BIOHYDROMETALLURGY AND THE ENVIRONMENT TOWARDS THE MINING OF THE 21ST CENTURY

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Influence of bacteria and sulphite ions on the transformation of pyritic tailings: shake flask tests

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Mining and metallurgical tailings, once dumped, are exposed to changing weather conditions. The action of natural agents such as water, oxygen, bacteria and other complex factors, transforms and, at the same time, modifies the composition and causes deterioration of the tailings. In the present study, the affect of two factors on the transformation of pyritic tailings from the flotation of a complex sulphide ore is analyzed. Shake flask tests were carried out to determine the behaviour of the tailings under the influence of bacterial catalysis and the reducing conditions induced by the presence of sulphite ions in the system. As expected, the presence of lithotrophic bacteria in the system catalyzed the tailings (pyrite) oxidation. On the other hand, during the flotation of these ores, the sulphite ion is used to condition the medium in which the solid is concentrated resulting reducing conditions. The presence of sulphite ion influenced the subsequent bacterial action and controlled the final transformation of the solid residue.

1. INTRODUCTION

An abandoned mine, a mine waste, or any heap of ore exposed to the changing environmental conditions suffers important transformations giving rise to a phenomenon named as weathering. When the residue is a sulphide ore these transformations produce acid mine drainages (AMD). To study these transformations different tests have been designed to describe the changes concerning an abandoned residue using controlled laboratory conditions. To design the weathering tests it is necessary to know previously several geological and climatic factors as the rain frequency, temperature fluctuations, etc. with the aim of deciding the appropriate and specific tests to follow the deterioration of a specific residue. Although there are no concrete rules defining this experimentation, different tests have been proposed which lay down the guideline and determine, in general, the capacity of a residue either to produce acid or to neutralize it. Among them, several static and kinetic tests are included.

From the beginning of the studies related to the generation of acid waters, it has been shown that different acidophilic bacteria of the genus Thiobacillus (T. ferrooxidans and T. thiooxidans) and Leptospirillum (L. ferrooxidans) are the responsible for the oxidation of
pyrite and other metal sulphides with the consequent production of acidity. Another important group of microorganisms related to the transformation of the compounds appearing in the mine waters are sulphate reducing bacteria (SRB) which, at the same time, reduce the sulphate of the medium to sulphide, increase the pH and, as a consequence, facilitate the precipitation of the dissolved metals.

In the present work, a study was carried out of different factors involved in the transformation when mining solid residue derived from a polymetallic sulphide flotation plant, was placed in a pond. As starting point, the final consequences of these transformations were known but the different factors influencing the system and the mechanism behind the observed phenomena were unknown. For this, several factors influencing this type of system were studied to prove, in comparison with the real data, their implication on the process. In this sense, two static weathering tests were used: the ABA test and the shake flask test. The ABA (Acid-Base Accounting) test was chosen because it produces quick results giving a first idea of the water quality after the residue is abandoned. On the other hand, the shake flask test permits performance of controlled experiments to determine the influence of particular variables such as temperature, microbiological activity, etc. In this case, the influence of two factors, the presence of bacteria and the sulphite ions, was studied. It is necessary to underline that the results presented in this paper are a part of a global study of the system in which kinetic weathering tests and column experiments were carried out.

2. MATERIALS AND EXPERIMENTAL METHODS

2.1. Site

The site studied was a real system consisting of a pond of pyritic residues belonging to a mining company from the pyritic belt in the Southwest of Spain. The residues came to the pond from a flotation plant producing three differential concentrates from a polymetallic complex sulphide.

2.2. Sampling

To characterize the system, the pond was sampled as a whole. The samples from the bottom were collected using a special device named Ballcheck KB. All the samples were taken in duplicate and introduced into sterile flasks which were completely filled in to avoid the presence of oxygen and the possibility of future oxidation.

2.3. Chemical and mineralogical analysis

Solid samples

For the characterization of the solids contained in the samples, X-ray diffraction (Philips X’Pert-MPD) and granulometric analysis (Microtac FRA) were carried out. The chemical composition was determined after acid digestion of the samples; the subsequent analysis of the metals in solution was performed by Atomic Absorption Spectrophotometry (AAS) (Perkin-Elmer 1100B). The sulphur content was determined using an automatic analyser from Leco.

Liquid samples

The pH evolution was followed using a Crison 2001 electrode. For sulphate analysis a photocolorimeter (Metrohm 662) was used which determined the turbidity of BaSO₄ precipitates formed by reaction of sulphate ion with barium chloride (1). The concentration of sulphite ion was measured using an automatic titrapparatus from Metrohm and registered the potential at which the reaction between sulphite ion and iodine took place (1,2).

The concentration of metals in the samples taken in the water pond and from the shake flask tests was measured by means of AAS.

2.4. Microbiology

Samples taken from the different points of the pond as well as the solid sample used to carry out the weathering experiments were microbiologically characterized. For microorganism isolation the following culture media were used:

Aerobes

- 9K medium without iron: (NH₄)₂SO₄, 3 g.L⁻¹; KCl, 0.1 g.L⁻¹; K₂HPO₄, 3H₂O, 0.618 g.L⁻¹; MgSO₄, 0.5 g.L⁻¹; Ca(NO₃)₂.4H₂O, 0.013 g.L⁻¹.
- Heterotrophs (bacteria, yeasts, and fungi):
  - Medium A: 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.
  - Medium B: 9K medium without iron at pH 3 with 0.5 g.L⁻¹ bacto-tryptone; 10 g.L⁻¹ malt extract; 10 g.L⁻¹ glucose; and 15 g.L⁻¹ agar.
- Chemolithotrophs
  - Medium containing one of the following compounds as energy source: iron, tetrahydrothionate, thiosulphate or elemental sulphur. pH was adjusted to 2, 3 or 6 with sulphuric acid.

Anaerobes

Postgate’s C medium was used (ASTM D 4412-84). The same medium but with sodium molybdate was also used as a control test.

All the cultures were maintained at 30°C. Bergey’s Manual of Determinative Bacteriology was used for the identification of the isolated microorganisms. To count the microorganisms the most probable number (MPN) method was used (1,3).

2.5. Experimental tests

ABA Test

The ABA test was carried out in duplicate in accordance with the Method 1312 introduced by EPA (Acid-Base Accounting, 4).

Shake flask tests

To perform these tests 25 g of ore, previously dried and ground, were put in an Erlenmeyer flask together with 250 mL of either distilled water or 9K medium at pH 2 tests in the presence of bacteria. To carry out the sterile tests, in addition, 20 mL of a 2 % thymol solution were added. The cultures were maintained during one month with orbital agitation and at 35°C. Periodically a 2 mL sample was taken out to determine pH, metals (Fe, Cu and Zn) in solution and sulphate concentration. Furthermore, in each sample, the number of bacteria was checked. In the tests carried out in the presence of sulphite ion, 0.1 g.L⁻¹ of this were added as sodium sulphite was added.
In addition the behaviour of the pulp leaving the flotation plant was also studied. In this case 100 g of sludge from the plant were placed in the 250mL flask as initial ore.

3. RESULTS AND DISCUSSION

3.1. Chemical and microbiological characterization of the tailings

The residue discharged from the flotation plant was a basic pulp of pyrite with 30% of solids. The chemical composition of the water contained in this pulp is shown in Table 1 in the column "Initial discharge". From a mineralogical point of view the sample was a pyritic ore with the following chemical analysis: 0.5% Cu; 2.83% Pb; 0.05% Zn; 38.8% Fe; and 37.6% S. The mineralogical composition of the solid contained in the pulp was: chalcopyrite, 1.4%; sphalerite, 0.8%; galena, 0.5%; pyrite, 63.4%; carbohydrates and silicates, 34.0%. The particle size of the solid was between 25 and 30 μm. The number of microorganisms contained in the pulp in the initial conditions was: 18,300 cells/mL of sulphur-oxidizing lithothrophic bacteria and 8,060 cells/mL of total heterotrophs. Anaerobic SRB and iron-oxidizing lithothrophic bacteria were not detected.

During the storage of the pulp in the pond two different phenomena took place. On the one hand, the discharged basic pulp transformed producing a new residue, an acid water in which the concentration of metals and sulphate ion had appreciably increased (Table 1, column "Final AMD"). Only one ion, the sulphite ion, virtually disappeared during the transformation. On the other hand, a chemical and microbiological gradient appeared when the system was tested from the surface to the bottom. This gave rise to a new distribution of chemical species (Table 2) and a microbiological partitioning as a function of these new conditions (Table 3). As the data show, in the pond surface the conditions were clearly oxidizing with a potential of 405 mV (versus SHE) which favoured the decrease of the pH in the medium up to 3 and the consequent release of metals to the aqueous solution. These conditions were uniform in the surface layer of pond water.

Iron requires a special attention because the ferric/ferrous relationship can be a reference to the bacterial activity. The iron contained in the surface samples of the pond water was basically ferric iron: of the 50 mg/L of total iron contained in the water sample only, 1.7 mg/L corresponded to ferrous iron. This reveals the possible microbiological contribution to the surface oxidation in the pond water, because the chemical oxidation of ferrous iron in an acid medium is a very slow process.

At the same time a microbiological selection took place which was characterized by the presence of acidophilic lithothrophic bacteria in the surface water: T. ferrooxidans and T. thiooxidans were identified. In the samples taken from the pond bottom, SRB were found. A number of heterotrophs, including fungi and bacteria, in different parts of the system. (5). The microorganism distribution in the pond is shown in Table 3. To carry out the experiments in shake flasks a pyrite pulp from the discharge point in the flotation plant was used.

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<th>Table 1: Chemical analysis of the initial tailings discharge and the final residue (mg/l)</th>
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<th>Table 3: Microbiological gradient in the pond (MPN g⁻¹)</th>
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3.2. Weathering tests on the tailings

3.2.a. ABA test

For the digestion of the samples, 80 mL of 0.5N HCl were used with orbital agitation for 48 hours. The volume of NaOH consumed by the sample was 20.65 mL. The initial and final pH values were 0.6 and 8.3, respectively.

The calculated neutralizing potential of the samples was 12.22 and the acid potential of the samples obtained from the total contained sulphur was 1,775. Therefore, the net neutralizing potential (NNP) was -1,163 kg CaCO₃/t of sample, that is to say, negative and the conclusion was that the sample had capacity to produce acidity. This first approximation was later confirmed by means of other static and dynamic tests which showed the conditions and rates these acid producing samples were attacked. It is important to take into account that the interpretation of these types of results requires experience and care, because, samples showing a positive NNP did not always exhibit this behaviour either in the kinetics tests or in the field.
experiments. However, this does not mean that under certain conditions these samples could not produce some acidity.

3.2.b. Bacterial catalysis

To study the influence of bacteria on pulp weathering three different experiments were prepared in which pulp was used as starting solid residue. In one test, sludge alone was used. In a second test, 10 mL of an inoculum of iron-oxidizing bacteria isolated from the pond were added. In a third test, a sludge with 20 mL of thymol was used with the aim of knowing the chemical evolution of the solid in the absence of bacteria. The inoculum (10 mL) was taken from a T. ferrooxidans culture which was previously centrifuged to avoid incorporating iron into the experiment.

The pH evolution in the three tests (Figure 1) proved that the presence of bacteria influences acid generation. Chemical factors decreased the pH to about 4, but microbiological activity was necessary to bring the pH closer to 2. The pH behaviour of the pulp only and the pulp with bacteria was similar. The explanation for this is that bacteria, indigenous to the sample, grew. Growth did not occur when a bactericide was added.

The changes in the pulp among the three tests were very evident when the behaviour of the total iron in solution was analyzed (Figure 2). In the presence of the bactericide practically no iron was solubilized, whereas in the presence of bacteria the iron concentration increased remarkably both when the bacteria populations were natural and when adapted bacteria were inoculated.

The presence of bacteria associated with the initial ore was proven during system characterization and microorganism isolation. This test work determined whether the bacteria adhered to the solids or if they were free in the pulp water, and ascertained the bacterial activity in each case. Duplicate experiments were performed using dry ore obtained from the pond pulp. The ore, once dried, was finely ground before beginning the test; 25 g of ore in 250 mL of distilled water were used. Five different experiments were prepared: (a) ore only (O), (b) ore plus thymol (OT), (c) ore in 9K medium at pH 2 with 33.3 g/L of ferrous sulphate and a 10% inoculum of a T. ferrooxidans culture (OB), (d) ore in 9K medium at pH 2 with iron and thymol (OBT), and (e) ore in 9K medium at pH 2, 33.3 g/L, ferrous sulphate and a 10% inoculum from a mixed culture of bacteria grown from a pulp of the same ore (OMC). The inocula were centrifuged before addition to the flasks to avoid the addition of ferric ion to the solutions. The pH in the test carried out with only ore (test O) evolved to acid values. This is difficult to explain if only chemical factors are considered (Figure 3). In inoculated tests, pH change paralleled cellular growth, which was monitored by bacterial counts. Cell numbers appreciably increased as the conditions became more acid, hence, more suitable for the bacterial growth. This sharp decrease in the pH was not observed when a bactericide was used (test OT), although with thymol evaporation, some acidification was detected (Figure 3). Thymol was not replaced during the test. Bacterial monitoring using optical microscopy was carried out in the test with thymol. No appreciable cell growth was observed. In test O 10^6 cells/mL were counted; this number increased with time. The catalytic effect of bacteria on the ore oxidation was clearly detected. This effect was more evident in the tests carried out in the presence of a mixed culture as well as an iron-oxidizing culture (Figure 3). The pH decrease was much more evident in the tests carried out in the presence of bacteria (Figure 3) than in the sterile test. The main difference between the experiment OMC, using a mesophilic mixed culture, and the experiment OB, using a T. ferrooxidans culture, was that pH values were much lower in the first case than those reached in the presence of T. ferrooxidans. The reason could be that in a mixed culture it is possible to find bacteria oxidizing both iron and sulphur. T. thioreducens is common and can reduce the pH of the medium to 0.6 (6). In the test OMC pH values of 1.0 were reached.

Figure 1: Influence of microbical oxidation on pH

Figure 2: Influence of microbial oxidation on soluble iron concentration

The variation of the total iron concentration observed in these tests reflected an important bacterial activity both in the inoculated tests (OB and OMC, Figure 4) and in the tests where indigenous bacteria grew (test M). Solubilization of iron in test M was paralleled to the bacterial growth curve. This trend was much more evident in the case of the tests inoculated with T. ferrooxidans (test OB, Figure 4). In tests performed in the presence of thymol (tests OT and OBT), oxidation of the ore was not detected. Sulphate concentration in the tests showed the same trend as iron (Figure 5). A higher concentration of sulphate in solution was observed in the test inoculated with T. ferrooxidans. Based on soluble sulphate, the tests carried out in the presence of thymol again showed the absence of ore leaching. In test OT, in which the ore bacteria evolved freely, the sulphate concentration also increased.

Figure 3: pH variation in shake flask tests. Legend: ore (O); ore + thymol (OT); ore + T. ferrooxidans + thymol (OB); ore + T. ferrooxidans + mixed culture (OMC)

Figure 4: Solution iron concentration in shake flask tests. Legend: ore (O); ore + thymol (OT); ore + T. ferrooxidans + thymol (OB); ore + T. ferrooxidans + mixed culture (OMC)
appreciably in comparison with the test where the bacterial activity was non-existent although the oxidation level was always lower than that detected in the experiment inoculated with T. ferrooxidans.

Figure 5: Changes in sulphite ion concentration in shake flask test. Legend: ore (O); ore + thymol (OT); ore + T. ferrooxidans + thymol (OS); ore + mixed culture (OMC)

Figure 6: Influence of bacteria and sulphite ion on pH during weathering

3.2.3. Sulphite ion affects

The effect of sulphite ion in the pond waters was reproduced by performing different experiments in shake flasks. A new test was designed whereby changes in the pulp in the presence of sulphite ion were compared with changes in the pulp in the presence of bacteria. For this, two flasks each containing 150 g of partially leached pulp were prepared. Fresh solid was acid conditioned for 18 days at pH 3. The reason for using this type of raw material was to avoid the adaptation phase of the microorganisms to the extremely basic conditions of pulp freshly discharged from the flotation plant. In the first flask 10 mL of an inoculum of T. ferrooxidans were added to 150 g of acid conditioned pulp; in the second flask 0.1 g/L of sulphite ion as sodium sulphite was added to the acidic pulp. Changes in pH and total iron concentration in solution as a consequence of ore weathering are shown in Figures 6 and 7. Sulphite ion diminished (oxidation), as evidenced by a higher pH (Figure 6) and less iron being solubilized (Figure 7). In the presence of bacteria the final pH was 2 (Figure 6) and more iron was released (Figure 7). These results strongly indicate that sulphite affected the global chemical condition of the system because the final pH of the tailings pond was similar to the final pH of about 3 reached in the laboratory test with sulphite ion (Figure 6). The moderately reducing character of sulphite minimizes metals dissolution as demonstrated by iron behaviour illustrated in Figure 7.

4. CONCLUSIONS

The effect of microbiological oxidation of solid residue in the tailings pond is an important factor; however the final state of the system depended on all the factors (physical, chemical and microbiological) acting jointly. In laboratory, the bacteria oxidized the ore and produced waters with pH lower than 2; nevertheless pH values measured in the pond were higher. In the tailings pond there was a concomitant relationship between the reducing conditions imposed by the presence of sulphite ion, and the microbiological activity. In the absence of sulphite ion the bacterial activity occurred and was responsible for both low pH values and the increase of metals and sulphate ion in solution. In the presence of sulphite ion moderately reducing conditions resulted in higher pH values in the tailings, decreased metals dissolution and minimized pyrite oxidation.

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