Portugal, constitutes a case study and several studies have thus been conducted on it and reported (Report of the Mineo Project, 2000; Alves, 1997; Canteiro, 1997; CONASA, 1990–1994; Gonçalves *et al.*, 1995).

The experiments described herein examine the viability of using SRB in a semi-continuous process, where column bioreactors filled with solid matrices of zero or negative cost (easily available in the region where the mine is located), were used. In addition, the use of a supplementary carbon source (lactose) contained in effluents of zero or negative cost, also largely available in the mine region, is analyzed.

As in the present case, many current bioreactors use a simple passive to semi-continuous flow through design, where the bioreactor is filled with a solid matrix that serves as a physical framework for microbial attachment and metals sulphide precipitation (Tsukamoto *et al.*, 2004; Drury, 1999; Lyew *et al.*, 1999; Lyew *et al.*, 1997; Steed *et al.*, 2000). These reactors are considered promising as a less expensive treatment in comparison with the traditional lime treatment (Tsukamoto *et al.*, 2004). In this work solid sewage and sludge, both from an effluent treatment plant, were tested together with an acidic soil from the mining area, collected near the pit lake. The use of soil from the mining area had two objectives: as a source of potential bacterial populations specially adapted to the conditions of the mine site, and to test the possibility of using the process for the simultaneous decontamination of waters and sediments. Two columns (columns 1 and 2) were filled with those three materials according to the description made in the experimental section. Moreover, it is also expected that these matrices serve as a source of carbon for the growth and maintenance of SRB.

3.1.1. *Column 1*

In column 1 the medium acidity caused by the soil utilized was previously neutralized, by the addition of NaOH (until pH~6.5) to the mixture of the soil and nutrient medium, before putting them into the column. In this way it was created a suitable pH condition inside the column to favour the growth of SRB.

The concentrations of metals (Fe, Cu and Zn), sulphates and the pH in the effluents of column 1 as a function of time are represented in Figures 2a and 2b.

After an acclimatization period of 20 days, the nutrient medium started to be gradually replaced by the AMD (composition showed in Table I). After that period, a black precipitate formed on the top of the column (where the pH was close to 7), which indicates the bacterial growth and the generation of FeS according to Equation 3. As time goes by, all column gradually became black from the top to the bottom, being totally black by the end of the experiment. The nutrient medium was completely changed by the AMD after 70 days. The concentrations of metals and sulphate at time zero correspond to the amounts contained in the Postgate C solution. Both the nutrient medium and the AMD have similar sulphate concentrations (Table I). The pH at time zero was also the pH of the nutrient medium.
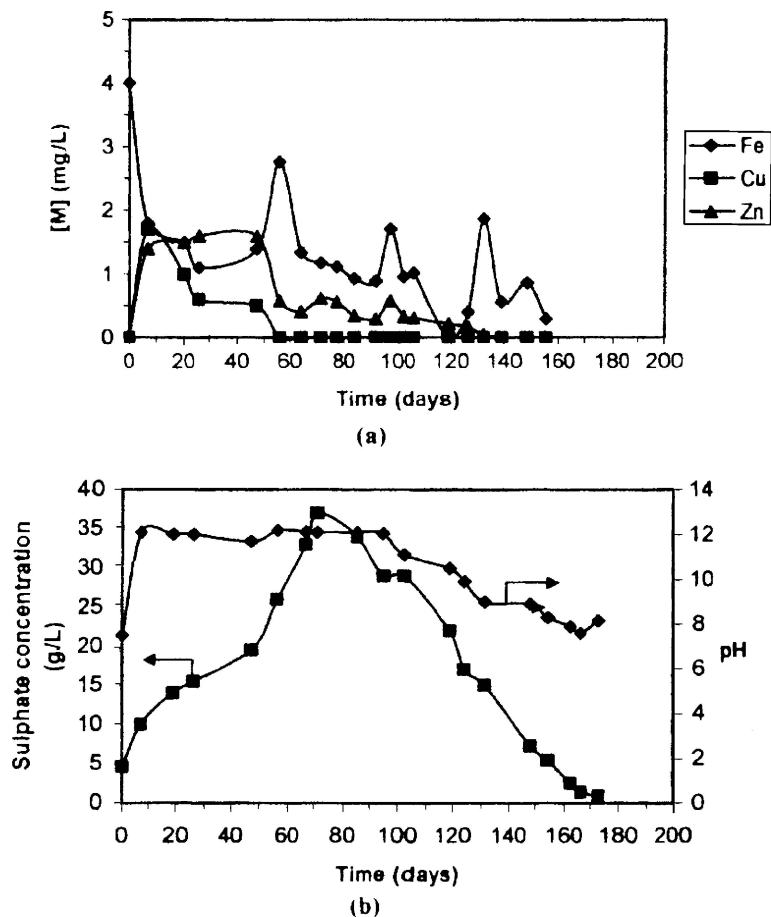
Figure 2. (a) Iron, copper, zinc and (b) sulphate concentrations and pH in the effluents of column 1 vs. time.

Taking into account the initial concentrations of Fe, Cu and Zn in the AMD (Table I) and the concentration of those metals in the column effluent (Figure 2a), it is possible to verify the efficiency of the process to remove them. In fact, the concentration of all these metals in the effluent is very low, being lower than 1 mg/L for Cu and Zn, and lower than 2 mg/L for Fe. Both values are below the limit allowed for Fe and Cu in waste water discharges, which, according to the Portuguese legislation, are 2.0 mg/L and 1.0 mg/L, respectively. The concentration of Cu and Zn in the effluent is also below the maximum values admitted for irrigation waters (5 mg/L and 10 mg/L, respectively), as well as the concentration of Fe, which is below the maximum recommended value (5.0 mg/L).

The precipitation of iron as oxides and hydroxides, due to the basic pH values particularly observed at the bottom of the column, should also be taken into consideration.

As it can be seen in Figure 2b, the process is also efficient to decrease the water acidity due to the generation of CO_2 during the reduction of sulphate by SRB (Equation 2). However, during the first 100 days the pH of the effluent was kept very high (about 12), demonstrating that there is no need to neutralise the initial pH of the medium. After 120 days the pH started to decrease and kept between 9 and 7, which is acceptable for the possible utilization of the treated water for irrigation (values between 4.5 and 9.0 are allowed by the Portuguese legislation). The initial formation of black precipitates only at the top of the column indicates that the bacterial growth just occurs in zones where the pH is neutral and not basic.

Sulphate concentration (Figure 2b) showed a considerable increase during the first 70 days, reaching values more than seven times higher than that existing in the influent medium. This must be due to the massive leaching of the sulphide ores contained in soil of the column matrix. The inefficiency of the process to reduce the sulphate concentration at this stage was certainly due to an unbalance between sulphate production and reduction. This can also be due to the depletion of the carbon source within the matrix, or to its insufficiency to remove the high sulphate concentrations involved. To improve the process of bacterial sulphate reduction, the addition of a supplementary carbon source (lactose) to the influent was made at day 72. Immediately after that, the sulphate concentration in the effluent started to decrease indicating that the rate of sulphate reduction seems to be limited by the amount of an appropriate organic substrate for the SRB growth within the matrix. Thus, in order to improve the efficiency of sulphate removal, 2g of lactose were then added on a weekly basis. At day 155 the sulphate concentration reached a value lower than those existing in the AMD and after day 170 it became lower than 1 g/L, which corresponds to about 80% of sulphate removal from the AMD water. According to the Portuguese legislation, 2 g/L is the maximum concentration admitted for this anion in waste water discharges, while 750 mg/L is the limit for irrigation applications.

3.1.2. *Column 2*

After the acclimatization period (20 days) the Postgate C medium initially added to the column was gradually replaced by the AMD. As in column 1, during that period of time a black precipitate started to form at the bottom of the column (while in column 1 this occurred at the top) where the pH was close to neutral, indicating the bacterial growth and the generation of metals sulphide. This gradient of pH naturally established inside the column (acidic in the top and near neutral at the bottom), defined a zone of SRB growth without the need for an initial addition of an alkaline agent to the column. During the experiment the whole column gradually became covered by this black precipitate starting from the bottom to the top. After a 70-day period the nutrient medium was considered to be totally replaced by the AMD.

The time dependence of metals concentration (Fe, Cu and Zn) in the column effluent is shown in Figure 3a. In the first 20 days of the experiment the amount of iron increased probably as a consequence of the leaching of the iron sulphides
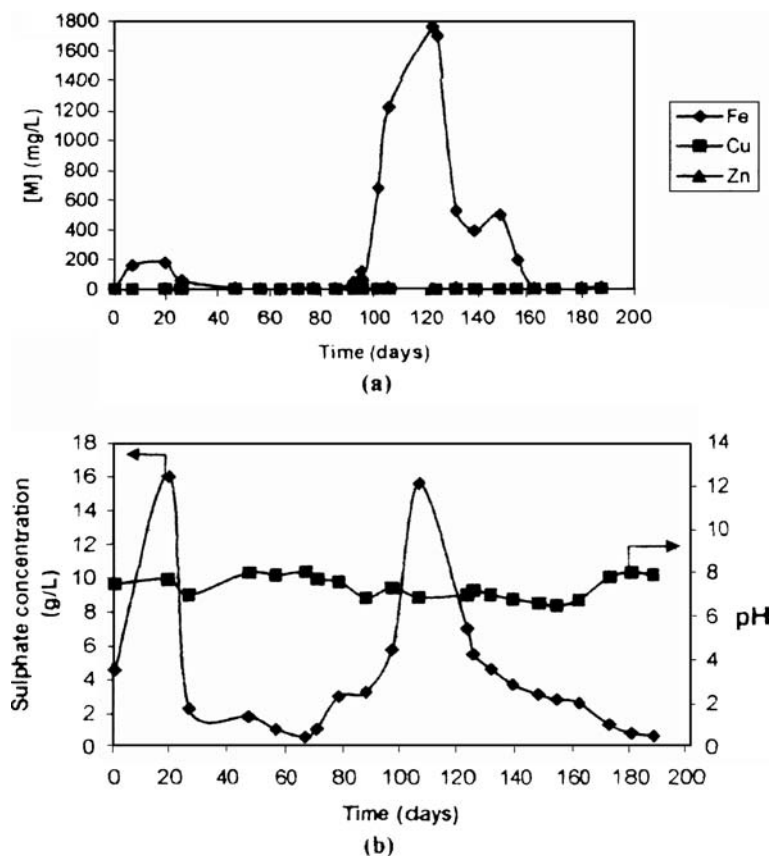
Figure 3. (a) Iron, copper, zinc and (b) sulphate concentrations and pH in the effluents of column 2 vs. time.

contained in the soil used as a layer of the column matrix. In fact, pyrite was detected in the soil by X-ray diffraction (XRD). In this case, contrary to what happened in column 1, due to the lower pH values inside the column the leached iron was not precipitated as oxides or hydroxides and its concentration thus increased in the effluent. The much higher values of iron frequently observed in the effluents of column 2 in comparison to those observed in the effluents of column 1, can be explained by the occurrence of this precipitation phenomenon in column 1.

The concentration of sulphate in the effluent also increased at the beginning of the experiment (Figure 3b) due to leaching of sulphide minerals contained in the soil. However, between 25 and 75 days it reached values lower than those presented in the AMD, thus confirming the efficiency of the SRB process for sulphate removal from both AMD and soil.

For copper and zinc up to 100% efficiencies of metals removal was almost always obtained (values below 0.5 mg/L and 2 mg/L were almost always attained

during the treatment of those metals respectively), which means the production of sufficient sulphide for their complete precipitation. Between 45 and 85 days the iron concentration decreased to acceptable values (below 10 mg/L) and thereafter it started to increase again, reaching a maximum value at about 120 days. At about between 100 to 150 days the amounts of iron in the outlet stream are even much higher than those existing in the AMD due to the aforementioned leaching of iron sulphides from the soil. A similar behaviour was observed in the same period for sulphate. Thus, the increase of iron concentration is concurrent with the increase of sulphate concentrations (Figure 3b), and they are likely due to the depletion of the carbon source. As also happens for iron, the concentrations of sulphate in the outlet stream at about 95 and 140 days are even much higher than that in the AMD. Once the carbon source is exhausted from the matrix, SRB will no longer sufficiently reduce sulphate to sulphide and the treatment efficiency decreases (Castro *et al.*, 1999; McConchie, 2003; Burgess and Stuetz, 2002). For extending the bioreactor lifetime, the addition of an alternative carbon source (lactose) to the spent matrix was carried out on a weekly basis after day 123. Immediately afterwards, both iron and sulphate concentrations started to decrease continuously until the end of the experiment, confirming the exhaustion of the reducing agent within the matrix and the need of its periodic addition to maintain the treatment efficiency. After day 180 until the end of the experiment, values of sulphate removal from 80% to 90% (based on the AMD content) were achieved.

The process was also efficient to neutralize the AMD, since during the whole experiment pH values between 6.5 and 8 were achieved in the column effluents, the CO_2 produced by SRB being responsible for this pH increase.

Some problems related with the percolation of the AMD through the columns occurred occasionally in columns 1 and 2.

A multielemental analysis of the effluent of column 2 obtained after 95 days experiment was carried out by TXRF. According to the results presented in Table II, it is possible to conclude about the ability of the process to decrease the concentration of most of the metals contained in the AMD. At present, with the exception of Ca, K and Sr, the concentration of all the other metals decreases significantly. In addition to the reduction in Fe, Cu and Zn contents, the decrease in concentration of other very toxic elements such as Pb, As, Cd and Cr is very important in terms of the decontamination and possible utilization of these waters. In accordance with Portuguese legislation for irrigation waters the elements Mn, Co, As, Pb, Ni, Cu and Zn are below the maximum acceptable values.

The increase in calcium, strontium and potassium concentrations can be explained by the fact that these elements belong to the IV (Ca and Sr) and V (K) groups according to the "Qualitative Analysis of Cations" scheme (the V group is also called the soluble group), (Vogel, 1981) and therefore, they do not precipitate as sulphides, either in acidic or basic media, as also happens with the other cations that belong to the previous groups (with the exception of selenium).

TABLE II

Multielemental analysis of the column 2 effluent
obtained after 95 days experiment by TXRF

Element	Concentration (mg/L)	Removal (%)
Fe	123	75
Cu	<0.03	>99.9
Zn	0.14	99.9
Ca	1220	0
K	25	0
Mn	3.8	95
Co	0.6	85
Ni	<0.1	–
As	0.14	65
Se	<0.02	–
Sr	2.0	0
Cd	<0.1	>80
Cr	<0.2	–
Pb	<0.08	>73
Rb	0.11	–

3.1.3. *Column 3*

The nutrient medium (Postgate C) started to be gradually changed by the AMD after the acclimatization (batch) period (about 20 days). As it happened in column 2, during that time a black precipitate appeared at the bottom of the column (where the pH and the anaerobic conditions favoured the growth of SRB), and thereafter all the column gradually became black from the bottom to the top, being completely black by the end of the experiment. Semi-continuous feeding started after day 20 and the initial nutrient medium was completely replaced by the AMD by day 70.

In this column, the percolation of the liquid through the column was easier and thus, in the last week (after 150 days) a free continuous flow was used (20–25 mL/h).

The efficiency of the treatment to remove the main metals of the AMD is shown in Figure 4a. Throughout the treatment the concentration of the metals in the effluent was very low. The concentration of iron never exceeded 10 mg/L, while the concentrations of copper and zinc were almost always below 1 mg/L. The increase in the iron concentration from day 140 is related to the increase of the flow through the column. However, even during the continuous flow the treatment is efficient enough to remove these metals from the AMD.

The rate of sulphide production was enough to precipitate almost completely the heavy metals, but was not sufficient to decrease sulphate during the feeding period (Figure 4b). After a first period in which the sulphate concentration decreased to a
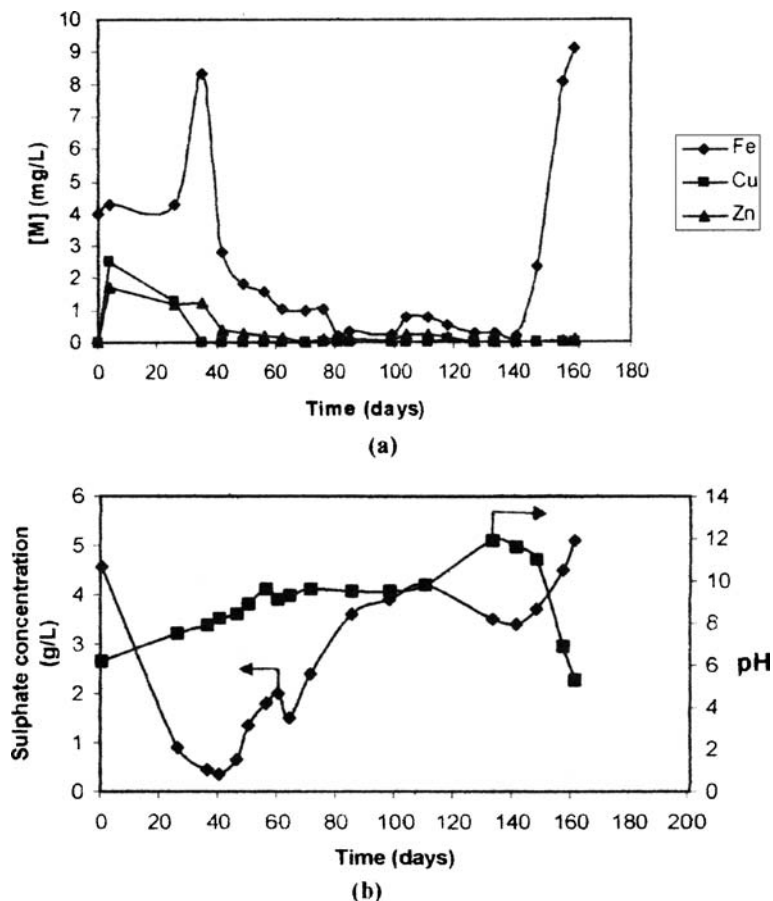
Figure 4. (a) Iron, copper, zinc and (b) sulphate concentrations and pH in the effluents of column 3 vs. time.

value lower than 0.4 g/L (that corresponds to about 90% of removal), it started to increase to values near that on the feeding, most probably due to the exhaustion of the carbon source from the matrix. Because of this, lactose started to be added to the feed and the sulphate concentration decreased again, but it has never reached values below 3.4 g/L, which corresponds to a maximum efficiency of only 15%. Afterwards, the sulphate concentration increased once again until reach a maximum value of 5.1 g/L near the end of the experiment during the continuous flow. This concentration is even higher than that one existing in the AMD, probably due to the presence of aluminium sulphate (a material frequently used as flocculent on waste water treatment plants) in the solid materials used as the column matrices (sewage and sludge from a waste water treatment plant). Due to the absence of soil as solid support of the column matrix, the sulphate amounts in the effluent did not

reach the high values obtained in columns 1 and 2. Nevertheless, the efficiency of sulphate removed by this column was lower than that achieved in the other columns, although in column 3 the treatment of higher volumes of AMD, as a consequence of higher percolation rates, was achieved. However, the addition of lactose provided a slight increase in the efficiency of sulphate removal.

As occurred with the other columns, in this case the treatment was also efficient enough to neutralize the AMD (Figure 4b). However, higher pH values (11–12) than those obtained in the column 2 were achieved what may be one of the factors responsible for the reduction in sulphate removal efficiency. To reduce alkalinity, a continuous flow was implemented after 150 days until the end of the experiment, and immediately the pH of the effluent started to decrease, reaching a value of 5.3 in the end of the experiment. As a result of the continuous flow, much higher volumes were treated during this period, and, as a consequence, a slight increase in iron and sulphate concentrations was observed (the last two points of Figure 4a and 4b). At this stage column 3 was probably already poisoned due to SRB death caused by the high pH values.

3.1.4. *Sludge Characteristics*

The black sludge produced by SRB and collected from column 2 at the end of the experiment was analysed by stereo and scanning electron (SEM) microscopy, this last technique being associated with an energy dispersive system (EDS). Before analysis by SEM/EDS the sludge was first observed using a stereo-microscope. Three distinctive precipitates presenting different colours (black, white and orange) were detected. A sample of each of these precipitates was then coated with gold and analysed by SEM/EDS.

The three EDS spectra of each coloured sample are presented in Figure 5. Although the presence of sulphur was observed in all spectra, they present different metallic compositions depending on the colour of the precipitate. The EDS analyses do not give any information about the oxidation state of metallic species, nor do they make it possible to know which ion is associated with the precipitates. However, in the absence of other elements it may be concluded that iron was retained in the black sludge precipitate (Figure 5a) presumably mainly as iron sulphide. The white precipitate (Figure 5b) is likely to be associated with the main presence of $CaSO_4$, since, as mentioned previously, calcium does not precipitate as sulphide in acidic or basic media. In addition, the presence of calcite ($CaCO_3$) should also be considered. The EDS system used does not detect elements with an atomic number lower than or equal to 16, and this is why it did not detect the presence of C and O in this precipitate. The orange precipitate (Figure 5c) can be a calcium jarosite mineral, which contains iron and calcium in its composition.
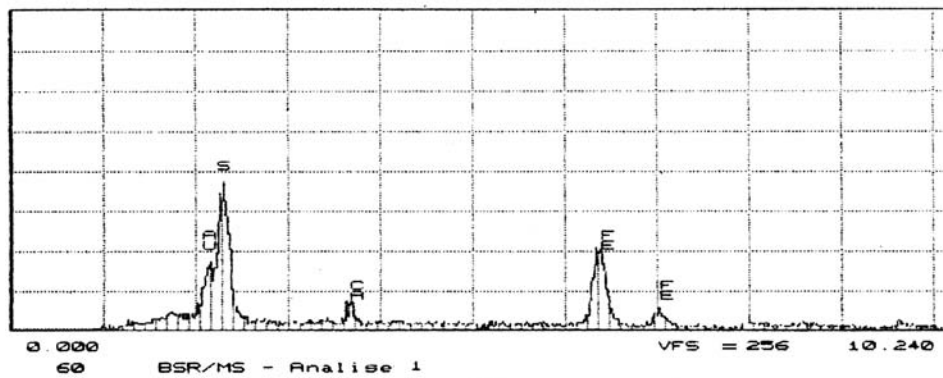
Figure 6 presents the three SEM micrographs obtained, which show clearly different structures. Figure 6a illustrates the presence of some smooth spherical and rod-shaped objects that, according to the literature (Postgate, 1981; Lima and co-workers, 2001), can be attributed to the presence of sulphate-reducing bacteria,
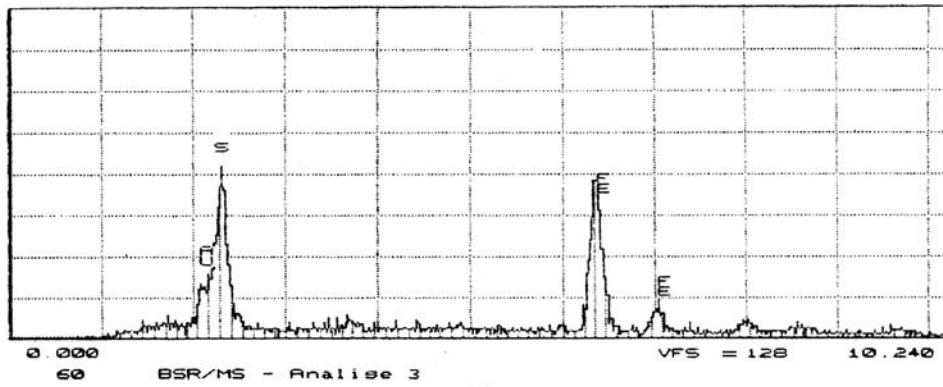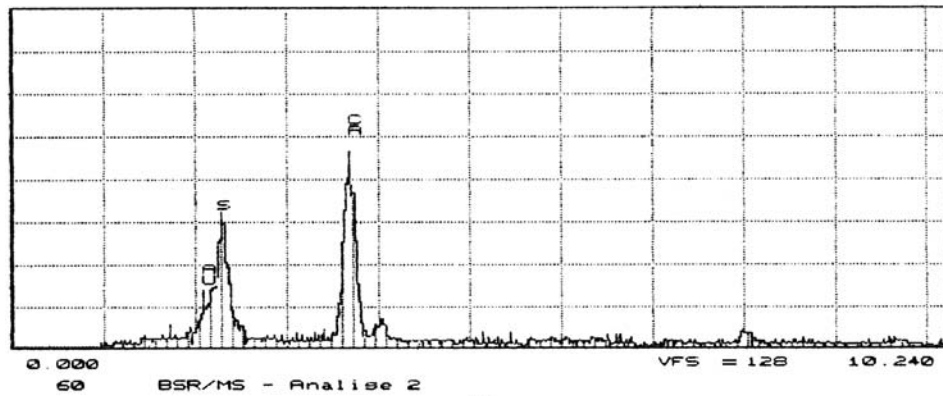
Figure 5. EDS spectra of the sludge: (a) black precipitate, (b) white precipitate and (c) orange precipitate.
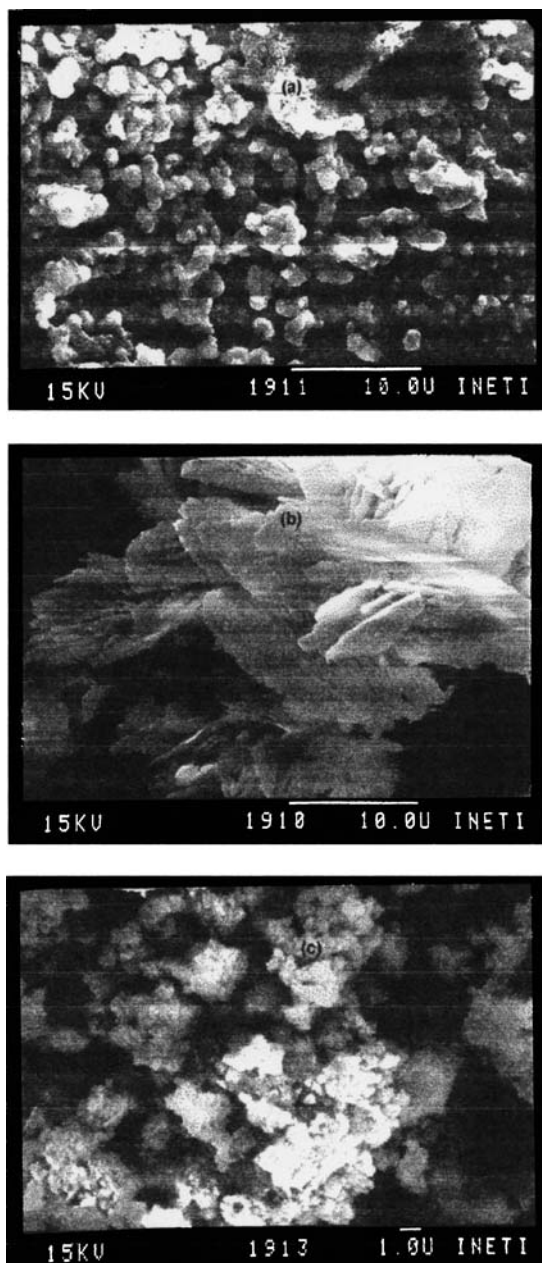
Figure 6. Scanning electron micrographs of the sludge: (a) black precipitate, (b) white precipitate and (c) orange precipitate.

TABLE III

Semi-quantitative analysis of a sludge sample obtained by TXRF

Element	(%*)
S	36
Ca	34
Fe	28
Zn	1.41
Cu	0.280
Co	0.107
Mn	0.054
Sr	0.026
As	0.003
Cr	0.007

*This percentage is reported to the concentration of each element in relation to the whole mass of all the elements detected.

covered with deposits of iron sulphide minerals. This being true it means that SRB may act as a nucleation site for the formation of iron sulphide minerals, as it was reported by Lima and co-workers (2001). This phenomenon can be responsible for some inhibition of bacterial activity, thus explaining why the treatment is not more efficient in the removal of sulphate from the AMD. Figure 6b shows a completely different structure, most probably of a calcium sulphate or a calcium carbonate crystal, while Figure 6c appears to have a mixed structure.

A semi-quantitative analysis of the sludge was also performed by TXRF. The results obtained are presented in Table III and confirmed the main presence of sulphur, calcium and iron in the sludge. The analysis also allowed the identification of other minor elements such as zinc, and copper, confirming their removal from the AMD by precipitation.

4. Conclusions

The results presented highlight the viability of a simple and semi-continuous column process, based on the activity of SRB and on the use of sewage, sludge and soil as solid matrices (and/or inocula), for the treatment of the AMD of the abandoned S. Domingos mine.

Efficient metals removal was obtained, which means that the process generates enough sulphide to almost completely precipitate the main metals (Fe, Zn and Cu) and also other metals (the most toxic: As, Cd, Pb, Cr) presented in lower

concentrations. Most of the results in terms of the main metals concentration in the columns effluents were lower than 10 mg/L, and were therefore below the levels required by Portuguese legislation either for irrigation or discharge waters.

Satisfactory biological sulphate removal was also achieved by this process.

In addition the process generates enough alkalinity to increase the pH of the AMD from 2.3 to near neutral values without the need of any addition of alkaline agents, when soil from the mine place was incorporated into the column matrix. However, this soil is also a source of metals and sulphate and is thus responsible for some of the peaks of concentration observed.

The addition of a complementary carbon source (lactose) to the spent matrices showed to be necessary (after the depletion of the initial complex organic sources present in the matrix) to maintain high sulphate and metal removal levels. These experiments provided evidence that the addition of lactose as carbon and energy source appear to be determinant in the rate of biological H_2S production, thus enhancing the reduction in sulphate and the removal of metals.

This work also demonstrates that the incorporation of soil from the mining area in the column matrix can be advantageous for the process since the best results could be obtained, particularly for sulphate removal and pH control. Therefore, according to this investigation it is possible to extend the application of this column process to the simultaneous bioremediation of AMD and metal contaminated soils.

Further studies need to be carried out to control and maintain a stable sulphate removal for longer periods at values suitable for discharge on nature or for irrigation purposes.

Acknowledgments

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