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Qualitative Assessment of the Biogeochemical Reactivity of Sabodala Mine Tailings by Iron- and Sulphur-Oxidizing Bacteria

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Abstract

The reactivity of sulphide mine tailings is generally only considered from a geochemical point of view. However, the biological aspect plays an important role in natural cycles containing carbon and sulphur. Metal mobility in mine tailings is often controlled by sulphides. Chemical reactions or bacterial processes can promote sulphide alteration followed by heavy metal leaching. The aim of the present study is to conduct a qualitative assessment of the biogeochemical reactivity of Sabodala mine tailings. The qualitative assessment in shake flasks consists of a double test: a biotic test containing a mixture of a mass of mine tailings and a substrate with an initial pH adjusted according to the targeted bacterial population, and an abiotic or control test containing the same complete culture medium with sterilized tailings. Following incubation on a rotary shaker, the electrochemical parameters (pH and Oxidation-Reduction Potential (ORP)) of the biotic and abiotic tests were monitored over time. The results of this study show that the activities of iron-oxidizing and sulphur-bacteria were not detected in the Sabodala tailings, even though they can survive in these environments. In other words, the absence of these natural occurrences protects the mining environment from sulphide oxidation, *i.e.*, from the effects of Acid Mine Drainage (AMD).

Keywords

Biogeochemical Reactivity, Sulfur Cycle, Sulfur-Oxidizing Bacteria, Iron-Oxidizing Bacteria

1. Introduction

Sulphidic mine tailings generally have an acidic pH and high levels of toxic trace metals such as lead, zinc, arsenic, antimony, and cadmium … [1]-[4] Mining environments containing carbon and sulphur are often favourable to the proliferation of acidophilic oxidizing bacteria. In these environments, indigenous bacterial populations and geochemical transformations involving the carbonates and sulphides present react like a biogeochemical reactor [5] [6]. These biogeochemical conditions inhibit plant establishment and vegetation cover, exposing residues to wind and water erosion, which can transport contaminants in the form of fugitive dust or colloidal and/or dissolved metals [7] [8]. Physico-chemical interactions and biological activity also play an important role in the mobility of TMEs within mine tailings [9]-[12]. The formation of acid mine drainage is due to biogeochemical reactions in which sulphide minerals dissolve, and iron (II) is oxidized to give iron (III) [13] [14] by acidophilic bacteria (pH < 2) that can oxidize iron, such as Leptospirillum ferrooxidans and Acidithiobacillus ferrooxydans [13]. Their catalytic action involves the regeneration of Fe(III) from Fe(II) in an acid medium (Equation (1)). The phenomenon is sometimes "spontaneous" on outcrops of sulphide minerals, which are naturally subject to oxidation by contact with air and water within a well-defined timeframe. In the Sabodala area, the main families are pyrite (FeS_2), arsenopyrite (FeAsS), and chalcopyrite ($CuFeS_2$). The cyclic oxidation process of pyrite leads to the generation of Acid Mine Drainage (DMA) according to Equation (2) [13].

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \tag{1}$$

$$FeS_2 + 8H_2O + 14Fe^{3+} \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \tag{2}$$

Sulphides are oxidized to sulphates [15]-[17] by Acidithiobacillus thiooxidans. Acidithiobacillus ferrooxidans oxidizes both sulphur and iron (II). These reactions are greatly accelerated by catalytic bacteria, which facilitate the oxidation and reduction of minerals both on the mineral surface and in the aqueous solution. The Sabodala mine is planning to exploit the refractory Massawa gold deposit by bio-oxidation using iron- and sulphur-oxidizing bacteria. This operation can generate these micro-organisms in the Sabodala environment. These micro-organisms result in low pH, high conductivity, the presence of a mineral acid such as sulphuric acid (H_2SO_4), high concentrations of heavy metals and soluble sulphates, and other toxic elements that can seriously contaminate soil and groundwater [18] [19]. It is therefore necessary to establish a reference situation for the presence or absence of these natural occurrences. Similar indirect pH/ORP monitoring studies have been used by several authors to determine the presence or absence of ferrous and sulfide-oxidizing bacteria [17] [20]-[22]. The aim of this study is to assess the activity of iron- or sulphur-oxidizing bacteria in mine tailings by biogeochemical reactivity. The effect of aerobic bacteria on physicochemical parameters was studied by controlling pH and oxidation-reduction potential (ORP), to assess the impact of bacterial populations on mine tailings. Double tests were carried out on biotic and abiotic samples, with and without pre-acidification, to assess the impact of neutrophilic and acidophilic micro-organisms. The study of this reactivity will enable us to predict the acid generation of mine tailings and the mobility of metal ions.

2. Materials and Methods

2.1. Presentation of the Study Area

Located in southeastern Senegal, 700 kilometers from Dakar, Sabodala lies within the Mako greenstone belt, which forms the western part of the Birimian gold province. From an administrative standpoint, the study area is located in the municipality of Sabodala, which is part of the district of Sabodala, the department of Saraya, and the region of Kédougou [23] (**Figure 1**).

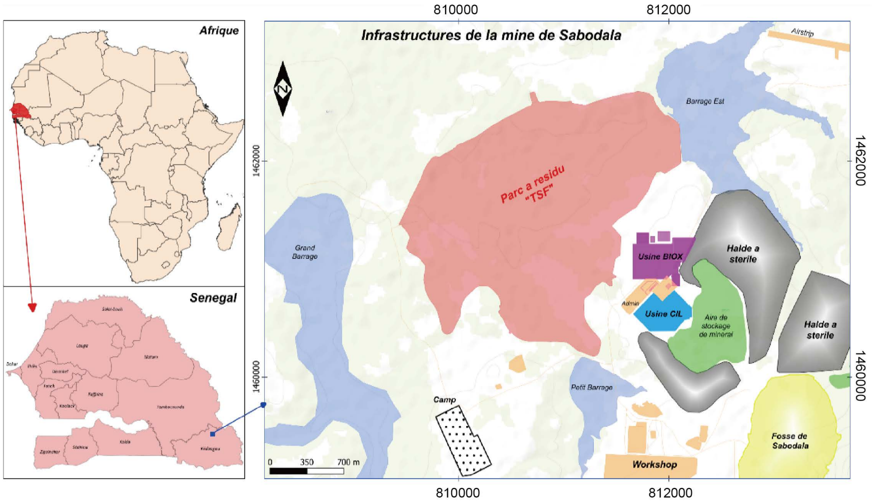

Figure 1. Location of the study area.

2.2. Sampling

Tailings from the Sabodala mine consist mainly of easily erodible, metal-rich, unconsolidated fine materials of various colorations stored in the tailings pond. Tailings samples are collected from the tailings pond with a pre-cleaned shovel to a depth of between 0 and 20 cm. At each point, a 0.5 to 1 kg soil sample was collected. A total of ten (10) samples were taken. Collected tailings are placed in Ziploc bags and stored at 4˚C until analysis, with a specific code. For each sampling point, the geographical coordinates were determined using GPS. The sampling map shows the spatial distribution of the samples (**Figure 2**).

2.3. Biogeochemical Methods

Aerobic bacterial populations were assessed indirectly in mine tailings. This aerobic microbial activity was assessed by a classical qualitative method, using the protocol presented in Zagury *et al.* (1997) [20] and modified according to Ouangrawa (2007) [21]. In this test, iron- or sulfur-oxidizing bacteria were monitored

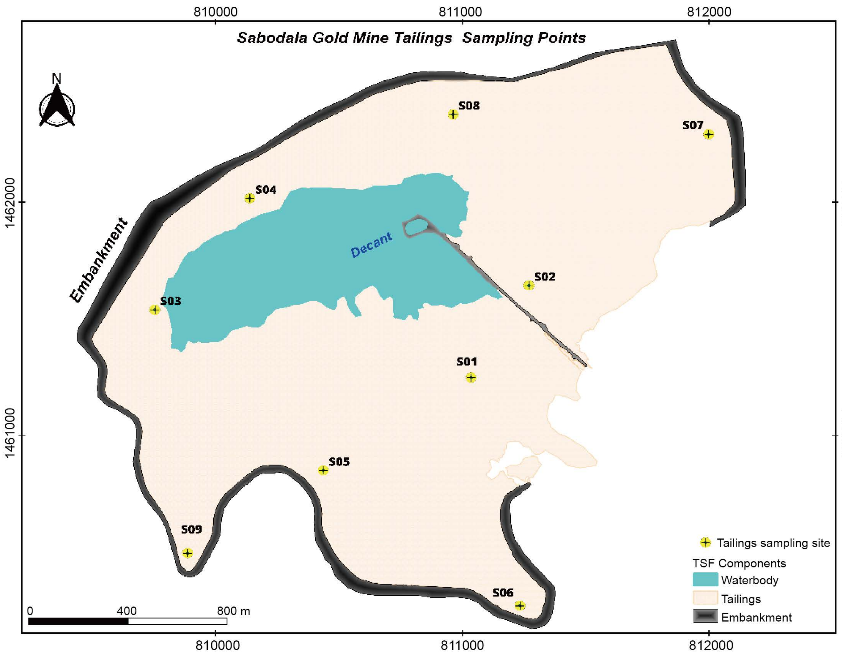

Figure 2. Mining residue sampling map.

by pH measurements, and the evolution of ORP over a given period. Qualitative assessment in shake flasks consists of a biotic test and an abiotic or control test. In each test, 2.25 g of dry mine tailings are mixed in an Erlenmeyer flask containing 150 ml of demineralized water and a substrate appropriate for the target population (**Table 1**). The initial pH of each sample was adjusted to an optimum value using 1M H_2SO_4 or 1M NaOH, depending on the bacterial population. After incubation on a rotary shaker, electrochemical parameters (pH and oxidation-reduction potential (ORP)) were monitored in parallel with a control. The control is autoclaved at 121˚C for 25 minutes and by adding sodium azide. The control allowed us to distinguish between chemical and biological processes. Incubation on a rotary shaker (250 rpm) was carried out for 24 days at 25˚C. pH and ORP values were measured every 2 - 3 days until stabilization, for approximately one month as described by [24]. The experimental protocol is described in **Figures 3-6**.

Table 1. Growth conditions for bacterial populations with substrate and initial pH [21]-[23].

Bacterial populations	Substrate	Initial pH adjusted
Acidophilic ferro-oxidizing bacteria (AFOB)	- 1.5 g $FeSO_4·7H_2O$ in 150 ml distilled water - 3 g/L $(NH_4)_2SO_4$	3.5 ± 0.1
Neutrophilic sulfo-oxidizing bacteria (NSOB)	- 1 g of S0 in 100 ml of distilled water - 3 g/L of KH_2PO_4 - 3 g/L of $(NH_4)_2SO_4$	$7.0 ± 0.1

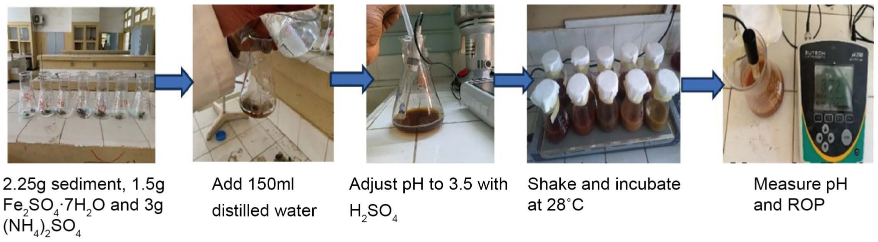

Figure 3. Methodology for analyzing the activities of acidophilic ferro-oxidizing bacteria (AFOB) in biotic environments.

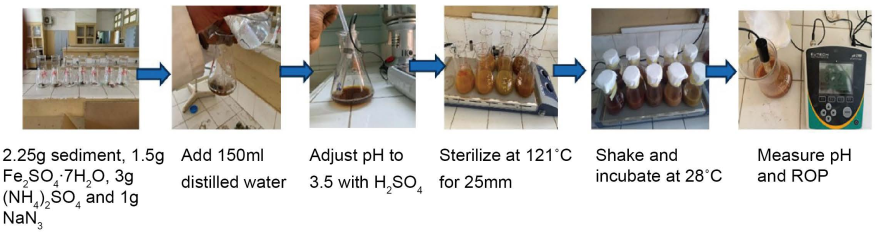

Figure 4. Methodology for analyzing acidophilic ferro-oxidizing bacteria (AFOB) in abiotic environments.

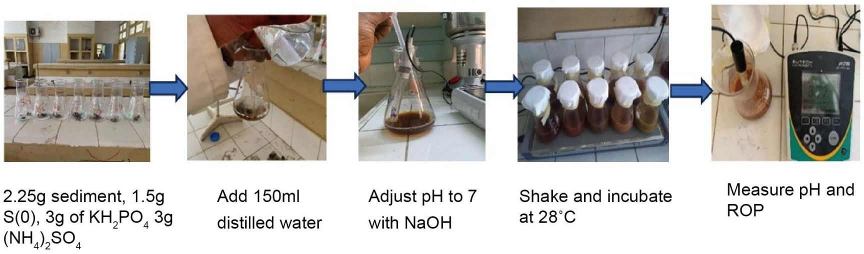

Figure 5. Methodology for analyzing neutral sulfooxidizing bacteria (NSOB) in biotic environments.

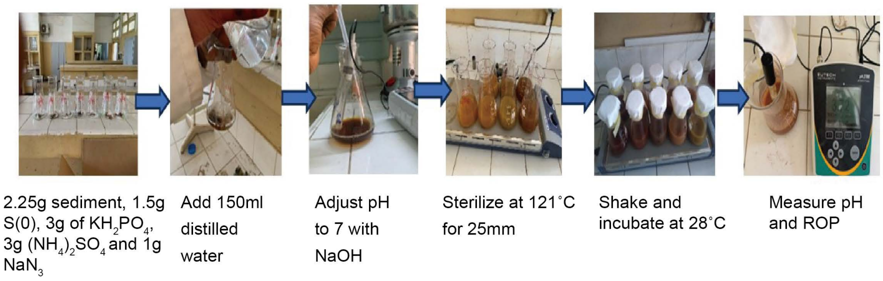

Figure 6. Methodology for analyzing sulfate-oxidizing bacteria (SOB) in abiotic environments.

2.4. Statistical Analysis of pH and ORP Variations

The Shapiro-Wilk test is used to assess the normality of the data distribution, while the Bartlett test is used to check the homogeneity of variances between groups. When the data meet the conditions of normality ($p \geq 0.05$ for the Shapiro-Wilk test) and homogeneity of variance ($p \geq 0.05$ for the Bartlett test), an analysis of variance (ANOVA) is applied to compare variations in pH and oxidation-reduction potential (ORP) based on the days and different experimental media. If the ANOVA test indicates a significant difference ($p < 0.05$), a post-hoc test such as Tukey's test is used to make pairwise comparisons between the days. If the data do not meet normality ($p < 0.05$ for the Shapiro-Wilk test) or homogeneity of variance ($p < 0.05$ for the Bartlett test), the non-parametric Kruskal-Wallis test is used to compare variations in pH and ORP as a function of time and media, offering a suitable alternative for non-normal distributions.

3. Results and Discussion

3.1. Qualitative Assessment of the Biogeochemical Reactivity of Mine Tailings by Iron-Oxidizing Acidophilic Bacteria

3.1.1. pH Monitoring of Biotic and Abiotic Tests

The activities of iron-oxidising bacteria on sulphide mine tailings are based on a decrease in pH and an increase in oxidation-reduction potential (ORP) over time in the reaction medium. To study acidophilic bacteria, the initial pH was rigorously adjusted to 3.5 to create conditions conducive to their development. In the 10 samples, the temporal changes in pH are almost identical. As an example, Figure 7 shows the change curves for samples 2 and 6. The pH curves for both biotic and abiotic environments show a slight increase over the first few days, followed by stabilisation at around pH = 4.5 for around five days (Figure 7). This increase could be explained by the reactivity of the carbonates (calcite and ferrodolomite) present in the tailings [25] [26]. These carbonate minerals are likely to react with the acid in the environment, increasing the pH by neutralisation mechanisms. After this initial phase, a slight drop in pH was recorded until the end of the experiment. This acidity of the medium can be attributed to the chemical oxidation of iron (II) to iron (III), followed by the precipitation of iron (III) to form iron hydroxides ($Fe(OH)_3$) (Equation (3)) [27]. This phenomenon was corroborated by the appearance of a reddish-orange suspension in certain samples a few days after the start of the experiment. This colour change reflects the formation of iron (III) hydroxides, supporting the hypothesis that oxidation reactions caused the acidification [27].

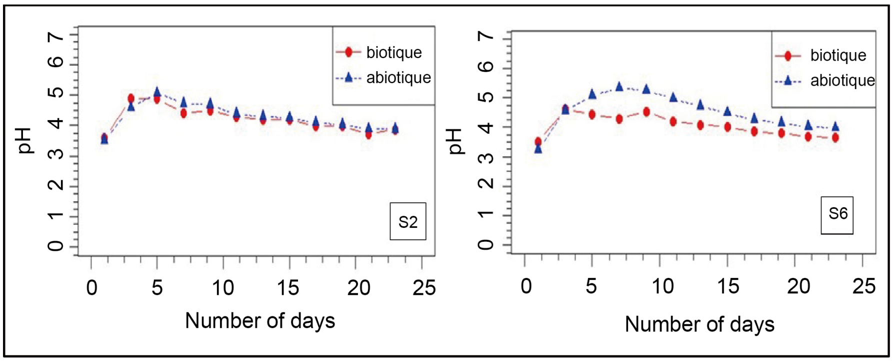

Figure 7. Temporal evolution of pH for the group of iron-oxidizing bacteria in samples (2 and 6) from the biotic test compared to the sterilized abiotic test.

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad (3)$$

The ANOVA test showed that the pH variations observed in the two media were not statistically significant ($p = 0.21$). While differences in pH evolution were observed (S6) at the start of the experiment, these variations were not significant according to the ANOVA results. A different behaviour was observed in the case of acid mine tailings, with a greater decrease in pH in the biotic sample than in the control, which stabilised at pH 3.0 [21]. This same behaviour has also been observed elsewhere in the case of bioleaching experiments on marine sediments dredged by Fe and/or S [25]. The small difference between the biotic sample and the control sample shows the absence of ferro-oxidising acidophilic bacteria.

3.1.2. Monitoring the Oxidation-Reduction Potentials (ORPs) of Biotic and Abiotic Tests

The temporal changes in POR for the identification of iron-reducing bacteria in mining residues from the biotic test are identical to those from the sterilized abiotic test. As an example, **Figure 8** shows the temporal changes in POR for samples 2 and 6. The oxidation reactions can be confirmed by the evolution of the redox potential. The tailings suspensions show oxidising behaviour from the start of the test, with an initial Eh value of +200 mV (**Figure 8**). Both trials follow a similar trend, with a phase of decreasing ORP followed by a phase of stabilisation. However, the initial drop is more noticeable in the abiotic environment than in the biotic environment. The ANOVA test comparing the two trials revealed no statistically significant difference in the evolution of ORPs ($p = 0.21$). The non-significant increase in redox potential observed afterwards can be attributed mainly to chemical oxidation processes involving the Fe^{3+}/Fe^{2+} redox couple [23]. This redox system plays a key role in regulating the oxidation-reduction potential in iron-rich environments. The transition from ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) via chemical oxidation is responsible for these observed variations, as shown in equation (Equation (4)).

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (4)$$

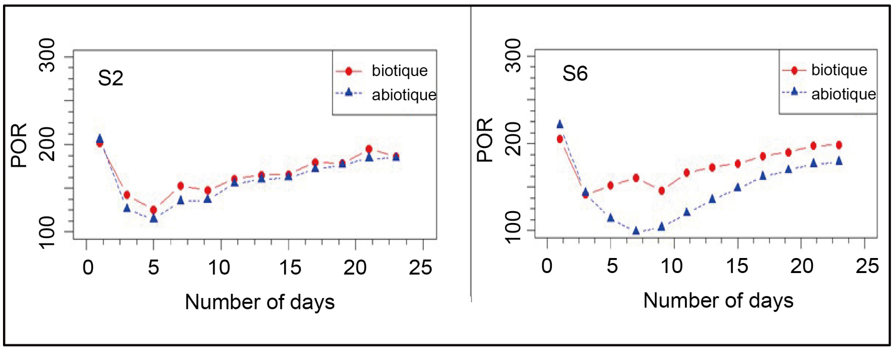

Figure 8. Temporal evolution of POR for identifying ferro-oxidizing bacteria in mining residues from the biotic test compared to the sterilized abiotic test.

In all the experiments, the differences observed in pH and oxidation-reduction potential (ORP) values between biotic and abiotic samples were small and statistically insignificant. The absence of significant differences between the two samples suggests a lack of iron-oxidising bacterial activity, which would otherwise have resulted in measurable changes in pH and redox potential. The small variation observed in the pH and ORP values could be due to the use of an autoclave for sterilising the abiotic samples.

3.2. Qualitative Assessment of the Biogeochemical Reactivity of Mine Tailings by Neutrophilic Sulphur-Oxidising Bacteria (NSO)

3.2.1. Monitoring the pH of Biotic and Abiotic Tests

In the 10 samples, the temporal changes in pH are almost identical. As an example, **Figure 9** shows the change curves for samples 3 and 4. The biotic test initially showed similar pH values to the abiotic environment, with stabilization around neutrality during the first few days of the experiment. The temporary variation between the two trials was not statistically significant, as confirmed by the Kruskal-Wallis test (p = 0.27). The small variation in pH can be explained by geochemical reactivity within the system. The dissolution of carbonates, such as dolomite and calcite, buffers the environment. In the absence of biogeochemical processes or biological activity affecting pH, as in the case of NSO bacteria, a slight oscillation in pH is observed, as noted above. The documented effects of SNO (sulphur and sulphide-oxidising) bacteria have been observed in previous studies [21] to promote acidification of the media, with a drop in pH from 7.0 to 4.0. However, in our study, the absence of significant variations between biotic and abiotic samples shows that biological processes are not the main driver of the acidification observed, and that it is rather the result of chemical and geochemical mechanisms inherent in the system (**Figure 9**).

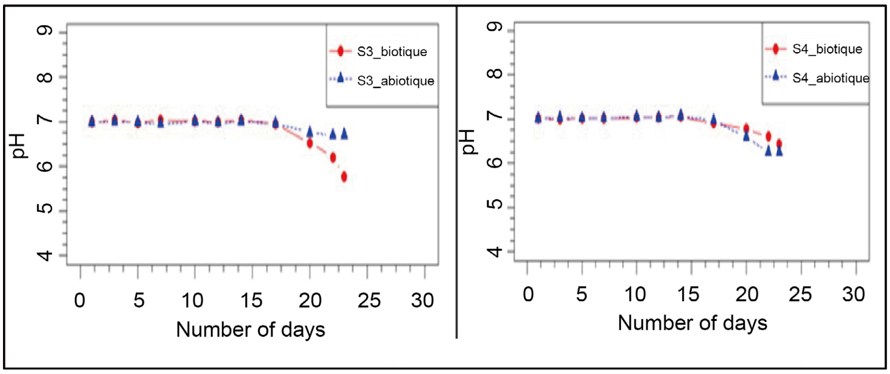

Figure 9. Temporal evolution of pH for the functional group of sulphur-oxidising neutrophilic bacteria in the biotic and sterilized abiotic test tailings.

3.2.2. Oxidation-Reduction Potential (ORP) Monitoring

The temporal changes in POR for the identification of iron-reducing bacteria in mining residues from the biotic test are identical to those from the sterilized abiotic test. As an example, **Figure 10** shows the temporal changes in POR for samples 3 and 10. At the start of the tests, the ORPs of the reaction media were similar. From day 17 for S3 and day 14 for S10, there was a more rapid increase in ORP in the biotic environment than in the abiotic environment. Biotic ORP values reached higher levels (around 80 mV) than in the abiotic environment (around 60 mV) towards the end (**Figure 10**). This difference is not significant (p = 0.08) with the Kruskal-Wallis test carried out in both media.

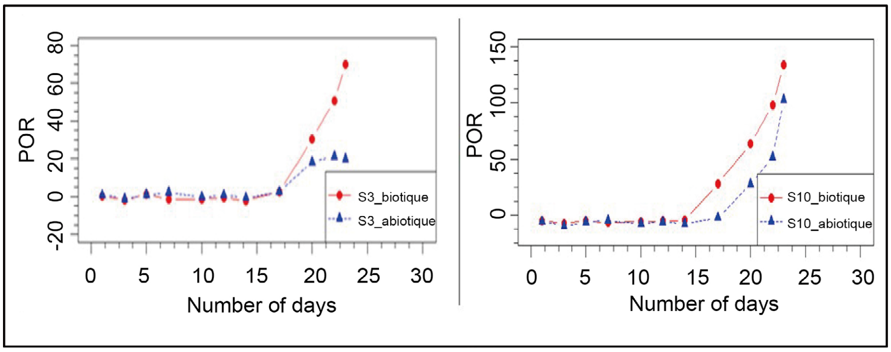

Figure 10. Temporal evolution of POR for the functional group of sulfate-oxidizing neutrophils in mining residues from the sterilized biotic and abiotic tests.

The graphs show a non-significant increase in oxidation-reduction potential (ORP) over time in both conditions. These variations can be attributed mainly to abiotic chemical processes. In sulphur-enriched environments, chemical reactions can play a crucial role in the evolution of the ORP. For example, the oxidation of sulphur in the presence of oxygen can lead to an increase in ORP. The general equation for sulphide oxidation can be represented as follows (Equation (5)):

$$2S + 2H_2O + 3O_2 \rightarrow 2H_2SO_4 \tag{5}$$

This reaction leads to the formation of sulphuric acid (H_2SO_4), increasing the acidity of the medium, which can indirectly affect the ORP. Under oxygen conditions, sulphide oxidation is rapid and may be the main reason for the increase in ORP in both systems, even in the absence of significant microbial activity. The variations in ORP could be linked to a difference in the initial chemical composition of the samples with the addition of sodium azide and heating at 120° for 25 minutes.

4. Conclusion

The biogeochemical method is based on the evolution of parameters such as pH and oxidation-reduction potential (ORP) in two distinct environments (biotic and abiotic). This method was used to diagnose the presence or absence of iron-oxidising acidophilic bacteria and sulphur-oxidising neutrophilic bacteria. Indirect measurements of bacterial activity show the absence of significant variations between biotic and abiotic tests, which would otherwise have led to measurable changes in pH and redox potential. In other words, iron-oxidising acidophilic bacteria and sulphur-oxidising neutrophilic bacteria, involved in the generation of acid by oxidation of sulphide minerals, are absent in the Sabodala mine tailings. The absence of iron- and sulphur-oxidising biological activity prevents the formation of acid mine drainage (AMD) and reduces the mobility of trace metal elements (TMEs).

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Conflicts of Interest

The authors of this work declare that they have no conflict of interest.

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