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(Bio)leaching Behavior of Chromite Tailings

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Abstract: Chromite beneficiation operations in Sukinda valley (India) produce large amounts of tailings, which are stored in open air. In this study, bioleaching experiments were carried out in batch reactors with *Acidithiobacillus thiooxidans* or *Pseudomonas putida* in order to determine the potential leachability of metals contained in these tailings due to biological activity. Acidic and alkaline pH resulted from the incubation of tailings with *A. thiooxidans* and *P. putida*, respectively. Tailings were characterized by X-ray diffraction (XRD), X-ray fluorescence (XRF), and scanning electron microscopy (SEM), and chemical extraction of Cr(VI) with KH_2PO_4 was performed. Mineralogical investigations showed that tailings are mainly composed of chromite, hematite, lizardite, chlorite, and goethite, which are all known as Cr-bearing phases. During the leaching with *A. thiooxidans* and *P. putida*, total Cr was initially extracted as Cr(VI) due to the presence of phosphates in the medium, and subsequently decreased because of Cr(VI) adsorption and reduction to Cr(III). Reduction was associated with bacterial activity, but also with the presence of ferrous iron. Despite the occurrence of siderophores in the tailings after incubation with *P. putida*, under acidic conditions, Fe extracted remained higher. Extracted Ni, Mn, and Al concentrations also increased over time. Given the significant amount of chromite tailings produced every year, this study shows that tailings storage and leachability represent a potential source of chromium. However, our findings suggest that the presence of bacterial communities, as well as physicochemical processes, favor Cr(VI) reduction.

Keywords: tailings; bioleaching; chromite mine; Cr(VI) reduction

1. Introduction

In India, there are 9900 mining leases, spread over an area of 7453 km², covering 55 minerals other than fuel. The Sukinda valley in Jajpur District, Orissa, known for its chromite (FeCr_2O_4) ore deposits, produces nearly 8% of the chromite ore in India [1]. Chromite can be found in stratiform deposits, with concentrations of up to 50%. Because of the high chromite content, no beneficiation process is required and the material can be directly extracted and used for metallurgical purposes. However, when the chromite concentration is lower than 33%, beneficiation with grinding and gravity methods is applied to concentrate the chromite ore [2]. Both in the mining and in the beneficiation process, a variety of waste residues are produced, including waste rock, overburden, and tailings. The inconsistent feed

quality during the beneficiation process results in a loss of efficiency and an increase of wastes, as is the case for tailings. Approximately 50% (by weight) of the total feed is discarded as tailings which still contain significant amounts of chromite (13–15%) [2].

Several studies have been carried out in order to increase the chromite extraction yield and consequently decrease the tailings production [3,4]. A maximum of 83% recovery of chromite was reached with 23% Cr₂O₃ content. Despite the efforts that have been made to reduce chromite losses or reprocess stockpiled tailings, the ultrafine particle size makes chromite recovery and management difficult. Therefore, several tons of tailings per year are still produced and stored in open air conditions [5,6].

Chromium (Cr) has several oxidation states from −2 to 6, and the trivalent (Cr(III)) and hexavalent (Cr(VI)) states are primary oxidation states in nature. Hexavalent chromium is known to be highly mobile, soluble, and toxic, while trivalent chromium is not toxic and largely immobile in the environment [7]. Nevertheless, Cr(III) can be mobilized by forming complexes with dissolved organic carbon [8]. Because of its toxicity, Cr(VI) has been shown to have several negative environmental impacts [9,10]. In groundwater, the natural occurrence of Cr(VI) is linked to the hydrolysis of feldspar, some common mafic minerals such as Cr-bearing pyroxenes and chromite, together with calcite, which causes alkaline groundwater conditions. This, coupled with the absence of natural reducing agents like Fe(II), organic matter, or reducing organisms, may allow the oxidation of Cr(III) to Cr(VI) [9]. In rocks, chromite grains are locked either within the iron ore minerals (goethite/hematite) and silicates or the chromite with inclusions of silicate [6]. Silicates are more susceptible to weathering than chromite, which represents a source of chromite nanoparticles (containing mainly Cr(III)), that after dissolution, could be oxidized to Cr(VI) [10,11]. According to Beukes et al. [9], Cr(VI) containing wastes generated by chromite mining are classified by three groups: (i) recycled within the process, (ii) re-purposed or re-used in other applications, and (iii) considered hazardous. Because tailings are not recycled or re-used and due to their potential to release Cr(VI), they belong to the third, hazardous, group of waste generated by chromite mining.

Consequently, all the efforts are focused on the understanding of Cr(VI) dynamics [7,12–18] and the prevention or at least mitigation [19–23] of Cr(VI) production. Tiwary et al. [24] studied the migration of metals leached from an overburden dump of chromite ore to groundwater. This study revealed that due to the poor permeability of the seepage, the migration of metals occurs at a low rate and could only take place in the first layers down to 10 m in 10 years. Godgul [25] performed leaching experiments of chromite according to pH and highlighted that there is no relation between the total Cr in the solid sample and in the leached solution, because of the distribution of Cr in different mineral phases besides chromite. It was also observed that the maximum of Cr leached is obtained at pH 8 and all the elements (Cr, Fe, Al, Mn, and Ni) are leached from the same mineral phase.

A great variety of microorganisms have been identified in mine waste, and microbial processes are usually responsible for the environmental hazard created by mine wastes. Microorganisms can, in principle, influence metals leaching from mine waste in several ways. However, they can also be used to retard the adverse impact of mine wastes on the environment [26]. Given the fact that some microorganisms contribute to metal immobilization, several studies have focused on the use of microorganisms such as bacteria for metal recovery or Cr(VI) reduction from mining residues. Das et al. [27] studied the selection of Cr(VI) reducing bacteria from soils impacted by tailings. *Bacillus amyloliquefaciens* was isolated from chromite mine soil and exhibited Cr(VI) tolerance and a Cr(VI) reduction rate of 2.2 mg Cr(VI)/L/h. Alleghretti et al. [28] investigated the presence of intermediary compounds, produced by *Acidithiobacillus* and *Thiobacillus*, that were responsible for Cr(VI) reduction. They identified several polythionates associated with sulfur particles between 0.45 and 3 µm. Wang et al. [29] evaluated the leachability of Cr(VI) from tannery sludge with *A. thiooxidans*. After five days in a bubble column bioreactor at 30 °C, 99.7% of Cr was leached out from the tannery sludge. Kumar and Nagendran [30] also observed the efficient removal of metals, including Cr (95%), in soils through bioleaching with *A. thiooxidans*. Cr in the organic and residual fractions was unaltered

during the course of the experiment, while the Fe-Mn oxide bound chromium was transformed to the exchangeable and carbonate fraction. Metal solubilization mechanisms by *A. thiooxidans* can be direct or indirect. In the direct mechanism, metal sulfides are directly oxidized to SO_4^{2-} by the bacteria, while in the indirect mechanism, H_2SO_4 , a strong leaching agent, is produced by *A. thiooxidans* [31]. *Acidithiobacillus* species have also been used in both the aerobic and anaerobic reductive dissolution of iron rich nickel laterite to remove target metals [32,33] and in laterite tailings [34] to recover heavy metals. Most of these studies target industrial applications. However, neither the leaching nor the bioleaching behavior of chromite tailings stockpiled in open air in the environment has been studied so far and the effect of bacteria on chromite tailings bioleaching has not yet been identified.

Given the increasing importance of chromite ores for Cr world production and the huge amount of tailings produced (5×10^5 ton/year—average production between 2016 and 2017 in all the chromite mines in Sukinda valley) [35], it remains of key interest to fill the knowledge gap regarding the tailing discarded after the chromite beneficiation process. In the present study, the influence of pH on the bioleaching of chromite tailings was evaluated in batch experiments. Acidic and alkaline conditions were obtained through the incubation of tailings with *Acidithiobacillus thiooxidans* and *Pseudomonas putida*, respectively. The bacterial influence on tailings leachability was evaluated and the potential release of Cr(VI) from tailings was estimated. Fresh and old tailings were compared to estimate the potential risk of chromite tailings to the environment.

2. Materials and Methods

2.1. Field Settings

Superficial fresh chromite tailings and concentrate ore, as well as a core in the stockpiled old chromite tailings, were collected in May 2017 from materials generated in a typical beneficiation plant of Sukinda valley, Jajpur district, Odisha, India (Figure 1). The Sukinda valley is located in the eastern part of the Indian peninsula, where the average annual precipitation is about 2400 mm. Most of the rain occurs during the monsoon season between May and October. It is primarily drained by the Damsala Nala River. The river flows westward through the central valley to meet the major river, Brahmani, which flows at a distance of 15 km downstream of the mining belt [36].

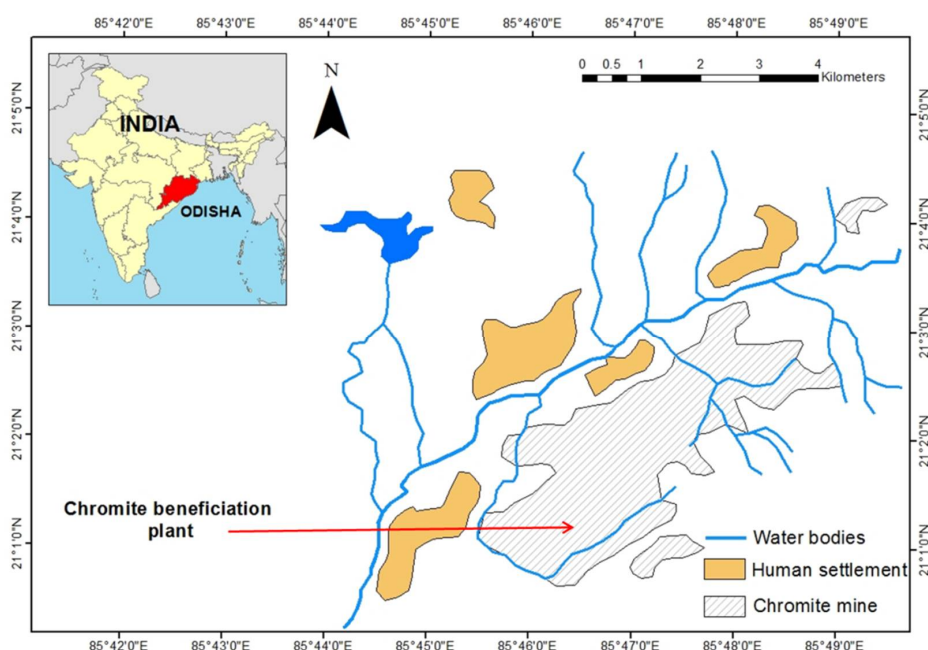


Figure 1. Chromite mine in Sukinda valley (Odisha, India).

Most of the mines are located in the central part of the valley and are operated by 12 different companies [37]. The Sukinda ultramafic complex is composed of alternate bands of chromite, dunite, peridotite, and orthopyroxenite, which are extensively lateralized [38,39]. The chromite bands in the Sukinda valley present various compositions. When the Cr_2O_3 content is higher than 48%, the ore is “high grade” and is directly marketable, while between 32% and 45%, the ore is “medium grade”, and finally, lower than 30% is “low grade” [37]. For the last case, the presence of gangue minerals such as goethite, serpentine, olivine, and talc has led to the utilization of lean ore after beneficiation. The beneficiation process majorly consists of comminution to physically liberate the minerals followed by physical separation to generate the concentrate of the chromite ore [4]. During the comminution process, large quantities of fines are generated. The fines are commonly de-slimed using hydro-cyclone and cyclone underflow processed using gravity concentrators like spirals and tables. As a result, chromite concentrated ore is obtained, and roughly 50% to 70% of the chromite losses are in the fine fractions (tailings), where the fraction below $75\ \mu\text{m}$ contains about 9–20% Cr_2O_3 [2,6].

2.2. Chemical and Biological Leaching Experiments

The effect of bacterial bioleaching activity of fresh tailings was tested at three pulp densities of 5, 10, and 30 g/L in batch reactors. Batch reactors were selected in the present work, because they have been systematically used to study the kinetics of chromium transformations under typical environmental conditions [13], in order to establish the leachability of metals from laterite tailings [40] and to study the leaching characteristics of chromite ore processing residue (CORP) [41–43]. Also, batch experiments have been used to establish the microbial effect on chromium mobility [44,45].

All the material was autoclaved at $121\ ^\circ\text{C}$ for 20 min prior to use. The 500 mL reactors were closed using a cotton plug, both previously sterilized, and placed in an orbital shaker at 190 rpm and $30\ ^\circ\text{C}$. Samples were collected after 4 h, 1, 2, 5, 6, 8, 10, 12, 15, and 30 days. A total of 20 mL of leachate was collected and replaced by fresh sterile medium to maintain the solid liquid ratio. In the samples collected, dissolved oxygen, pH, and electrical conductivity were monitored using a WTW 3410 Set 2 multiparameter probe (Xylem Inc., Rye Brook, NY, USA). In the case of bio-leaching with *P. putida*, approximately 5 mL of sample was frozen at $-20\ ^\circ\text{C}$ to determine the protein content and siderophores concentration later. The rest of the sample was filtered through $0.45\ \mu\text{m}$ pore size nitrocellulose syringe filters, for the determination of major and trace element concentrations in the leachate. Samples were acidified with 16 N HNO_3 and stored at $4\ ^\circ\text{C}$ until their analysis. Biological and chemical leaching experiments were done in duplicate to verify reproducibility. For this reason, the results presented in this work are an average of the duplicates with its associated standard deviation.

2.2.1. Bio-Leaching with *A. thiooxidans* and *P. putida*

The bioleaching experiments of fresh tailings with *A. thiooxidans* and *P. putida* were performed in solutions containing the same components of the growth media where bacteria were pre-grown. The initial pH of the growth media was set at 3.5 for *A. thiooxidans* and 7 for *P. putida*, with 1 N H_2SO_4 and 1 N NaOH, respectively. The batch reactors were inoculated with one percent (v/v) of pre-grown bacterial culture. The sterilized fresh tailing sample plus culture media were used as a control and distilled water plus sterilized tailing sample as a blank. In both cases, sodium azide (NaN_3) was added to avoid bacterial growth.

Acidithiobacillus thiooxidans

The gram-negative bacterial strain *A. thiooxidans* (DSM 9463) was grown in a medium containing 2 g ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 0.25 g magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.1 g dipotassium hydrogen phosphate (K_2HPO_4), and 0.1 g potassium chloride (KCl) per liter, in addition to 11% (wt/v) of elemental sulfur that was previously autoclaved at $121\ ^\circ\text{C}$ for 20 min. The medium pH was adjusted to 3.5 before sterilization, and the sterilized sulfur was added subsequently together with the inoculum.

The culture was maintained at 30 °C. The volume of the culture was increased gradually from 5 mL to 500 mL in separated Erlenmeyers.

Pseudomonas putida

The gram-negative bacterium *P. putida* (WCS 358) bacterial strain was kindly provided by Peter Bakker, University of Utrecht (The Netherlands). For siderophore production, iron free succinate medium (SM) consisting of (in g/L): K₂HPO₄ 6.0, KH₂PO₄ 3.0, MgSO₄·7H₂O 0.2, (NH₄)₂SO₄ 1.0, and succinic acid 4.0, pH 7.0 was used to inoculate *P. putida* at a concentration of 1% (v/v) inoculum.

2.2.2. Chemical Leaching

In addition to the experiments done with *A. thiooxidans* and *P. putida*, chemical leaching of the fresh tailing sample with distilled water at pH 2 (adjusted with H₂SO₄) and distilled water at pH 9 (adjusted with NaOH) was carried out at a pulp density of 30 g/L. The leachability of chromite was also studied through the leaching of the concentrated ore coming from the beneficiation plant.

2.3. Chemical Extraction

In order to determine the chemically exchangeable pool of Cr(VI) in the fresh and stockpiled old tailings and chromite ore, chemical extraction was performed using distilled water and 0.1 M KH₂PO₄. The principle of the extraction with KH₂PO₄ is based on the fact that chromate ions can be desorbed by reactions of the soil with other specifically desorbed anions, such as phosphates (PO₄^{3−}) and sulfates. A suspension of 1 g of soil was agitated with 25 mL of the reactant (distilled water or 0.1 M KH₂PO₄) for 1 h [46]. The supernatant was separated by centrifuging for 15 min at 2500 rpm, followed by filtration with a 0.22 µm pore size PES filter. Finally, the total Cr concentration in the supernatant was determined with ICP-AES. Cr(VI) was analyzed by the colorimetric technique (diphenylcarbazide (DPC) method) [47,48] and was measured with a Shimadzu UV-2550 spectrophotometer with a 1 cm quartz cell at λ of 540 nm.

2.4. Analytical Methods

2.4.1. Chromite Tailings Characterization

The mineralogical composition of the fresh tailing sample and the concentrated ore was determined using X-ray diffraction (XRD) analysis on a PANalytical diffractometer and Cu Kα radiation (at 45 kV–40 mA) in the grazing incidence angle in the 5°–70° 2θ range with a scan step of 0.013°. In addition, total metal concentration was determined by X-ray fluorescence (XRF), using an X fluorescence PANalytical spectrometer (Malvern Panalytical, Malvern, UK) equipped with Energy Dispersive Minipal 4 (Rh X-ray tube 30 kV–9 W) at a resolution of 150 eV (Mn Kα). The fresh tailing samples were thin-coated with carbon prior observations with a Zeiss Auriga Scanning Electron Microscope (SEM) (Zeiss, Jena, Germany). The SEM was equipped with a Field emission Electron Gun (FEG) at 15 keV with an SE-Inlens detector.

2.4.2. Leachates Composition

The concentration of major elements in filtered samples was determined using ICP-AES (ICAP 6200 Thermo Fisher, Thermo Fisher Scientific, Waltham, MA, USA), whereas HR-ICP-MS (Thermo Scientific Element II, Thermo Fisher Scientific, Waltham, MA, USA) was used for trace elements analysis. Detection limits were typically between 0.6 and 74 ng/L and the standard deviation associated with the measurements was smaller than 5%. Fe speciation in the leachate was measured with the ferrozine method [49].

2.4.3. Protein Determination

In order to establish the growth of the bacterial community, the Lowry protein test [50,51] was performed in the supernatant collected in the leaching experiments. For the calibration curve, protein standards were prepared with bovine serum albumin concentrations between 10 and 500 µg/mL. A total of 0.1 mL of 2 N NaOH was added to 0.1 mL of sample or standard. The mixture was hydrolysed at 100 °C for 10 min in a boiling water bath. After cooling the hydrolysate to room temperature, 1 mL of the complex-forming reagent was added and the solution was left for 10 min at room temperature. After that, 0.1 mL of Folin reagent was added to the mix using a vortex mixer. After 30 to 60 min, the absorbance was read at 750 nm for protein concentrations below 500 µg/mL [50].

2.4.4. Siderophores Assay

Quantitative estimation of siderophores produced by *P. putida* was done by the CAS-shuttle assay [52]. This method is based on the high affinity of siderophores for iron(III) [53]. The CAS reagent was prepared using: (1) 0.06 g of CAS in 50 mL of distilled water; (2) 0.0027 g of FeCl₃·6H₂O in 10 mL of 10 mM HCl; and (3) 0.073 g of HDTMA in 40 mL of distilled water. Solution (1) was mixed with 9 mL of solution (2) and after with solution (3). The CAS reagent was autoclaved and stored in a plastic bottle [54]. For the quantification of the siderophores, 0.5 mL of supernatant (sample) was mixed with 0.5 mL of CAS reagent, and absorbance was measured at 630 nm. The siderophore content was calculated by using Equation (1), proposed by Sayyed et al. [55].

$$\% \text{ siderophore units} = \frac{A_r - A_s}{A_r} \times 100 \quad (1)$$

where A_r and A_s correspond to the absorbance of the reference and sample at 630 nm, respectively.

2.5. Geochemical Modeling

The visual MINTEQ 3.1 equilibrium modelling program was used to identify the major mineral(s) controlling the chemistry of fresh tailings. The Saturation Index (SI), which is defined as the logarithm of the ratio of the ion activity product and the solubility product (K_{sp}) ($SI = \lg IAP/K_{sp}$), was calculated with the program. Positive SI values indicate that the solution is oversaturated and precipitation is possible, negatives values indicate that it will tend to dissolve, and zero shows equilibrium of the solution with a mineral phase. The input data for MINTEQ 3.1 included the pH, temperature, total cation (Ca, Mg, Na, K, Ni, Fe(II), Al, Cr(VI)), and anion (SO_4^{2-} , NO_3^- , Cl^- , PO_4^{3-}) concentration. No adsorption parameters were included within the calculations.

3. Results

3.1. Tailings and Concentrated Ore Characterization

A detailed chemical composition of the samples is given in Table 1. X-ray Fluorescence analysis shows that in the fresh tailing sample, 18 wt % is chromium oxide and 56 wt % is iron oxides. For the concentrated ore sample, the proportion of Cr₂O₃ increases up to 33.17 wt %. The XRF composition is consistent with XRD results, which shows the presence of goethite (α -FeO(OH)), hematite (Fe₂O₃), gibbsite (Al(OH)₃), chlorite ((Fe,Mg,Al)₆(Si,Al)₄O₁₀(OH)₈), and chromite (FeCr₂O₄), as the main crystallized mineral phases in the fresh tailing sample.

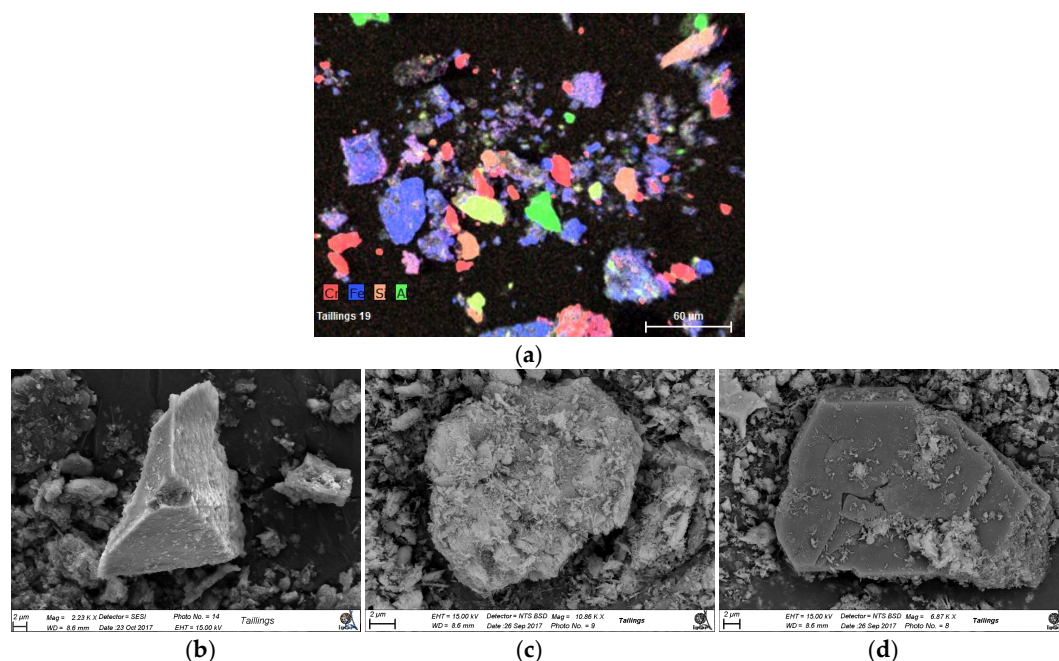
Table 1. XRF results of the fresh tailing and chromite concentrated ore sample collected in the beneficiation plant (Sukinda valley) in May 2017.

Oxide Rate (%)	Fresh Tailings	Chromite Concentrated Ore
Al ₂ O ₃	8.7	9.4
SiO ₂	13.4	3.7
Cr ₂ O ₃	18.1	33.2
Fe ₂ O ₃	56.7	52.0
Total	96.9 *	98.4 *

* The 3.1% and 1.6% leftover for tailings and concentrated ores, respectively, correspond to traces of Mn, Mg, V, Ti, Ca, P, and S.

Dwari et al. [6] found additional mineral phases in chromite tailings from Sukinda valley, including kaolinite (Al₂Si₂O₅(OH)₄), quartz (SiO₂), and magnesioferrite (Mg(Fe)₂O₄). The concentrated ore sample is enriched in chromite with some traces of lizardite, hematite, and goethite.

SEM-EDX images collected on the fresh tailing sample are displayed in Figure 2. As was previously shown by the XRF results, the tailings are dominated by the presence of Fe, followed by Cr, Al, and Si oxides (Figure 2a). In the fresh tailing sample, Cr is found as chromite (Figure 2b).

**Figure 2.** Chromite tailing (a) elementary map of EDS showing the dominance of Cr, Fe, Si, and Al. SEM images of (b) chromite (Cr 63 wt %), (c) iron oxide (Cr 9 wt %), and (d) aluminum oxide (Cr 6 wt %).

In Sukinda chromite tailings, Tripathy et al. [3] found two different types of chromite grains: the first one is rich in Cr with a minimum amount of Fe, Al, and Mg; whereas the second one is rich in Fe, Al, and Mg, along with Cr. The grain display in Figure 2b corresponds to the second group, where there is 63 wt % of Cr₂O₃, 18 wt % of FeO, 9 wt % of MnO, 6 wt % of Al₂O₃, and 4 wt % of MgO. Other Cr-bearing phases are goethite, FeO(OH), aluminum oxides, and other colloids with a positively charged surface where hexavalent Cr species, which are negatively charged (e.g., CrO₄²⁻ and HCrO₄⁻), can be adsorbed [56]. In the tailings, gangue minerals enriched in Fe oxy(hydroxides) (81 wt %) are identified with the presence of Cr (Cr₂O₃ 9 wt %), Si (SiO₂ 5 wt %), and Al (Al₂O₃ 5 wt %) (Figure 2c). Al oxide is also present in the fresh tailing sample, with a lower Cr content (6 wt %) (Figure 2d).

3.2. pH Profiles

The changes of pH observed in the biotic and abiotic leaching experiments in distilled water and growth medium with *P. putida* and *A. thiooxidans* are presented in Figure 3. The leaching of the fresh tailing sample with distilled water shows a stable pH close to 7, and is 0.2 units higher for the pulp density at 30 g/L compared to 5 and 10 g/L (Figure 3a).

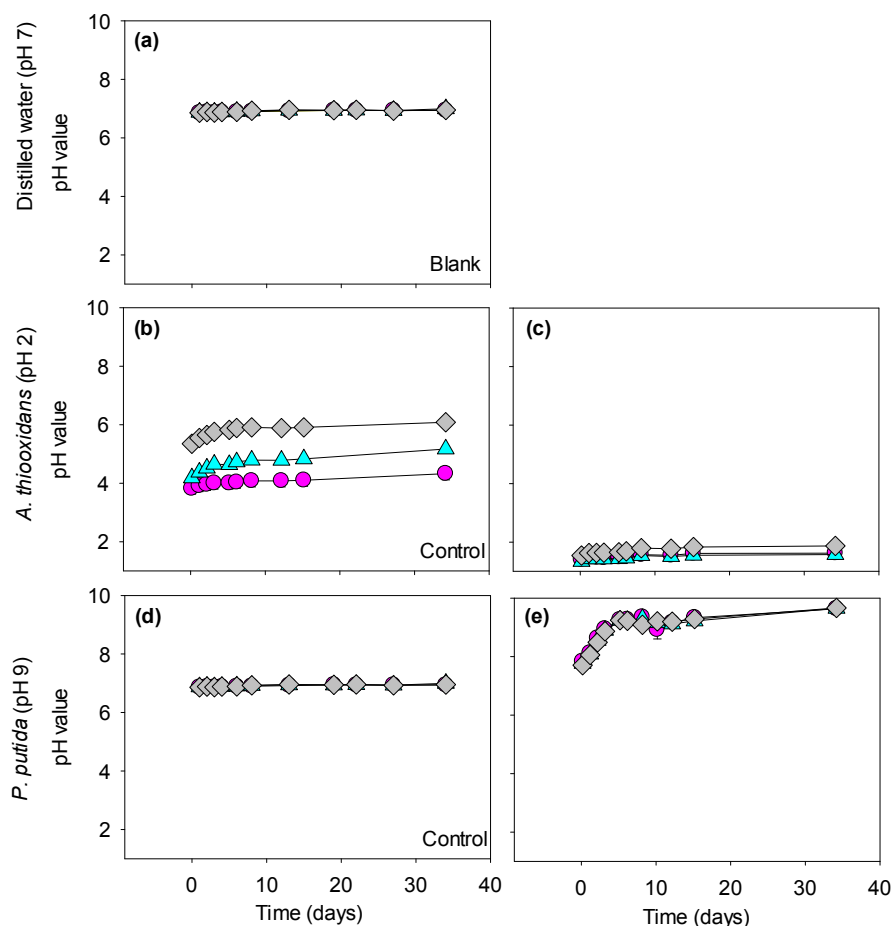


Figure 3. pH evolution in leaching experiments of the fresh tailings sample with distilled water (blank) (a), and incubation with *A. thiooxidans* (c) and *P. putida* (e). Controls for *A. thiooxidans* and *P. putida* are displayed in (b,d), respectively. Three pulp densities were evaluated: 5 g/L (—●—), 10 g/L (—▲—), and 30 g/L (—◆—).

For *A. thiooxidans*, in the control (growth medium pH adjusted to 3.5), pH is higher at a higher pulp density. For 5 g/L the pH varies from 3.8 to 4.3, for 10 g/L it varies from 4.1 to 5.2, and for 30 g/L from 5.3 to 6.0. When *A. thiooxidans* is inoculated, the pH is consistently low (1.6 in average) for all pulp densities. The low pH is the result of the sulfur oxidation by *A. thiooxidans* using dissolved oxygen as the electron acceptor, and the resulting H_2SO_4 production [57]. In the case of *P. putida*, the control (growth medium pH adjusted to 7) has a pH close to 7 and no differences were observed according to the pulp densities. However, in the reactors where *P. putida* is inoculated, the pH increases significantly from 7 to 9.2 in the first five days, whatever the pulp density. Van Hullebusch et al. [58] explained the pH increase in slag leaching as a result of the initial dissolution of cations such as Ca^{2+} and Mg^{2+} , followed by neutralization by H^+ from H_2O . Potysz et al. [59] also reported a pH increase in Cu slags leaching due to H^+ replacing Fe^{2+} during fayalite dissolution. In batch reactors inoculated with *P. putida*, dissolved oxygen increases from 0 on the first day to 4.7 mg/L on the fifth day, and remains constant for the rest of the sampling time (Figure S1).

3.3. Release of Elements during Leaching Experiments

The behavior of Cr, Fe, Ni, Mn, and Al during the bioleaching of the fresh tailing sample is presented in Figures 4–8 and the units are expressed in mg of element released per kg of tailings. The use of *A. thiooxidans* as a leaching agent facilitates Fe dissolution. At a pulp density of 5 g/L, Fe extracted from the fresh tailings sample increases from 421 to 1234 mg/kg, and for 10 g/L, the range is narrower, with values between 510 and 940 mg/kg (Figure 4). For 30 g/L, no significant fluctuations were observed, with an average value of 190 (± 21) mg/kg over time. In the reactors with *A. thiooxidans* for the three pulp densities, the pH is always below 4 and for the first day, Fe^{2+} is the dominant species; however, after that, Fe(III) species are more abundant, caused by the oxidation of Fe^{2+} by *A. thiooxidans* [60]. The same Fe speciation is observed in a leaching test using fresh tailings sample conducted with distilled water acidified with H_2SO_4 and without bacteria (Figure S2). Liu et al. [60] performed leaching experiments of chalcopyrite with *A. ferrooxidans* (pH 2–2.5) and they found total Fe concentrations up to 0.6 g/L, which were present as Fe^{2+} and Fe^{3+} for the first six days, but afterwards, only Fe^{3+} was detected. In the control of *A. thiooxidans*, at the higher pH (6; pulp density 30 g/L) the Fe extracted is lower, compared with the lower pH (4; pulp density 5 g/L), where total iron reaches 7 mg/kg on the sixth day of leaching. This result suggests that the medium effect is negligible compared to the leaching produced by the presence of *A. thiooxidans* and more specifically by the sulfuric acid produced.

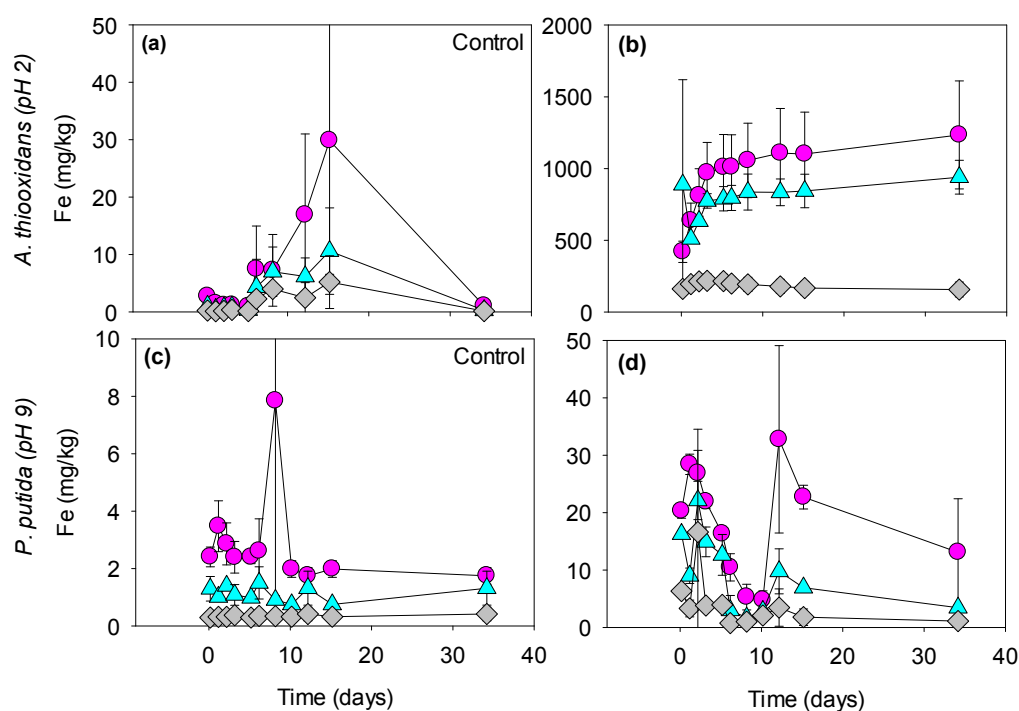


Figure 4. Fe concentration in the leachate as a function of time during (bio)leaching experiments of the fresh tailing samples with *A. thiooxidans* (b) and with *P. putida* (d). Controls for *A. thiooxidans* and *P. putida* are displayed in (a,c), respectively. Three pulp densities were evaluated: 5 g/L (—●—), 10 g/L (—▲—), and 30 g/L (—◆—).

Because of its toxicity, mobility, and enrichment in chromite and other Cr bearing phases in chromite tailings, the leaching behavior of chromium is of particular interest. With distilled water, a maximum of chromium is leached from the fresh tailing sample in the first three days for all pulp densities, reaching values of 94.8 (± 1.41), 74.45 (± 0.35), and 62.70 (± 8.87) mg/kg for pulp densities of 5, 10, and 30 g/L, respectively. After the third day, the chromium extracted slowly increases until day 34. When *A. thiooxidans* is present in the batch reactor, chromium is leached from the fresh tailing sample

in the first hours and then the curve decreases. The pulp density at 5 g/L presents the highest Cr content (174–268 mg/kg), followed by 10 (159–236 mg/kg) and 30 g/L (130–191 mg/kg). In the control of *A. thiooxidans*, the maximum chromium extracted for the 5 (259 mg/kg) and 10 g/L (245 mg/kg) pulp density is similar to the batch incubated with *A. thiooxidans*, for the same pulp densities. For the pulp density at 30 g/L in the control for *A. thiooxidans*, the chromium concentration slightly increases in solution from 170 to 202 mg/kg in the first two days and then remains stable (Figure 5).

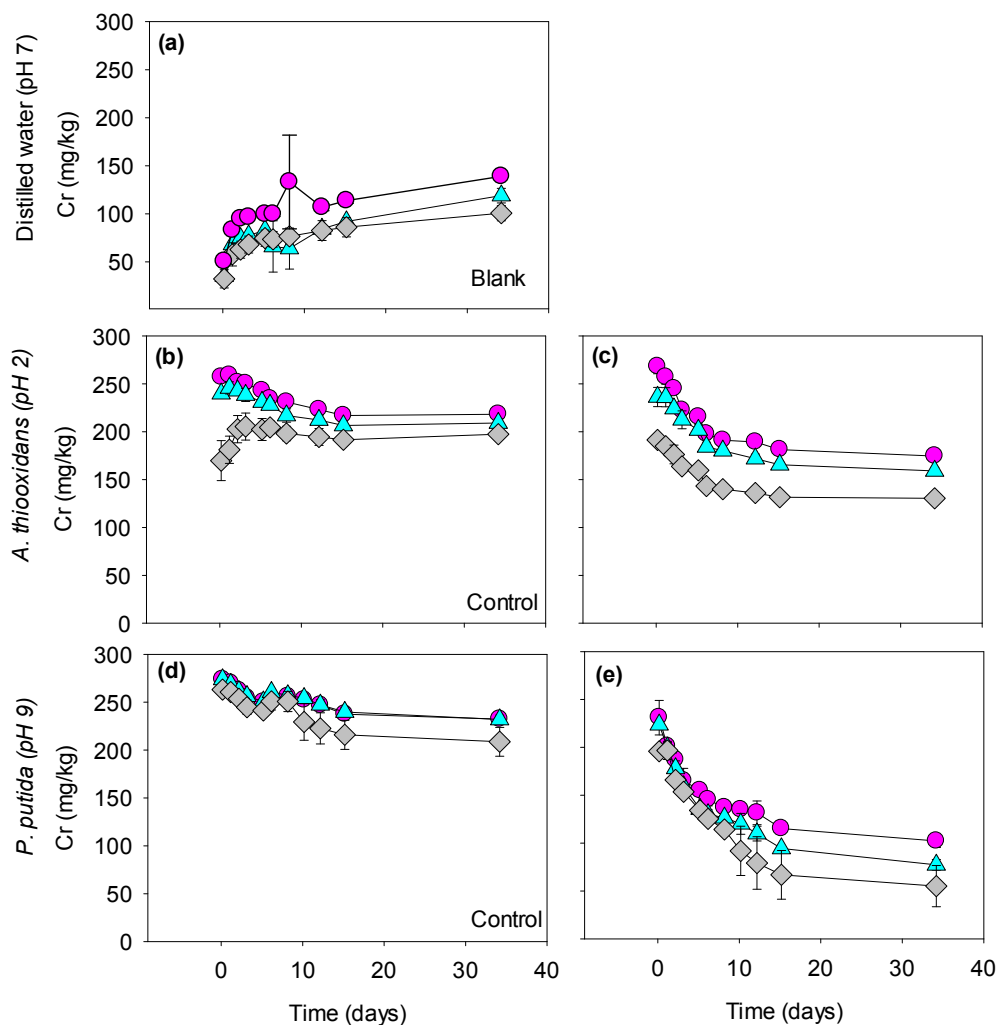


Figure 5. Cr concentrations in the leachate as a function of time during (bio)leaching experiments of the fresh tailing sample with distilled water (a), incubated with *A. thiooxidans* (c) and *P. putida* (e). Controls for *A. thiooxidans* and *P. putida* are displayed in (b,d), respectively. Three pulp densities were evaluated: 5 g/L (—●—), 10 g/L (—▲—), and 30 g/L (—◆—).

In the case of the fresh tailing sample incubated with *P. putida*, for all pulp densities, the maximum amount of chromium extracted (217 mg/kg in average) is reached in the first hours and then the chromium concentration slowly decreases. Starting from day 10, the differences between pulp densities become relevant with higher amounts of Cr extracted for a pulp density of 5 g/L (135 mg/kg). In the control for *P. putida*, chromium decreases by 15% at 5 and 10 g/L and 20% at 30 g/L, from day 1 to 34.

For both *A. thiooxidans* and *P. putida*, the maximum Cr concentration is observed at the beginning of the incubation, and is influenced by the presence of KH_2PO_4 in the growth medium. Phosphates (PO_4^{3-}) can promote the desorption of anions including chromate ions, even if they are tightly bound compared with other anions such as chloride, nitrate, and sulfate [61,62]. However, not only the

presence of phosphates promotes the presence of chromium in solution, because almost 150 mg/kg of Cr is extracted during the leaching with distilled water after 34 days (Figure 5).

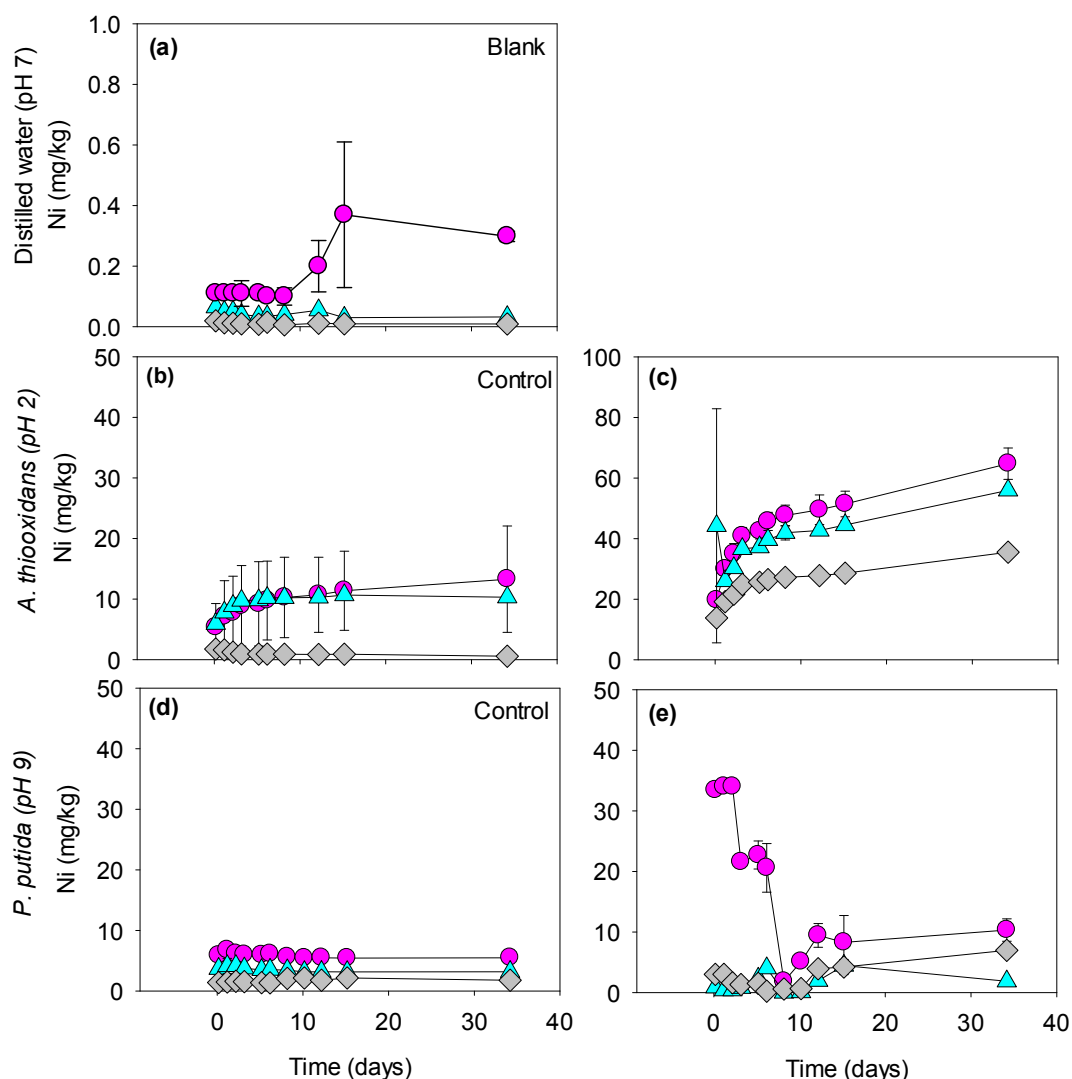


Figure 6. Ni concentrations in the leachate as a function of time during (bio)leaching experiments of the fresh tailing sample with distilled water (a), incubated with *A. thiooxidans* (c) and *P. putida* (e). Controls for *A. thiooxidans* and *P. putida* are displayed in (b,d), respectively. Three pulp densities were evaluated: 5 g/L (—●—), 10 g/L (—▲—), and 30 g/L (—◆—).

It is known that lateritic chromite overburden contains nearly 0.4%–0.9% Ni, which is entrapped within the goethite (FeOOH) matrix [63]. It is also known that Ni and Cr are elements characteristic of ultramafic areas, and both of them have species with a certain grade of toxicity. For this reason, the leachability of Ni is also evaluated. Figure 6 presents the Ni behavior in the leachate over time.

The leaching of the fresh tailings sample with distilled water showed that Ni in the leachate was lower than 1 mg/kg for all pulp densities. In the incubation with *A. thiooxidans*, the extracted Ni concentration increased over time. For a pulp density of 5 g/L, the Ni extracted goes from 20 to 65 mg/kg, for 10 g/L is between 44 and 56 mg/kg, and for 30 g/L is between 14 and 36 mg/kg. The *A. thiooxidans* medium composition (Figure 6b) induces Ni leaching from the fresh tailing sample; however, it only corresponds to the 20% of the Ni extracted under acidic conditions in the batch reactor incubated with *A. thiooxidans*. This indicates that the growth of *A. thiooxidans* promotes the leaching of Ni. In the leaching experiments with *P. putida*, Ni tends to decrease from the first day (33 mg/kg for

5 g/L, 6 mg/kg for 10 g/L, and 3 mg/kg for 30 g/L) to the sixth day (20 mg/kg for 5 g/L, 0.5 mg/kg for 10 g/L and 0.2 mg/kg for 30 g/L), and after this, part of it is re-solubilized and/or more Ni is leached from the tailings. The *P. putida* medium composition effect is negligible.

As occurs with other elements, manganese is released from the fresh tailing sample under incubation with *A. thiooxidans*. At a pulp density of 5 g/L, Mn increases at a rate of 21 mg/kg/day in the first three days and after this, continues to increase at a rate ten times lower (2 mg/kg/day). For 10 g/L, Mn extracted varies from 94 to 138 mg/kg. For the pulp density of 10 g/L, in the first three days, the Mn extracted increases at a rate of 8 mg/kg/day and in the period remaining, decreases to 1.6 mg/kg/day. For 30 g/L, the range of variation is smaller (25 to 56 mg/kg), and it also increases over time. The manganese chemically extracted by the medium without the incubation of *A. thiooxidans* corresponds to 10% of the total amount extracted after incubation. For the incubation with *P. putida*, the Mn concentrations are considerably lower than for *A. thiooxidans*, due to the instability of Mn^{2+} at a pH higher than 7. The Mn variation, during incubation of the fresh tailing sample with *P. putida*, is higher for the pulp density at 5 g/L, with values between 0.6 and 23 mg/kg (Figure 7).

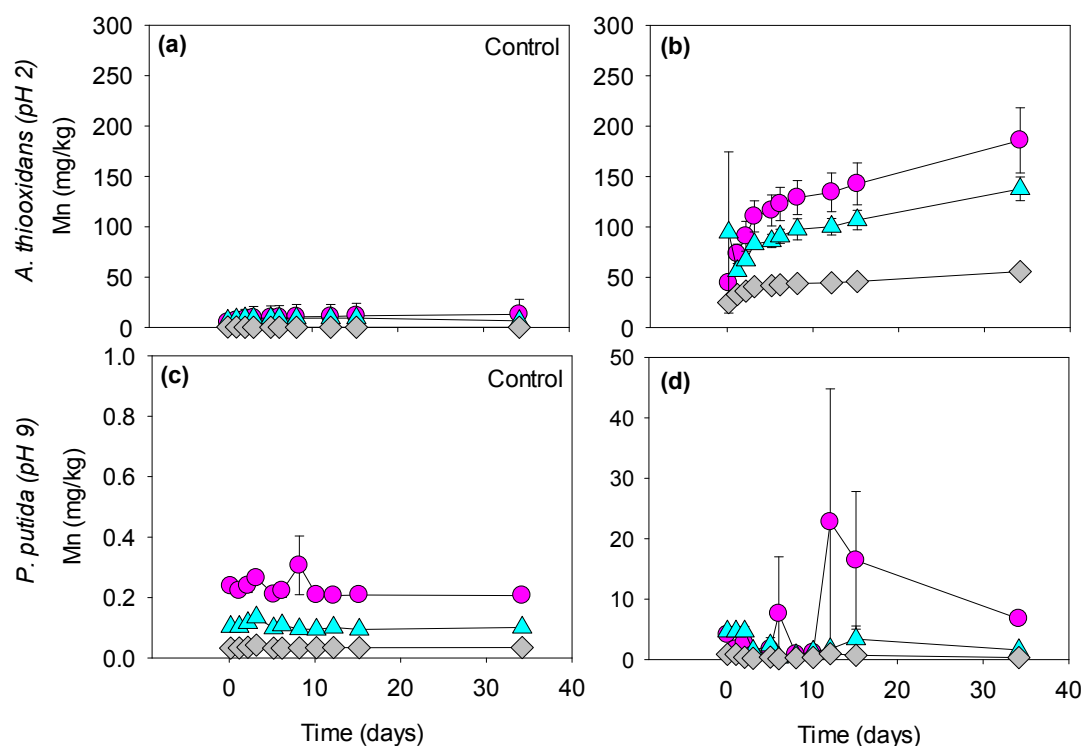


Figure 7. Mn concentration in the leachate as a function of time, during (bio)leaching experiments of the fresh tailing sample with *A. thiooxidans* (b) and with *P. putida* (d). Controls for *A. thiooxidans* and *P. putida* are displayed in (a,c), respectively. Three pulp densities were evaluated: 5 g/L (—●—), 10 g/L (—▲—), and 30 g/L (—◆—).

Aluminum is the fourth most abundant element present on the chromite tailings, and it remains present in the concentrated ore after the beneficiation process. Leaching of the fresh tailings sample with distilled water shows that Al in solution is released in the first hours during the first six days. However, after this period of time, Al starts to continuously increase within 40 days of incubation, except for the pulp density of 30 g/L, where the concentration remains constant.

When the fresh tailing sample is incubated with *A. thiooxidans*, there is a release of Al starting from 302 to 1784 mg/kg, from 725 to 1826 mg/kg, and from 239 to 1274 mg/kg, for the pulp densities of 5, 10, and 30 g/L, respectively. This is due to the production of H_2SO_4 by *A. thiooxidans* and the dissolution of amorphous mineral phases containing Al. Chromite tailings of a beneficiation plant in

India were characterized by Tripathy et al. [3] and they found kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$), in addition to the Al mineral phases, such as gibbsite and chlorite, in the chromite tailings used in the present work. The control of *A. thiooxidans* reveals no medium effect in Al dissolution. The incubation of the fresh tailing sample with *P. putida* shows notably less Al alteration compared with the incubation with *A. thiooxidans*. In addition, Al does not dissolve continuously; on the contrary, two phases were identified. An initial Al precipitation, with an incubation period that lasts approximately 10 days, was noted, followed by Al dissolution in the time remaining. In the first phase, 50% of Al precipitates for the pulp density at 5 g/L, 64% for 10 g/L, and 73% for 30 g/L. In the second phase, Al is dissolved again (Figure 8).

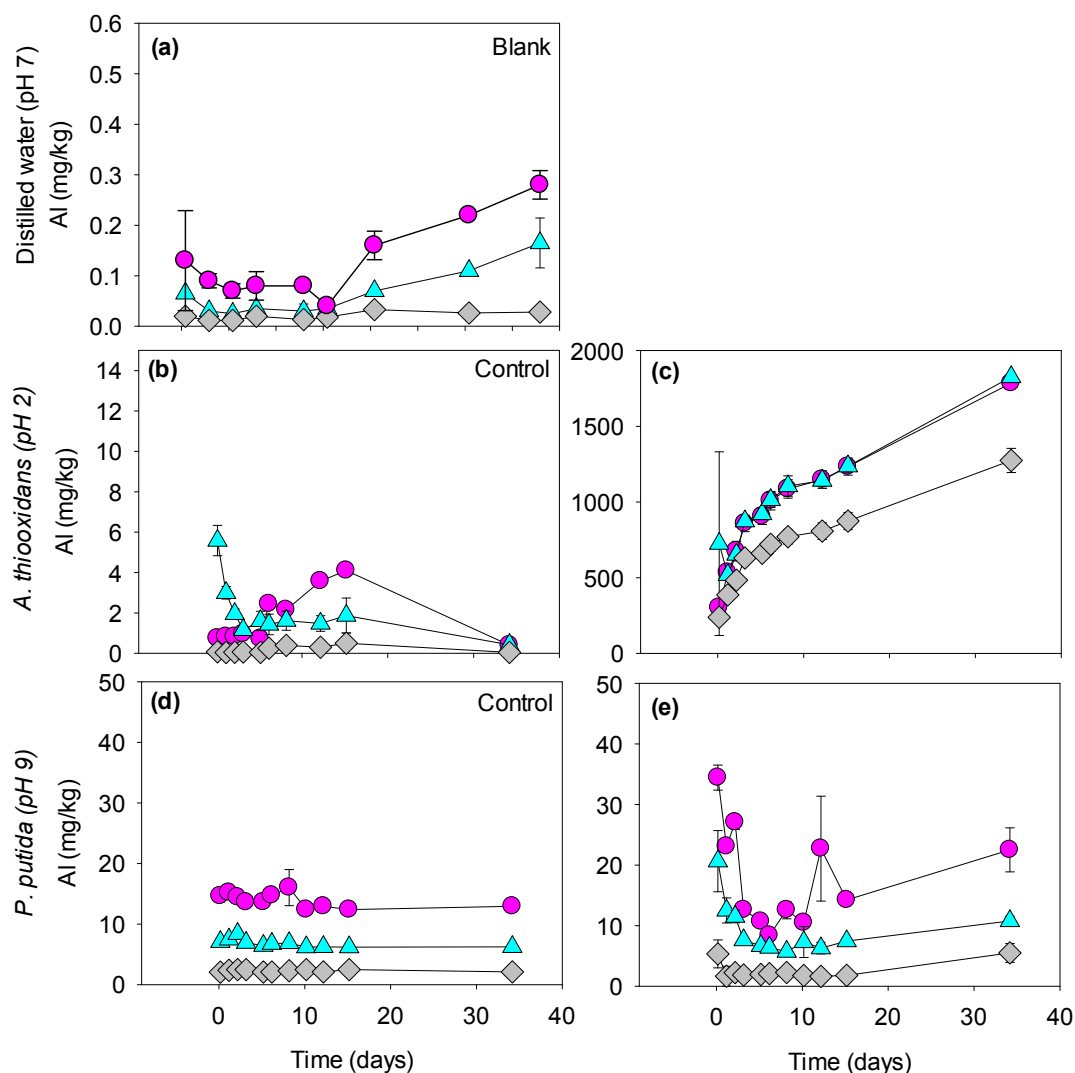


Figure 8. Al concentrations in the leachate as a function of time during (bio)leaching experiments of the fresh tailing sample with distilled water (a), incubated with *A. thiooxidans* (c) and *P. putida* (e). Controls for *A. thiooxidans* and *P. putida* are displayed in (b,d), respectively. Three pulp densities were evaluated: 5 g/L (—●—), 10 g/L (—▲—), and 30 g/L (—◆—).

3.4. Chemical Extraction

The chemically exchangeable pool of hexavalent chromium ($\text{E}_{\text{Cr(VI)}}$) for fresh and stockpiled old tailing and chromite ore, in mg of Cr(VI) per kg of tailing, is presented in Table 2. For the chromite ore samples, the total Cr content in the solid ranges between 203 and 236 g/kg. For the chromite ore 1, Cr(VI) extracted with water is equivalent to total Cr extracted with KH_2PO_4 , with an average value of

3.4 mg/kg, while the Cr(VI) extracted with KH_2PO_4 ($E_{\text{Cr(VI)}}$) remains lower (2.3 mg/kg). In the case of chromite ore 2, the Cr(VI) extracted with water and KH_2PO_4 has the same value (1.6 mg/kg) and corresponds to 50% of the total Cr extracted with KH_2PO_4 .

Table 2. Results of chemical extraction for the tailings and chromite ore sample.

Sample	Total Cr (g/kg)	Cr(VI) Water (mg/kg)	Cr(VI) KH_2PO_4 (mg/kg)	Cr(Tot.) KH_2PO_4 (mg/kg)
Chromite ore 1	203	3.5	2.3	3.4
Chromite ore 2	236	1.6	1.6	3.1
Fresh tailing	96	63	223	240
7–10 cm	99	36	149	158
Old tailing	105	19	85	89
>30 cm	101	3.7	31	31

In the fresh tailing sample, the total Cr content in the solid (96 mg/kg) is approximately 50% of the total Cr contained in the chromite ore sample. However, the amount of Cr(VI) extracted with KH_2PO_4 ($E_{\text{Cr(VI)}}$) (223 mg/kg) is 100 times higher compared with the chromite ore sample. In the fresh tailings sample, Cr(VI) extracted with KH_2PO_4 corresponds to 93% of the total chromium extracted with KH_2PO_4 .

Total chromium in the stockpiled old tailing sample does not change significantly with the depth (102 ± 3 g/kg); however, Cr(VI) extracted with KH_2PO_4 ($E_{\text{Cr(VI)}}$) decreased from the top to the bottom. For the Cr(VI) extracted with KH_2PO_4 ($E_{\text{Cr(VI)}}$), the range of variation goes from 36 and 149 mg/kg at the surface to 3.7 and 31 mg/kg in the deepest layer for extraction with water and KH_2PO_4 , respectively. Between 94 and 100% of extracted Cr with KH_2PO_4 is present as a Cr(VI).

4. Discussion

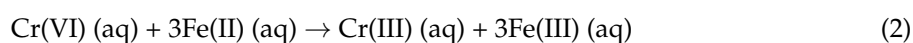
4.1. Cr Leachability and Interaction with Other Ions

Among the main chromium bearing phases in chromite tailings, iron and aluminum oxides play an important role in Cr mobility and leachability [7]. In leaching experiments of soils derived from chromium ore processing residue (CORP), Weng et al. [42] found that Cr(VI) was not detected in the leachate ($\text{pH} < 2.5$), because of the adsorption of Cr(VI) onto soil surfaces or the reduction of Cr(VI) to insoluble Cr(III). Significant amounts of Cr(VI) were leached between pH 4.5–12. Accordingly, Liu et al. [64] reported an optimal pH between 3 and 4 for hexavalent chromium leaching of chromium slag. Besides the importance of pH in the presence of Cr(III) and/or Cr(VI) in the dissolved fraction, pH also impacts the dominance of oxyanions species. According to visual MINTEQ simulation results, Cr precipitates during the leaching experiments and the Cr remaining in solution is mainly present as Cr(VI) oxyanions (Table S1). For leaching with *P. putida* growth medium (control) at a neutral pH, CrO_4^{2-} and HCrO_4^- are the main Cr forms in the leachate, accounting for about 86% of total dissolved forms. The dominance of those species remains over time (Table S2). When *P. putida* is inoculated, the pH increases up to 9.6 and CrO_4^{2-} represents 93% of the dissolved chromium. This occurs for all the pulp densities evaluated. In addition, visual MINTEQ simulation results revealed that at pH 3.5 (control of *A. thiooxidans*), HCrO_4^- dominates (94%), and at pH 1.6, when *A. thiooxidans* is inoculated, HCrO_4^- decreases (83%), while $\text{CrO}_3\text{SO}_4^{2-}$ represents 13% of dissolved chromium (Table S1). In the blank, when the fresh tailing sample was leached with distilled water ($\text{pH} = 7$), 66% of the Cr was present as a CrO_4^{2-} and the 34% remaining as HCrO_4^- . The oxidizing conditions and the pH explain the presence of Cr(VI) as HCrO_4^- or CrO_4^{2-} [14].

The leaching of Cr for chromite tailings can be explained by two main processes. The first main process for Cr leaching is associated with the presence of phosphate anions in solution, which can

exchange with chromate and bi-chromate anions, promoting the presence of Cr(VI) [46] in solution. According to the chemical extraction performed with KH_2PO_4 (Table 2), the chemically exchangeable pool of chromium in the fresh tailing sample is 223 mg of Cr(VI) per kg of tailing. Chemical extraction was also performed with concentrated ore and the value obtained is 100 times lower. Given that the concentrated ore is mainly composed of chromite, it can be concluded that chromite contained in the fresh tailings is not the main source of Cr(VI), at least in the short term. However, it is important to consider that in the chromite deposit, which is the raw material for the concentrated ore, serpentinization and magnesium (Mg) ion release during the deuteric alteration of ultramafic rocks (and associated laterization) creates alkaline pore water, which generates Cr(VI) formation [65]. Chromite and Cr-magnetite have also been proposed by Garnier et al. [66] as a source of Cr in ultramafic soils, in spite of r-spinel resistance to alteration. These findings are supported with XANES evidence for the oxidation of Cr(III) to Cr(VI) by Mn-oxides in a lateritic regolith in New Caledonia [67]. In other words, Cr(VI) is potentially produced in the chromite deposit, and if it is not removed during the chromite beneficiation process, it can be still present in the concentrated ore and tailings. However, given the Cr release during leaching experiments with concentrated ore, in the tailings, chromite does not seem to be the main source of Cr(VI). Consequently, Cr(VI) leached from the tailings is coming from other bearing phases, such as iron and aluminum oxides and silicates.

The second main process for Cr leaching that could explain the presence of Cr in the leachate is related to redox processes. Manganese oxide containing minerals acts as a transporter of electrons between dissolved oxygen and Cr(III), and therefore, Mn indirectly oxidizes Cr(III) to Cr(VI) [7]. On the other hand, Cr(VI) can reduce to Cr(III), which is less toxic. In this process, iron(II), reduced sulfur, and organic matter are the chief sources of electrons [56]. Reduction of Cr(VI) by Fe(II) (aq) can be expressed by the following general reaction [68]:



Given the visual MINTEQ calculations, during the leaching with *A. thiooxidans*, under acidic conditions (pH = 1.6), ferrous iron is mainly present in solution as FeSO_4 (aq) and as a free ion Fe^{2+} . The presence of ferrous iron in the leachate could explain the decrease of Cr over time, because of its iron dominant role in the reduction of Cr(VI) to Cr(III), a process which is 100 times faster than a biotic process [7]. In the case of Mn, it is mainly present as Mn^{2+} , which is a non-stable species, and it does not play a role in Cr(III) oxidation. At an acidic pH, Cr(III) precipitation is not expected to occur, as is the case at a neutral pH, so one can expect to see the precipitation of Cr(III) produced after Cr(VI) reduction in leaching with distilled water and *P. putida* [69]. In the batch experiments with *P. putida*, due to pH (6.6–9.6) and oxidizing conditions, Fe is present in solution at a really low concentration, forming FeHPO_4 . A low content of Fe indicates that no significant colloidal transport of particles occurred [70].

Other elements of interest leached from the fresh tailing sample are Ni and Al. Given visual MINTEQ results, in the presence of *P. putida* medium (control), Ni is complexed by phosphates, which are concentrated in the growth medium. In total, 77% is complexed as NiHPO_4 (aq), 14% is present as Ni^{2+} , and 5% as NiSO_4 (aq). When incubating the fresh tailing sample with *P. putida*, NiHPO_4 remains dominant until day 4, when the pH reaches a value of 8.9. However, the diversification of Ni species increases over time, and at the end of the leaching period, Ni complexed with ammonia becomes dominant. Those species include $\text{Ni}(\text{NH}_3)_2^{2+}$ (31%), $\text{Ni}(\text{NH}_3)_3^{2+}$ (18%), and NiNH_3^{2+} (17%). When incubating the fresh tailing sample with *A. thiooxidans* at pH 2, Ni is distributed as 68% Ni^{2+} and 31% NiSO_4 (aq) (Table S2). The main Ni species in solution, according to visual MINTEQ results, shows its strong affinity with sulfur, nitrogen, and phosphorus compounds. A negative significant correlation between Cr and Ni ($R^2 = 0.8$) in the leachate is observed for all pulp densities during the leaching experiment with *A. thiooxidans* (pH = 2).

For Al, the presence of KH_2PO_4 and K_2HPO_4 in the *P. putida* growth medium induces the formation of AlHPO_4^+ as the dominant (97%) species in solution. When *P. putida* was inoculated at

the beginning of the leaching experiment, when the pH was lower than 8, Al was present as three main species, including $\text{Al}(\text{OH})_3$, $\text{Al}(\text{OH})_4^-$, and AlHPO_4^+ , representing 3.4%, 49.4%, and 46.5%, respectively. After three days, Al started precipitating due to the high pH, which varied from 8.9 to 9.6 between day 4 and 34. Visual MINTEQ simulation results predict the precipitation of Al as Gibbsite ($\text{Al}(\text{OH})_3$) and kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$).

Under more acidic conditions (leaching with the *A. thiooxidans* medium), with pH = 4, during the whole experiment, Al distribution did not change. The main Al species are AlHPO_4^+ (50%), AlSO_4^+ (25%), and AlSO_4^{2-} (22%). Precipitation of Variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$) is expected according to the thermodynamical modeling results (Table S3). During incubation of the fresh tailings sample with *A. thiooxidans*, at pH 2, aluminum sulfate complexes (AlSO_4^+ and AlSO_4^{2-}) became the dominant species throughout the leaching period, accounting for about 90% of the dissolved forms. Due to the acidic conditions, no precipitation occurs. In the leaching experiment with distilled water, Al is mainly present as $\text{Al}(\text{OH})_3(\text{aq})$ and $\text{Al}(\text{OH})_4^-$; however, part of the aluminum hydroxide could precipitate as a Gibbsite. A significant negative correlation between Cr and Al ($R^2 = 0.8$) in the leachate is observed for all pulp densities during the leaching experiment with *A. thiooxidans* (pH = 2).

4.2. Bacterial Effect on Chromite Tailings Leachability

In the present work, two bacterial strains, *A. thiooxidans* and *P. putida*, were used for acidic and alkaline leaching conditions, respectively. In the previous section, the leaching of chromite tailings was discussed without including the possible bacterial effects. However, the differences in element concentrations observed over time (Figures 4–8) between blanks, controls, and batch experiments inoculated with both strains, indicate that bacteria may play an important role in tailings leachability, other than as a simple pH controller. Despite Schindler et al. [11] finding no direct bacterial effect on the release of Cr from altered chromatites, they affirmed that the presence of bacteria in tailings, mine waste piles, and soils may control pH and Eh and thus indirectly control Cr redox chemistry. Under this premise, the role of bacteria on Fe and Cr mobility, as the most abundant compounds on tailings, was selected to discuss this point.

Initially, the only role of *A. thiooxidans* on Fe leaching was considered to be associated with the production of sulfuric acid, through the oxidation of both elemental sulfur and sulfide. However, Marreno et al. [32] demonstrated that *A. thiooxidans* is able to mediate reductive dissolution of laterite overburden coupled to the acidolysis under aerobic conditions. This chemolithoautotrophic bacterium has also been used to leach iron from low grade cobalt laterite [71], tailings [40,72], and laterite overburden [32]. As shown in Figure 4c, in leaching experiments with *A. thiooxidans*, Fe is permanently leached for all pulp densities, with higher Fe concentrations for 5 and 10 g/L. Considering that the increase in total iron concentration does not evidence a bacterial effect, batch experiments inoculated with *A. thiooxidans* were compared with batch experiment with distilled water acidified at pH 2 with 1 N H_2SO_4 for a pulp density of 30 g/L (Figure S3b). In the batch experiment with acidified distilled water (pH 2), for a pulp density of 30 g/L, Fe extracted reached a total of 479 mg/kg in 11 days, while at the same pulp density, the batch experiment inoculated with *A. thiooxidans* total Fe only reached 213 mg/kg. Nevertheless, when fresh chromite tailings leaching with *A. thiooxidans* was performed with a pulp density of 5 and 10 g/L, the total amount of Fe extracted was 834 and 1108 mg/kg, respectively. This could suggest an inhibitory effect of *A. thiooxidans* at a pulp density of 30 g/L due to the release of toxic elements in the leachate such as Cr (946 $\mu\text{g/L}$) and Ni (843 $\mu\text{g/L}$). Jang and Valix [73] reported a bacteriostatic effect of Ni and Cu on *A. thiooxidans* for the leaching of saprolitic Ni laterite ores.

The amount of total chromium extracted in the batch experiment with *A. thiooxidans* decreases over time. It has been reported that *A. thiooxidans*, *A. ferrooxidans*, and *Thiobacillus thioparus* are capable of Cr(VI) reduction when they are growing on sulfur compounds due to the production of a series of sulfur compounds with a high reducing power [28,74]. According to Viera et al. [74], in *A. thiooxidans* cultures, reduced glutathione is a required intermediate for the oxidation of elemental sulfur. The

polysulfide formed is then successively oxidized to different compounds like sulfite, thiosulfate, and finally sulfate. Sulfite and polythionates could be responsible for reductive reactions. In addition, reducing compounds associated with sulfur particles (less than 3 μm) could promote Cr reduction in *A. thiooxidans* cultures [75]. Steudel [76] found that colloidal sulfur in cultures of *Acidithiobacillus* would be present as long-chain polythionates, forming micelles of globules of up to a few μm . Later, Viera et al. [74] observed that at pH 2 and 4, *A. thiooxidans* cultures reached higher free bacterial populations but lower chromium reduction values. Thus, the higher the pH, the higher the amount of reducing compounds associated with the colloidal sulfur and cells. Taking into consideration that in the present work the Cr(VI) leached out due to the presence of PO_4^{3-} in the medium, the Cr decrease could be explained by the reduction of Cr(VI) to Cr(III) mediated by the presence of *A. thiooxidans* and the low pH.

During the incubation with *P. putida*, the pH is above 8, where Fe does exist at very low concentrations in solution. Siderophores (Figure S4a) produced by *P. putida* during growth in low-iron conditions solubilize and bind iron, and transport it back into the microbial cell, usually through siderophore specific membrane receptors [52]. In spite of the presence of Fe in the leachate, the Fe extracted remains considerably low (0.73–76 mg/kg) compared with the incubation with *A. thiooxidans*. The cell growth of *P. putida* was monitored with the total protein content in the leachate (Figure S4b). In the first two days, the protein content doubles for all pulp densities with a lower protein content for a higher pulp density. *P. putida* has a high affinity for goethite. The bacterial adhesion increases with K^+ concentration and pH (2–7) [77]. Given the fact that goethite is one of the main mineral phases on the tailings studied, adsorption of *P. putida* on the solids is highly possible, which represents a limitation of its effect in the liquid phase. Other mineral phases that are positively charged or where the electrostatic repulsion force between them and the bacteria is overcome, could adsorb bacteria as well [77]. The lower leachability of the metals (Fe, Cr, Ni, Mn, Al) in the presence of *P. putida* at 30 g/L, compared to 5 and 10 g/L, suggests an effect of the pulp density on the culture growth.

To establish the role of *P. putida* in the leachability of fresh chromite tailings, a batch experiment with distilled water at pH 9 (adjusted with 1 N NaOH) was carried out (Figure S3). Total Fe concentration on the leachate of the distilled water at pH 9 reaches 1.4 mg/kg after 262 h of leaching, while in the batch experiment with *P. putida*, the Fe extracted is higher (3.5 mg/kg) for the same pulp density. For Cr, there is only a 9 mg/kg difference between the extraction with distilled water at pH 9 and the batch experiment with *P. putida*, being higher for the last one. Reduction of hexavalent chromium by *Pseudomonas dechromaticans*, isolated from industrial sewage, has been reported, as it uses the chromate or dichromate as a terminal acceptor during anaerobic respiration. Under aerobic conditions, *P. fluorescens* uses a variety of electron acceptors for chromate reduction [45]. Additionally, microbial metabolite extracellular polymeric substances (EPS) enhance Cr(VI) reduction efficiency and form organo-Cr(III) complexes to protect the cell and chromate reductase from inactivation [78].

Nevertheless, the presence of anions such as SO_4^{2-} , SO_3^{2-} , MoO_4^{2-} , VO_4^{2-} , PO_4^{3-} , and NO_3^- affects cellular chromate sensitivity [79]. Given the Cr(VI) reduction mechanisms by *Pseudomonas* spp. and the decrease of Cr concentration over time during incubation of the fresh tailing sample with *P. putida*, the reduction of Cr(VI) to Cr(III) is likely to happen [44,45]. Besides, the Cr decrease in the leachate is accompanied by an increase in protein and siderophore content, which ratifies the role of *P. putida* in Cr(VI) reduction.

Nonliving biomass can also reduce Cr(VI) to Cr(III) through two different mechanisms. In the first case, Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with electron-donor groups of the biomass, and the second consists of three steps: (a) binding of anionic Cr(VI) to the positively charged groups on the biomass surface; (b) reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups; and (c) release of the aqueous phase due to electronic repulsion [79].

In the present work, *A. thiooxidans* bacteria have been shown to have a potential impact on chromite tailings leachability. Under aerobic conditions, *A. thiooxidans* promote Fe reduction, and acidic conditions associate with the production of sulfuric acid, which promotes heavy metals dissolution.

On the other hand, *P. putida* plays an important role in iron dissolution thanks to the production of siderophores, but the influence on Cr leaching is negligible. The role of those types of bacteria could be seen not only as a potential pollution factor, but also as a remediation pathway, as well as the possible opportunity to recover leached metal from residues (tailings).

4.3. Does Chromite Tailing Represent an Environmental Risk?

From previous sections, it is concluded that due to chemical and/or biological processes, chromite tailings can release elements such as Fe, Mn, Ni, and Cr. Even if it has to be considered that some of the batch experiments do not represent the field conditions, previous studies have shown that the exposure of tailings to air, oxidation, and climatic conditions favored the release of heavy metals which are often a threat to the environment [80,81]. Meck et al. [82] studied the impact of mine dumps, including chromite mines, on river water quality. Based on the fact that the effluents were not acidic, this study concluded that chromite dumps do not bring major risks to river water quality. However, water bodies in the surrounding chromite mine areas are known to be impacted, in particular due to the presence of Cr(VI), which is toxic and highly mobile [1,36,38,83]. The extent and degree of heavy metal release around the mines vary depending on the geochemical characteristics and the mineralization degree of tailings [65].

The environmental risk is directly related to the mineralogical composition of chromite tailings and its susceptibility to release toxic elements into the environment. Typically, chromite ore process tailings contain a heterogeneous mixture of iron and chromium oxides, silicates, and aluminum silicate minerals [6]. In the tailings studies, mineral phases included chromite, which is the main Cr mineral on earth. In order to rule out chromite as a source of dissolved Cr, a leaching experiment with concentrated ore (product after the beneficiation plant) at pH 2, and a pulp density of 30 g/L, was performed (Figure S5). The results show three fold less Cr in the leachate of the concentrated ore sample (47 mg/kg) compared with the leachate of the fresh tailing sample (147 mg/kg), suggesting that chromite is not the main source of Cr release from the tailings. Chromite nanoparticles can be released as a consequence of silicate weathering; however, the production of Cr(VI) first requires a dissolution of the nanoparticle [10]. Other mineral phases such as iron and aluminum oxides are able to adsorb chromium, especially Cr(VI) anions. In the ring of Fire (Canada), various Cr bearing minerals occur such as clinocllore, phlogopite, amphibole, and clinopyroxene, with traces of Cr occurring in orthopyroxene, olivine, serpentine, and carbonates. Preliminary leaching tests indicated that Cr(VI) can be generated from those minerals in the presence of birnessite (Mn-containing mineral) [9]. In the present study area, birnessite was not detected.

Fresh and old tailings were compared in order to evaluate the potential release of Cr(VI) over time (Table 2). Chemical extraction with KH_2PO_4 showed that 93% of total Cr extracted with KH_2PO_4 is present as Cr(VI), with a total concentration of 223 mg/kg. In superficial tailings collected in a stockpile (old tailings), 94% of the total Cr extracted is present as Cr(VI), which has a concentration of 158 mg/kg. The proportion of Cr(VI) compared with total Cr does not change with the depth; however, the Cr(VI) concentration decreases from 158 mg/kg in the first 10 cm to 31 mg/kg at 30 cm. These results evidence that the chemically exchangeable pool of Cr(VI) is higher in the fresh tailing sample compared with the stockpiled old tailing sample, and in the stockpiled old tailing sample, the pool of Cr(VI) decreases with depth. Stockpiled chromite tailings are susceptible to storing CO_2 within the structures of minerals, a process known as natural carbonation, which is enhanced in mine tailings by a dramatic increase in mineral surface area from crushing during ore processing [84]. During accelerated mineral carbonation, Mg-carbonate mineral and Fe-oxyhydroxide phases sequester transition metals. Hamilton et al. [85] demonstrated that upon precipitation, $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ (a common product of mineral carbonation at Earth's surface conditions) rapidly sequesters transition metals (Cr, Ni, Mn, Co, and Cu) in solution. The trace metal uptake appears to occur by substitution of Mg^{2+} in the crystal structure, and also by incorporation into minor, metal-rich phases, such as Fe-oxyhydroxides. Additionally, the natural content of organic matter (NOM) in mine tailings exposed over long periods to atmospheric conditions

increases over time. The NOM can directly affect the extraction of metals, reducing the production of oxidizing agents required for metal dissolution [86]. The natural carbonation and the NOM enrichment in old tailings could explain the lower exchangeable pool of Cr(VI), compared with fresh tailings, where the content of organic matter is negligible [87]. Mg-carbonate and hydromagnesite are common weathering products of serpentine minerals, such as lizardite, which is present in the stockpiled old tailing sample in all depths. Further analyses have to be done to understand the possible role of new secondary phases, NOM, and the passive carbonation in Cr(VI) mobility on chromite tailings.

A broad estimation of the exchangeable pool of total Cr and Cr(VI) release from chromite tailings (for an average mine) could be estimated based on the average production between 2016 and 2017 in all the chromite mines in Sukinda valley [35] (Equation (3)).

$$\text{Cr(VI)} \left(\frac{\text{kg}}{\text{yr}} \right) = E_{\text{Cr(VI)}} \left(\frac{\text{mg Cr(VI)}}{\text{kg tailings}} \right) \times \text{Tailings produced} \left(\frac{\text{kg}}{\text{yr}} \right) \times 10^{-6} \quad (3)$$

A total of 37.28×10^5 tons/year with a feed grade of Cr_2O_3 (dry) is processed on the beneficiation plant, and 4.72×10^5 tons/year of concentrated ore is produced (~50%), together with 5×10^5 tons/year (tailings produced) of tailings containing ~15% of Cr_2O_3 [35]. If the chemically exchangeable pool of Cr(VI) ($E_{\text{Cr(VI)}}$) in fresh tailings is considered, potentially 111,500 kg of Cr(VI) could be released per year. If $E_{\text{Cr(VI)}}$ in the surface of stockpiled old tailings is considered, the Cr(VI) production decreases to 74,500 kg per year. This estimation suggests that the production and storage of chromite tailings could represent a risk for the surrounding soils and especially the water bodies, which could be severely affected by the presence of Cr(VI). Given the results of the batch experiments of this study, we can conclude that chromite is not the main source of Cr(VI) for tailings; however, other Cr bearing phases more susceptible to alteration could represent an important source of Cr(VI) in the environment. However, batch experiments with *A. thiooxidans* and *P. putida*, under the conditions studied, showed that the reduction of Cr(VI) to Cr(III) is a dominant process that is mediated by Fe and bacteria. In addition, the absence of Mn containing minerals such as birnessite, limits the oxidation of Cr(III) to Cr(VI). These results suggest the important role of bacteria in reducing the toxic hexavalent chromium, as a natural mechanism in stockpiled tailings.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2075-163X/8/6/261/s1>. Figure S1: Dissolved oxygen evolution throughout the incubation of the fresh tailing sample with *P. putida* at different pulp densities, Figure S2: Total Fe, Fe(II) and Fe(III) evolution throughout the leaching of the fresh tailing sample with acidified (H_2SO_4) MQ water for a pulp density of 30 g/L, Figure S3: Cr and Fe extracted from the fresh tailing sample, after leaching with MQ water at pH 7, pH 2 with H_2SO_4 and at pH 9 with NaOH, Figure S4: Siderophores content under incubation of the fresh tailing sample with *P. putida* and protein content in the leached, Figure S5: Cr and Fe extracted from the concentrated ores sample, after leaching with MQ water at pH 7 and pH 2 with H_2SO_4 , Table S1: Species distribution obtained in visual MINTEQ simulation, for the control and the leaching of the tailing sample with *A. thiooxidans* for a pulp density of 5 g/L, Table S2: Species distribution obtained in visual MINTEQ simulation, for the blank, the control and the leaching of the tailing sample with *P. putida*. Results are presented for a pulp density of 5 g/L and three subsamples collected at 0, 5 and 34 days in the batch with *P. putida*, Table S3: Ion Activity Product (IAP) and saturation index (SI) of MnHPO_4 and variscite, obtained in visual MINTEQ simulation, for the control and the leaching of the tailing sample with *A. thiooxidans*. Results are presented for two pulp densities (5 and 10 g/L) and three subsamples collected at 0, 5 and 34 days.

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