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Effect of Rhamnolipids and Lipopolysaccharides on the Bioleaching of Arsenic-Bearing Waste

Agnieszka Pawlowska [1,*], Zygmunt Sadowski [1] and Katarzyna Winiarska [2]

[1] Department of Process Engineering and Technology of Polymer and Carbon Materials, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland; zygmunt.sadowski@pwr.edu.pl
[2] Department of Analytical Chemistry and Chemical Metallurgy, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland; katarzyna.winiarska@pwr.edu.pl
* Correspondence: agnieszka.pawlowska@pwr.edu.pl

Abstract: The adsorption of biosurfactants and polysaccharides changes the surface properties of solid particles, which is important for controlling the release of arsenic compounds from the solid phase and preventing undesirable bioleaching. Microbial leaching and scorodite adhesion experiments, including pure and modified mineral material, were conducted in a glass column with a mineral bed (0.8–1.2 mm particle size) to test how rhamnolipids (Rh) and lipopolysaccharides (LPS) affect surface properties of mineral waste from Złoty Stok (Poland) and secondary bio-extraction products (scorodite). Adsorption tests were conducted for both solid materials. The adsorption of Rh and LPS on the solids was shown to modify its surface charge, affecting bioleaching. The highest bio-extraction efficiency was achieved for arsenic waste with adsorbed rhamnolipids, while the lowest, for the LPS-modified mineral. Under acidic circumstances (pH~2.5), the strongly negative zeta potential of arsenic-bearing waste in the presence of Rh creates conditions for bacteria adhesion, leading to the intensification of metal extraction. The presence of a biopolymer on the As waste surface decreases leaching efficiency and favours the scorodite's adhesion.

Keywords: biosurfactant adsorption; biopolymer adsorption; column bioleaching; scorodite adhesion; zeta potential

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1. Introduction

Environmental contamination by heavy metals is particularly problematic in former mining areas [1,2]. Natural bioleaching forms acid rock (mine) drainage. In places where As-bearing deposits are excavated, mine water becomes rich in arsenic. It encourages the growth of arsenophilic bacteria [3,4] and enriches bottom sediments with arsenites and arsenates [5,6].

In Poland, one of the old gold mining and processing centres is Złoty Stok (south-west), which, from the 17th century until 1962, was one of Europe's arsenic industry centres. Ore mining and processing, which took place in this area, produced large quantities of waste materials rich in As, such as mine waste rock, slag, and tailings [7]. Most of the mine spoils are hardly distinguishable from natural forms of the slopes. Ore smelting carried out in small facilities that produced gold was the primary source of As released into the atmosphere and deposited on the earth's surface. Other than Poland, there are regions where this problem is also significant (Chile, Bangladesh).

The main ore types mined at Złoty Stok are löllingite, löllingite-arsenopyrite, pyrite-magnetite, and pyrothine-arsenopyrite [8,9]. As-bearing sulphide ores are often decomposed by biological weathering as a result of the simultaneous oxidation of Fe(II) and As(III) by acidophilic bacteria, which leads to the formation of secondary products such as scorodite [10]. These—including scorodite—were investigated by Siuda and Macioch [9].

While arsenopyrite, the most common As-bearing mineral, has been extensively studied, bio-oxidation of löllingite has received less attention. The dissolution of löllingite is almost twice as fast as that of arsenopyrite [11]. As a result, ferrous iron, arsenite, and sulphate are released. Various products such as scorodite and iron hydroxides precipitate and form a passivation layer that prevents further oxidation, delaying bioleaching (Figure 1) [12]. The oxidation of löllingite under aerobic conditions can be described as follows:

$$FeAs_2 + 2O_2 + 2H_2O \rightarrow Fe^{2+} + 2H_2AsO_3^- \quad (1)$$

$$H_2AsO_3^- + H^+ \rightarrow H_3AsO_3 \quad (2)$$

$$H_3AsO_3 + Fe^{3+} + 0.5O_2 + 2H_2O \rightarrow FeAsO_4 \cdot 2H_2O + 3H^+ \quad (3)$$

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

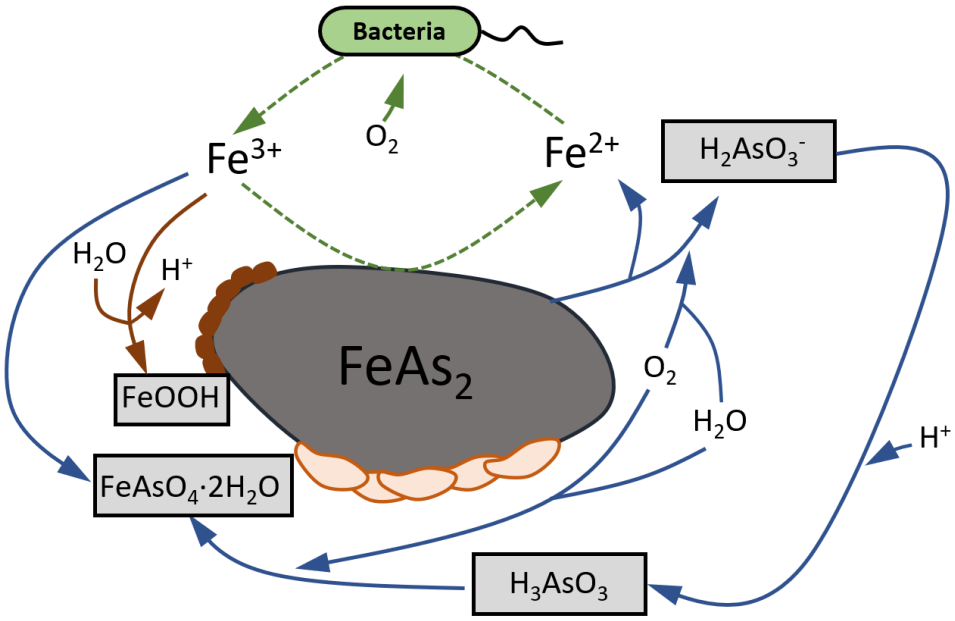

Figure 1. Conceptual scheme of oxidative dissolution of löllingite.

The formation of passivation products according to thermodynamics occurs in the order of As_2S_2 > As_2S_3 > $FeAsO_3$ > S^0 > $KFe_3(SO_4)_2(OH)_2$ [13].

The number of secondary phases affects the surface reactivity of bioleaching minerals. Chemoautotrophic bacteria actively involved in bioleaching produce biosurfactants and biopolymers, creating a specific microenvironment for bio-oxidation. These structures directly affect adhesion, which affects cell characteristics such as hydrophobicity, charge, or surface heterogeneity [14–16].

Rhamnolipids (Rh) are a group of glycolipid biosurfactants produced by the gram-negative bacteria *Pseudomonas aeruginosa*, fungi, and yeast [17,18]. Moreover, other isolates of the *Pseudomonas* genera, *P. putida* and *P. chlororaphis*, secrete various rhamnolipids [19,20]. As biosurfactants, they assist bacteria cells in adhesion, participate in biofilm formation, and change the wettability of the mineral surface [21]. The presence of rhamnolipids releases lipopolysaccharides (LPS) from the outer membrane, which increases bacterial cell surface hydrophobicity [22].

Extracellular polysaccharides are crucial for microorganism's adhesion to surfaces during the initial stage of biofilm formation. Lipopolysaccharides, a major component of the outer membrane of gram-negative bacteria, are secreted when they are in contact with minerals [23–26]. Partial removal of LPS from *T. ferrooxidans* has been shown to negatively affect the bioleaching of pyrite [27,28].

At present, there is only an incipient understanding of the interfacial interactions between biosurfactants/biopolymers, mineral surfaces, and microorganisms. Many current environmental and technological issues require a better understanding of the factors controlling microorganisms' immobilisation at solid–liquid interfaces. This work aims to show the influence of rhamnolipids and lipopolysaccharides on the bioleaching of arsenic waste where scorodite is present. The dissolution of secondary products can cause the release of arsenate and sulphate ions into mine waters, where bacteria can further reduce them. The mobility of toxic metals under aerobic conditions due to bacteria's activity in a porous bed is significant for environmental preservation. Therefore, the complex phenomena at the solid–liquid interface involving biosurfactants and polysaccharides need to be explained.

2. Materials and Methods

2.1. Solid Material

The mineral material was taken from a large stockpile of mining waste deposited in the area of the backfilled Jan shaft in Złoty Stok. The arsenic-bearing waste sample was ground and divided into several grain fractions, of which 50 g of 0.2–0.8 mm particle size was chosen. The XRD analysis revealed the presence of löllingite, tremolite, actinolite, phlogopite, albite, and nimite (Figure 2). An elemental analysis was performed by X-Ray Fluorescence (XRF). The arsenic waste consists of (m/m%): Si 34.3, Fe 20.3, As 8.29, Ca 7.69, Al 5.3, Mg 4.15, K 3.73, and 1.4 S.

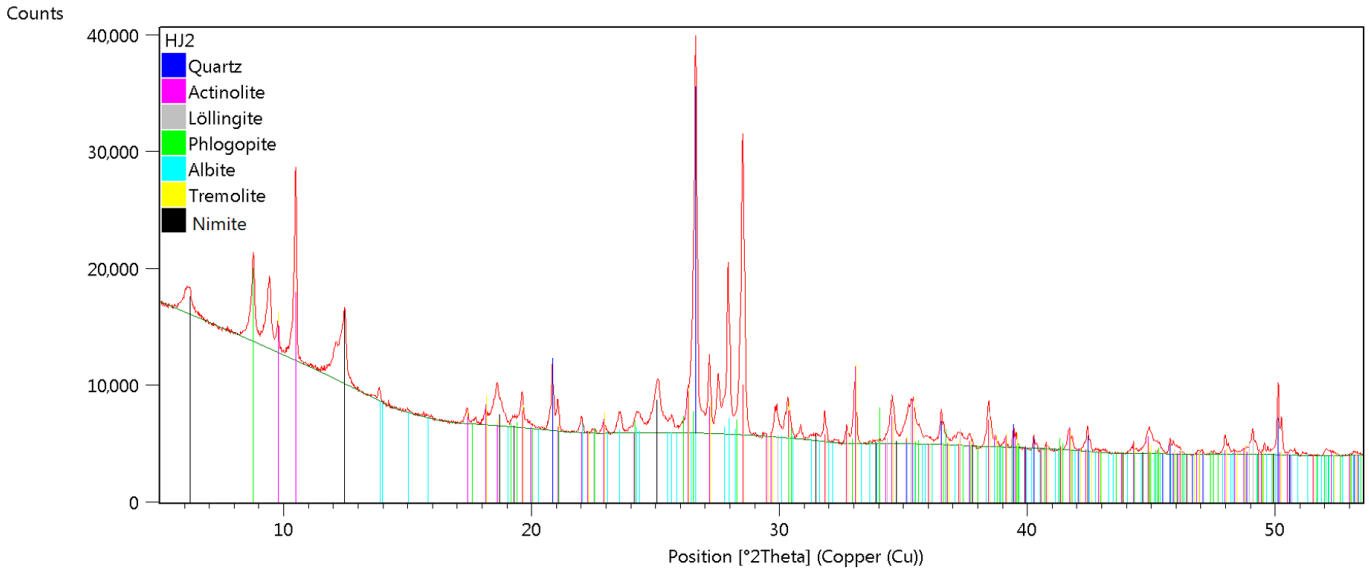

Figure 2. The XRD pattern of the arsenic waste.

The synthesis of the scorodite was performed according to literature data [29–31]. The scorodite was precipitated from a solution with Fe/As with a molar ratio of 1:1, pH equal to 1.2 and a temperature of 95 °C. The duration of the scorodite synthesis was 10 h. Figure 3 presents the XRD pattern of the synthesised secondary mineral.

The laser diffraction particle size analyser (LS 13 320 Beckman Coulter, Beckman Coulter Inc., Brea, CA, USA), in which the measurement is based on the principles of light scattering, determined the size distribution of the scorodite. The powder sample was suspended in a 10^{-3} M NaCl solution and introduced to the Universal Liquid Module (ULM), which measures the entire sample submitted to the system by recirculating it. The median particle size was 1.454 µm.

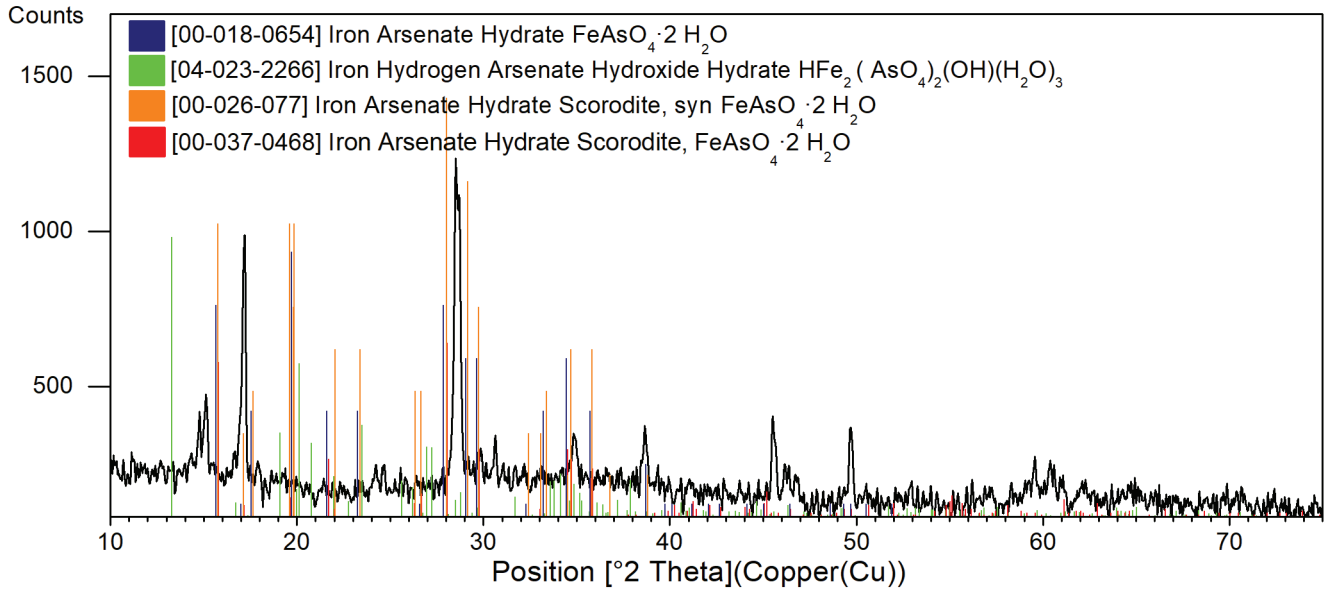

Figure 3. The XRD pattern of the synthetic scorodite.

2.2. Adsorption of Rhamnolipids (Rh) and Lipopolysaccharides (LPS) onto Solids

Adsorption experiments were carried out with 1 g of solid (As waste, scorodite) and 100 cm^3 of rhamnolipid solution. The concentration ranged from 10^{-4} to 10^{-3} M. Ninety percent pure Rh (AGAE Technology, USA) were used in the experiments without further purification. The suspensions were shaken for 24 h at room temperature to reach an equilibrium concentration. The solid phase was then separated by filtration. The Anthrone method determined the concentration of Rh [18]. Adsorption of LPS on the arsenic waste and the scorodite was conducted with an analogous procedure, with aqueous solutions of concentrations ranging from 1 to 6 mg/100 cm^3. The LPS were purchased from Sigma-Aldrich as a pure reagent. Its equilibrium concentration was attained with the phenolic method [26]. Adsorption experiments were conducted at pH 2.7 (for scorodite) and pH 2.6 for the arsenic waste.

2.3. Adhesion Experiments

Scorodite adhesion tests were carried out in a column filled with 50 g of arsenic waste of 0.2–0.8 mm particle size for: (i) arsenic waste; (ii) waste with adsorbed Rh, and (iii) arsenic waste previously in contact with LPS. The waste was conditioned with bio-compounds for 24 h, then separated from the solution and applied in adhesion. The column was uniformly packed and saturated with deionised water employing a peristaltic pump. After the flow rate (16.2 cm^3/min) had been stabilised, 100 mL of an aqueous suspension of scorodite of a given concentration (5 mg/100 cm^3; pH 2.6) and ionic strength of 10^{-3} M NaCl was continuously fed to the column. Samples were taken to determine the scorodite concentration employing the spectrophotometric method (30 min).

2.4. Zeta Potential Measurements

The zeta potential of solid samples was measured with the Zetasizer 2000 (Zetasizer, Malvern, United Kingdom) for: (i) pure arsenic-bearing waste and scorodite (without surface modification); (ii) for both solids previously in contact with rhamnolipids, and (iii) with adsorbed lipopolysaccharides. The suspensions were prepared by adding 1 g of solid (As waste or scorodite) to 50 mL water suspension of bio-compound at a 0.1–1 mM concentration. The effect of pH was investigated at a constant ionic strength of 10^{-3} M NaCl.

2.5. Column Bioleaching

A consortium of acidophilic microorganisms isolated from acid waters formed in excavations of former pyrite mines (Poland) was applied in the study. The 16S rRNA gene sequence analysis revealed the dominance of *Acidithiobacillus ferooxidans* (95.37%) and the presence of 4.63% of unclassified species [32]. The bacteria were cultured in a 9K medium, inoculum 10% *v*/*v*, pH 2 with the following composition (g/dm^3 deionised water): 44.8 g $FeSO_4 \cdot 7H_2O$, 3.0 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g KCl, 0.01 g $Ca(NO_3)_2$. The medium pH was maintained with 5 M H_2SO_4. The bioleaching was carried out in a column bioreactor to simulate the process occurring in a heap. The column had the following dimensions: a height of 68 cm, an inner diameter of 4.5 cm, with an outlet at the bottom and a second outlet at the height of 18 cm, which allowed a constant liquid level to be maintained above the mineral bed. 50 g of arsenic waste was taken to each experiment. The bio-extraction was carried out for three solid variants: (i) pure waste (without modifications); (ii) waste with adsorbed Rh, and (iii) waste with adsorbed LPS. To prepare the modified particles, they were conditioned in the bio-compound solution at a concentration of 10^{-3} M. After 24 h, the supernatant was separated, and the solid was taken for bio-extraction. The leaching solution was collected in a vessel placed under the column and then recirculated to the top with a peristaltic pump. 1 L of 9K medium at pH 2.0 was used. During bioleaching, pH, Eh, Fe^{2+} and Fe^{3+} concentrations were measured by titration. The arsenic concentration was determined through the inductively coupled plasma-optical emission spectrometry technique (Agilent 5110 ICP-OES Agilent Technologies Australia(M) Pty Ltd, Mulgrave, Australia). Measurements were made for the 188,980 nm As line, under the given conditions: a Synchronous Vertical Dual View (SVDV) plasma configuration; the RF power of 1.20 kW; gas flow rates of 12.0 (plasma), 1.0 (auxiliary), and 0.70 dm^3/min (nebuliser); the sample flow rate of 0.70 cm^3/min; stabilisation and sample uptake delays of 15 and 30 s; and three replicates. A five-point calibration was conducted in the concentration range of 0.1–10.0 μg/cm^3.

3. Results

3.1. Adsorption of Rhamnolipids and Lipopolysaccharides on Arsenic Minerals

The interaction between bacteria and mineral surfaces is a significant factor in bioleaching. Attachment can be mediated by surfactants secreted by microorganisms or, e.g., the formation of a biofilm, where different macromolecules, including LPS, play an essential role. Figure 4 presents the results of the adsorption of Rh and LPS on arsenic waste and scorodite. With an increasing rhamnolipid concentration, higher adsorption of scorodite was observed than for mineral waste. The highest value was 638 mg/g of scorodite (650 mg/dm^3) and 162 mg/g in the case of As waste (586 mg/dm^3). In experiments in which LPS were adsorbed, the differences between the waste and the secondary mineral were much less pronounced. For the highest initial polysaccharide concentrations, the adsorption density was 42 mg/g for mineral waste (61 mg/dm^3) and 51 mg/g for scorodite (58 g/dm^3).

3.2. Adhesion of Scorodite to Arsenic-Bearing Mineral Bed

Modifying the surface of mineral particles by adsorption of biopolymers and biosurfactants is a process that directly impacts the adhesion of scorodite to the mineral surface. This phenomenon can be observed during the bioleaching of minerals containing arsenic, where scorodite is synthesised and biosurfactants and biopolymers are present. Figure 5 shows the adhesion of scorodite on As waste without modification and after the adsorption of LPS and Rh. In the initial phase, the process is similar in all trials. After 10 min, stronger adsorption was observed for LPS than for Rh and pure waste material (the C/C_0 value equal to or less than 0.5).

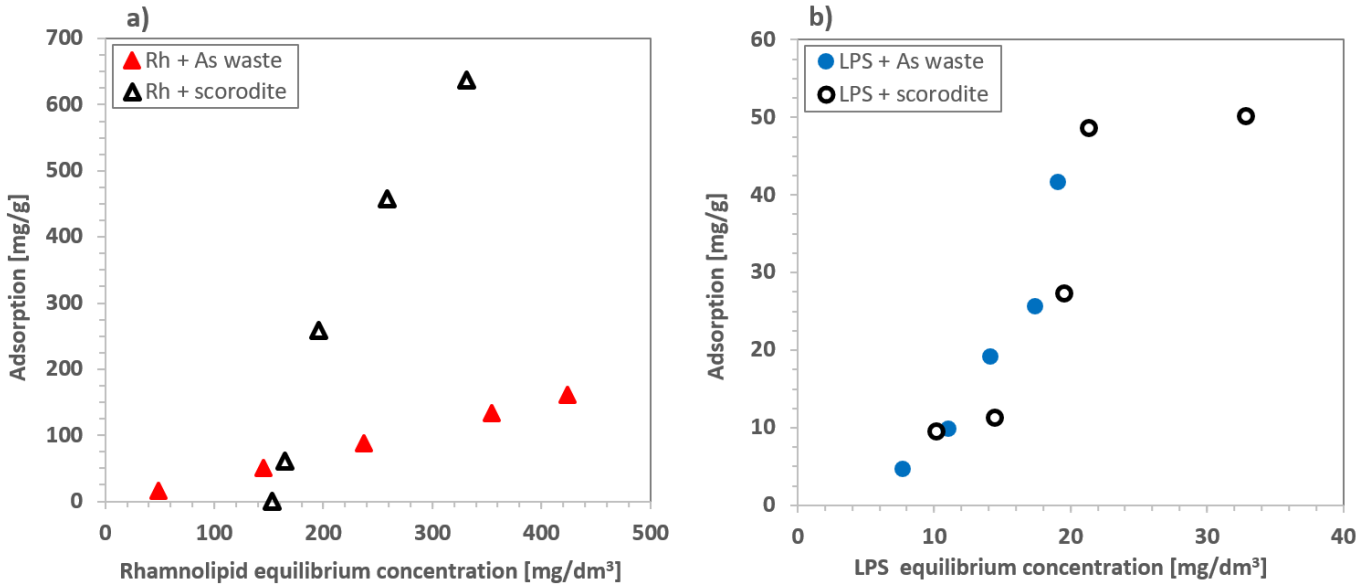

Figure 4. Adsorption isotherm of (**a**) rhamnolipids (Rh) and (**b**) lipopolysaccharides (LPS) on investigated solids.

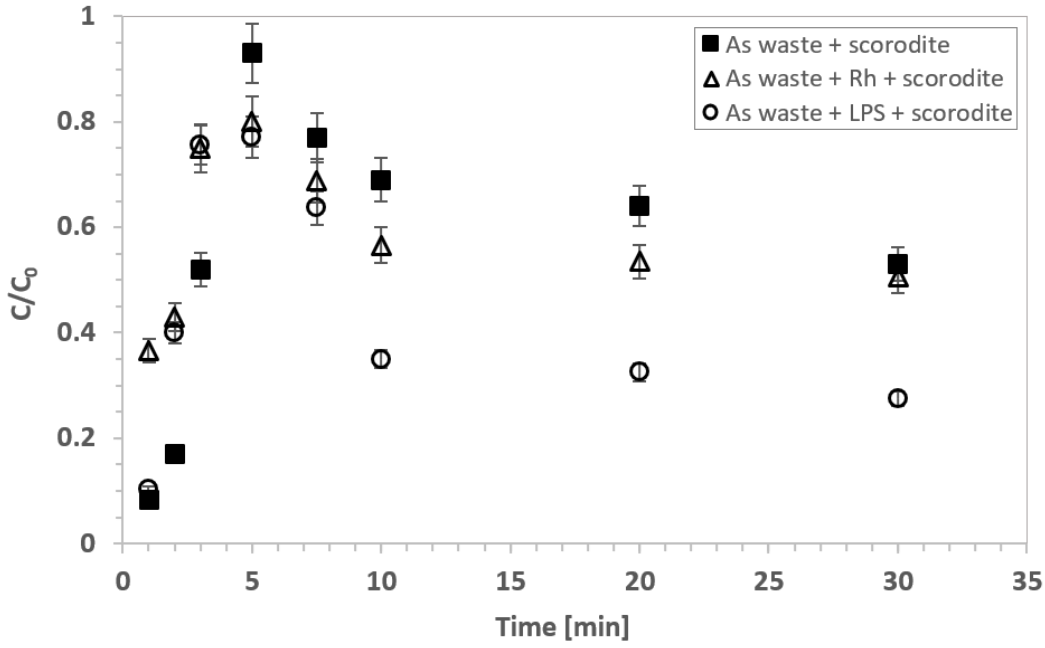

Figure 5. Adhesion of scorodite to non-modified and modified waste vs. function of time; rhamnolipids (Rh), lipopolysaccharides (LPS).

3.3. Electrokinetic Studies

3.3.1. Influence of Arsenic Waste Suspension pH on the Zeta Potential

The ability of bacteria to adhere to a mineral surface related to bio-extraction depends on the physicochemical properties of both the bacterial cell and the mineral surface and the presence of additional substances, e.g., surfactants. The characterisation of the zeta potential and, indirectly, the surface charge help provide a surface composition of both solids and defines the conditions feasible for adhesion. Figure 6 shows the variation of the zeta potential as a function of pH for the solid suspensions tested. The zeta potential of solid waste was negative throughout the tested pH range, with a maximum of −38.9 mV at pH 6.11. For scorodite, the isoelectric point was observed at pH 3.75. Above this value, the negative charge increased with increasing pH and reached a maximum of −49.2 mV at pH 9.06.

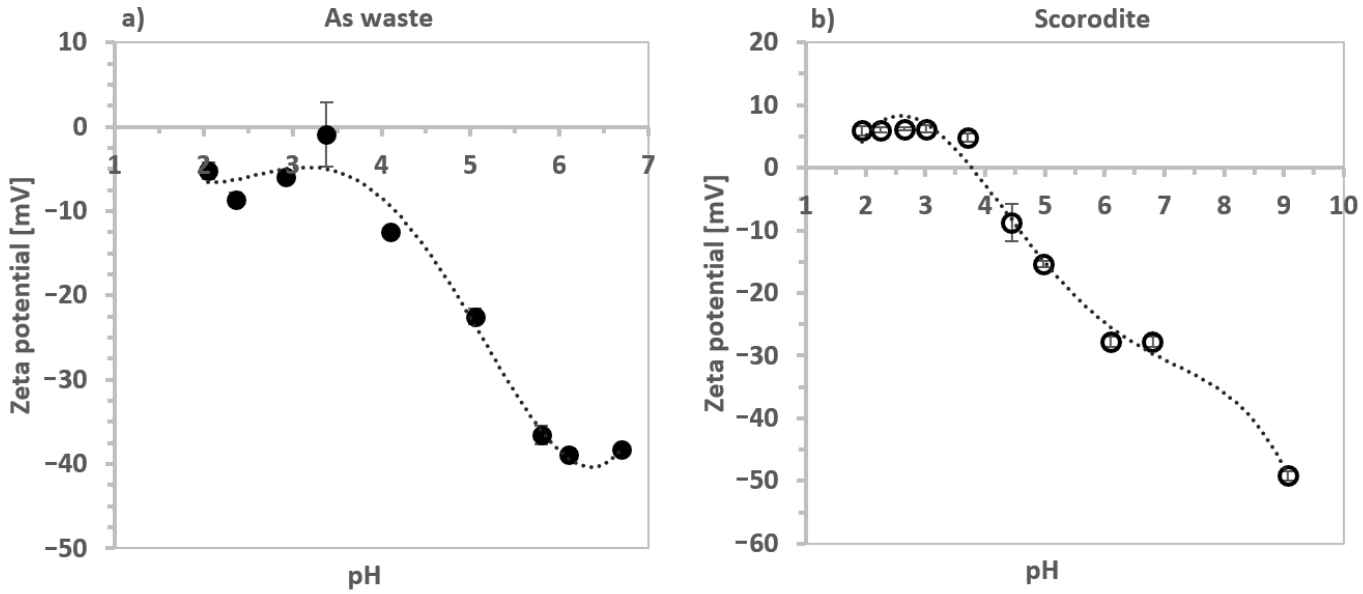

Figure 6. Zeta potential of (**a**) As waste and (**b**) scorodite suspension as a function of pH. Ionic strength 10^{-3} M NaCl.

3.3.2. The Effect of Rhamnolipid Concentration on the Zeta Potential

The adsorption of rhamnolipids and lipopolysaccharides changed the zeta potential of the solids. Figure 7a shows the zeta measurements of As waste for different concentrations of rhamnolipids and pH. An increase in pH changes the potential value towards negative values. At the pH at which bio-extraction occurs, significant differences were observed in the surface charge of the systems tested. For As waste, the zeta potential ranged from −5.2 mV to −18.6 mV (pH 2.6). In contrast, the scorodite presented in Figure 7b (pH 2.35) showed positive values throughout the examined concentration range (7.6 mV–23.1 mV). In both systems, the most significant changes in zeta potential were observed for low biosurfactant concentrations (0.1–0.5 mM).

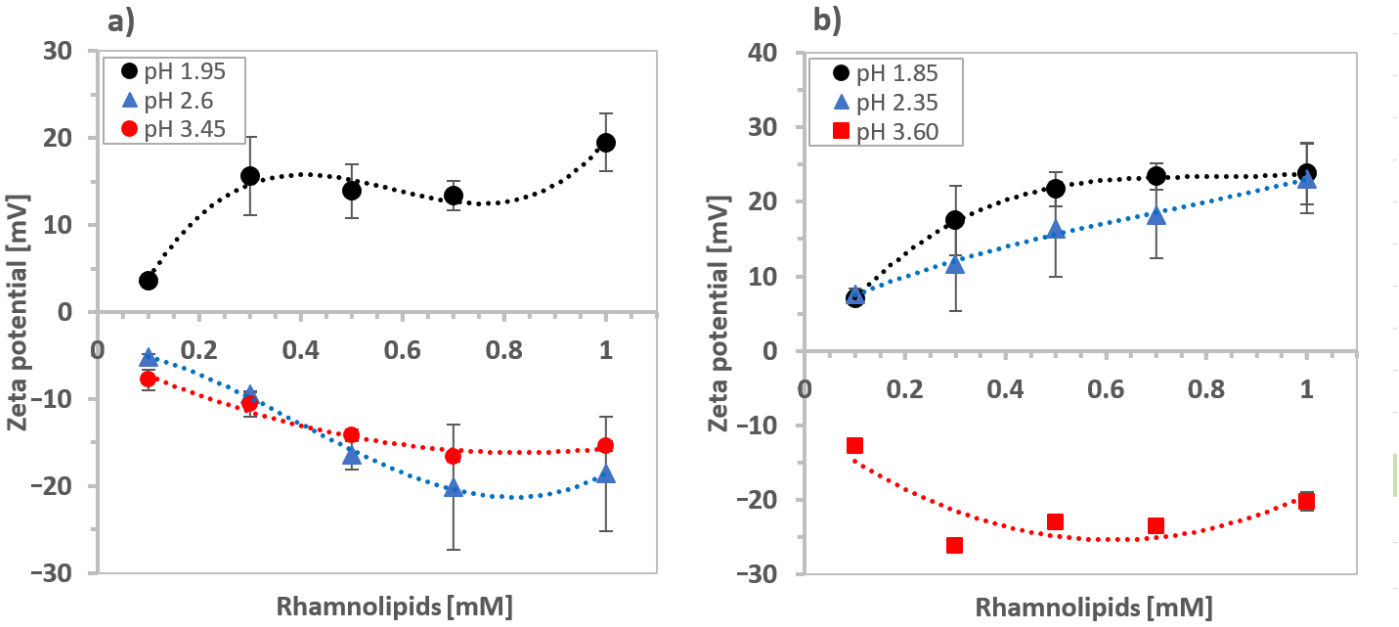

Figure 7. The zeta potential of (**a**) As waste in the presence of rhamnolipids and (**b**) scorodite in the presence of rhamnolipids.

3.3.3. The Effect of Lipopolysaccharides on Zeta Potential

Both surface and solution chemistry strongly influence LPS sorption at the mineral–water interface. For the adsorption of LPS on As waste and scorodite (Figure 8), a shift of zeta potential towards negative values was observed with increasing pH. Significant differences were noticed above a pH of 3.10. For mineral waste, an increase in LPS concentrations caused a change in the potential towards less negative values (−9.9 mV for 0.1 mM LPS, −2.3 mV for 1 mM LPS). For scorodite conditioned with 0.1 mM LPS solution, the zeta potential was 5.1 mV when the 1 mM solution was −13.9 mV.

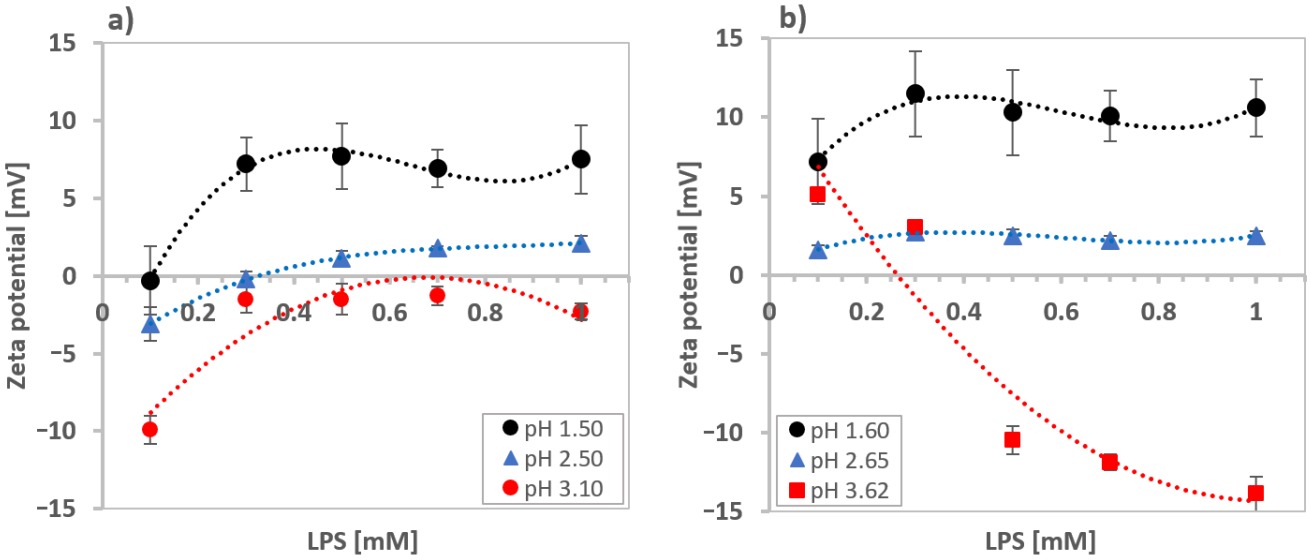

Figure 8. The zeta potential of (**a**) As waste in the presence of lipopolysaccharides (LPS) and (**b**) scorodite in the presence of LPS.

3.4. Bioleaching of Arsenic-Bearing Minerals

The effect of surfactants on the kinetics of arsenic bioleaching was tested. Figure 9a shows the changes in pH during the leaching of arsenic-containing waste. It can be seen that after 24 h of incubation, the pH values resulted in a decrease, which is caused by an acid-forming reaction triggered by bacterial activity. After that time, the pH remains constant, the lowest for Rh (2.10) and the highest for LPS (2.25).

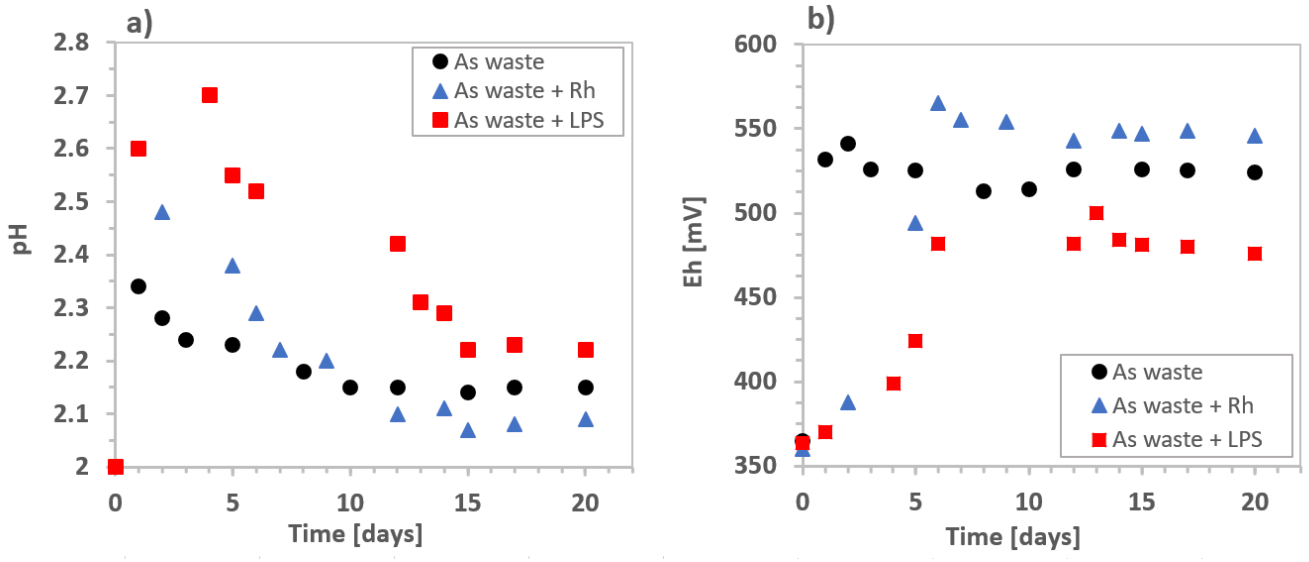

Figure 9. Changes in (**a**) pH and (**b**) redox potential during leaching.

One of the most critical parameters that govern bioleaching is the redox potential of the leaching solution. In all tested systems, the redox potential increased with time. For the bioleaching of As waste, a rapid establishment (in two days) of a higher Eh 541 mV was observed (Figure 9b). For the Rh-modified mineral, the maximum was reached after five days (565 mV). Slower Fe^{2+} oxidation occurred for waste with adsorbed LPS, where the value from day 6 fluctuated from 482–476 mV. The decrease in redox potentials was due to the consumption of Fe^{2+}.

Bioleaching results are presented in Figure 10. The highest concentration of As was obtained for waste modified by adsorption of rhamnolipids (1280 ppm). After 20 days of the process, the lowest efficiency was achieved for solid particles with adsorbed LPS (650 ppm). Escobar et al. [27] also observed the inhibitive effect of LPS on bioleaching, which was explained by low bacteria attachment, as part of the active sites of the mineral were occupied by LPS. The lower pH value and the higher redox potential result in a higher leaching rate of As.

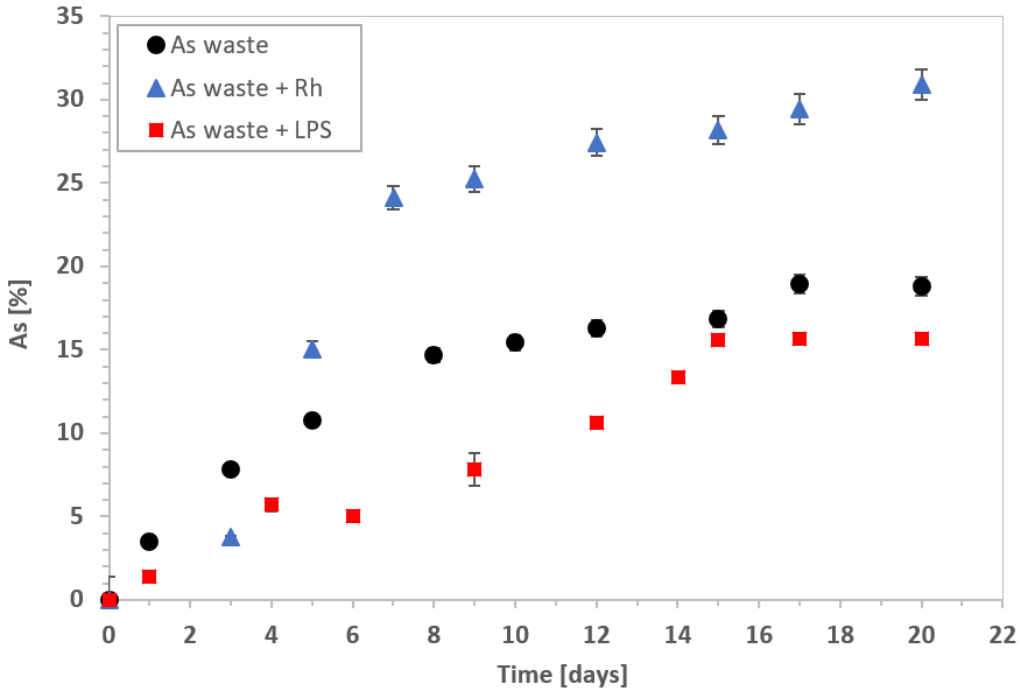

Figure 10. Bioleaching efficiency of As waste without and in the presence of rhamnolipids (Rh) and lipopolysaccharides (LPS).

4. Discussion

The bioleaching of minerals containing heavy metals inevitably leads to acidic leachates with a significantly increased concentration of toxic elements. The scorodite, the most stable form of arsenic, limits the release of this element from acid mine waters to the environment. However, its actual stability under varying environmental conditions is limited. In the case of heap leaching or in a column where the suspension of bacteria and secondary minerals circulates in a closed circuit, both secondary minerals and bacteria cells adhere to the surface of the primary mineral.

The activity of microorganisms results in the formation of macromolecules such as LPS or Rh. It has been shown that they take part in the adherence of microbial cells to a solid particle. Up to date, most studies investigated bioleaching and bacteria-cell interactions using pure bacterial strain and either cell-surface or cell-biomolecule, which does not fully reflect the conditions found in the natural environment. Moreover, mining wastes were examined to a much lesser extent. This study attempts to describe a system in which interactions occur between the solid particles, the biomolecules, and the bacterial cells. The

ability to control the behaviour of the particles in the solid–liquid interface might give a tool to counter the unwanted effects of toxic metal release to the environment.

It was shown that mineral waste and scorodite were sensitive to Rh and LPS presence in the acidic environment. Adsorption of Rh was slightly higher for scorodite than As waste (Figure 4). Rh are composed of two alkyl chains and either one or two rhamnose units and are weak acids due to the presence of carboxylic acid moiety [33]. Thus, increased interactions were observed between Rh and the positively charged scorodite (6.2 mV) than with the negatively charged surface of arsenic waste (−7.25 mV). Analogous results were for adsorption of LPS, as they exhibited an overall negative charge in tested pH due to the deprotonation of phosphate moieties of polymer backbone [34].

The presence of bio-compounds on solid surfaces induces changes in the electrical double layer. The adsorption of rhamnolipids on the arsenic-bearing waste at pH 2.6 increased the negative zeta potential, which shifted towards negative values from −7.25 mV to −18.6 mV, for a biosurfactant solution of 1 mM (Figure 7a). An increase in the negative surface charge density of Accusand caused by Rh adsorption was also reported by Bai et al. [35]. The interaction of Rh on scorodite caused a change in zeta potential compared to a pure secondary mineral from 6.2 mV to 23.1 mV at a concentration of 0.1 mM (Figure 7b). Such behaviour suggests that the surfactant structure was distorted, and negatively charged groups at the polar ends of the Rh molecules become protonated [36].

The conformation of the polymer molecules on the solid surface differs from that of the surfactant [37]. The LPS' structure presents various acid-base-reactive functional groups responsible for sorption, depending on pH. In acidic solutions, phosphate groups may control sorption. At moderate pH (4.5–6.5), carboxyl or carboxylate groups are involved. At higher pH, nitrogen-containing groups are reactive [34].

Consequently, its presence at the solid–liquid interface changes the electrical double layer structure and the zeta potential [38]. This phenomenon emerged in the case of LPS adsorption (Figure 8). The biopolymer presence on an As waste surface at pH 2.5 slightly affected zeta potential (from −7.2 mV for pure solid to 2.1 mV for modified). In the case of scorodite, values ranged from 6.2 mV for a pure mineral to 2.5 mV with adsorbed LPS (1 mM solution). Small values of the zeta potential cause the electrostatic interaction to be weak. Therefore, the loop-and-tail conformation of the LPS biopolymer might be responsible for the trapping of scorodite particles, as seen in the increase in adhesion (Figure 5).

The bio-extraction results should be interpreted according to the adhesion and zeta potential values obtained for the systems under study. The bacterial consortium applied in the present work showed a zeta potential value of 4.6 mV for pH 2.33 and 4.4 mV for pH 2.57, which was previously reported [32]. Moreover, other authors indicated that at acidic pH, the zeta potential of bacteria had positive values, as the bacterium surface properties depend on the growth conditions [39,40]. A positive surface potential results from the presence of protonated ammonium groups [41]. The highest bioleaching efficiency was observed for arsenic-bearing waste with adsorbed Rh. The porous medium's increased negative surface charge density (Figure 7a) favours bacteria adhesion, translated into high bioleaching efficiency for the waste–Rh–cell system (Figure 10). When the LPS previously extracted from the bacterial cell were incubated with As waste before leaching, there was an inhibition of cell adsorption to the particles, resulting in lower metal extraction. Similar observations were described by Escobar et al. [27] and Arredondo et al. [28], where cell attachment was reduced from 35–46% depending on the mineral used. It can be explained by a weak repulsion between particles colonised with LPS and microorganisms (Figure 8a) and reduced availability of sorption sites [27].

As the process progressed, the pH of the leaching solution decreased (Figure 9a). Figures 6 and 7 show that the surface charge of arsenic-bearing waste with adsorbed biomolecules changes towards positive values when the pH becomes more acidic. In such an environment, bacteria might detach from the solid, slowing down or terminating leaching. Higher positive values of zeta potential were observed when the LPS were

attached to As waste, resulting in a lower efficiency of As leaching. Later in the process, the presence of a secondary mineral enhances this effect. Figures 6b and 7b show that the lower the pH, the more positive are the values that have the zeta potential of scorodite–Rh and scorodite–LPS systems. If free Rh or LPS particles are present in the leaching solution, it may cause competition between the positive bacterial cells and the scorodite to adhere to the waste surface.

5. Conclusions

This study was conducted to explain the bacteria–mineral interactions that might occur during the bioleaching of As-bearing minerals, which occurs through (i) cell adhesion and biofilm formation or (ii) reaction of microorganisms and their metabolites with ore. It was shown that the adsorption of biosurfactant and lipopolysaccharides onto the mineral modifies its surface charge, affecting bioleaching. The highest bio-extraction efficiency was reached for arsenic waste with adsorbed rhamnolipids, while the lowest was reached when the LPS were adsorbed. The lower pH value and a higher redox potential resulted in a higher leaching rate of As. Electrokinetic studies showed that under acidic conditions (pH~2.5), adsorption of Rh causes a significant change in surface charge compared to LPS. The strong negative zeta potential of As waste in the presence of Rh provides conditions for bacterial adherence to the mineral surface, leading to intensifying As extraction and potential release to the environment. The presence of LPS on the surface of arsenic-bearing waste decreases the efficiency of metal bioleaching and favours the adhesion of scorodite.

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