Interaction Between Bacteria And Minerals In Post-Mining Heaps In View Of Metal(Loid) Release

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Abstract

Bioleaching of mining waste using acidophiles was studied, yielding 586.8 mg/L of arsenic. Bacterial mobility was analyzed in unmodified and surfactant-treated beds using hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), polyoxyethylenesorbitan monooleate (Tween 80), and rhamnolipids. Electrostatic interactions influenced adhesion, with mineral waste maintaining a negative zeta potential at pH 2–12. Bacterial cells exhibited a positive charge at highly acidic pH and a negative above pH 2.6, where the isoelectric point was determined. CTAB enhanced bacterial retention, SDS reduced it due to electrostatic repulsion, and rhamnolipids did not affect migration, suggesting additional adhesion mechanisms.

Keywords: Arsenic leaching, Bacterial migration, Surfactant adsorption, Zeta potential

Introduction

Releasing toxic elements from mining waste heaps presents environmental challenges, particularly in regions with a long history of ore processing. Waste heaps are generally located outside and exposed to weather conditions. For this reason, these areas risk becoming sites of enhanced microbial activity since the presence of acidophilic microorganisms accelerates the formation of acid mine drainage and acid rock drainage due to the sulfide and ferrous oxidation, which facilitates mineral dissolution.

Immobilization of bacterial cells on the mineral surface improves the bioleaching efficiency by creating a specific micro-environment for biooxidation (Watling 2006). Chemicals that prevent bacterial attachment and promote bacterial transport are important, e.g. for successful bioaugmentation (Zhong *et al.* 2017). Cell adhesion to the surface facilitates the colonization of bacteria, improving their ability to leach metals. On the other hand, increased mobility of microorganisms in mineral medium hinders their bio-oxidation capabilities and participates in subsurface migration of contaminants. Therefore, understanding interactions between bacteria and minerals is needed to explain the microbial cell deposition onto solid supports (Katsikogianni *et al.* 2004).

Surfactants were previously used for bacterial transport enhancement in geological media using Tween 20 and sodium dodecyl sulfate, SDS (Gross and Logan 1995) and rhamnolipids (Bai *et al.* 1997; Chen *et al.* 2004). The effect of Tween 20, Tween 80, Plurafac® LF 120, Plurafac® LF 600 and Lutensol® XL 90 on the growth of mixed thermophilic culture consisting mainly of *Metallosphaera hakonensis* was studied by Ghadiri *et al.* (2019). The influence of SDS on bacterial adsorption characteristics was also studied during the bioleaching process of vanadium-bearing shale by *Bacillus mucilaginosus* (Cai *et al.* 2022). Nevertheless, surface electrostatics is investigated to a much lesser extent. The presence of specific chemical groups on the particle surface determines its polar or hydrophobic character and possible electrostatic potential.

Our previous studies showed that the adsorption of lipopolysaccharides and rhamnolipids onto mineral surface changed in the electrical double layer structure and zeta potential, affecting bioleaching (Pawlowska and Sadowski 2020; Pawlowska *et al.* 2021). Therefore, insight into microbial-mineral interactions highlights the importance of controlling bacterial activity in mining waste management. An improved understanding of these processes could lead to more effective strategies for minimizing environmental contamination from mining heaps and optimizing metal recovery through biotechnological applications.

This study investigated the potential for arsenic leaching from mining waste using a microbial consortium isolated from a post-mining environment. There is still limited research on bacterial behaviour in porous media in the presence of surface active compounds, especially in multimineral beds. To address this gap, surfactants of different types were used to determine the conditions that promote bacterial adhesion to mineral surfaces and those that enhance microbial cell migration through the bed.

Methods

The mineral waste material used in the research originated from a heap in southwest Poland, where gold-bearing arsenic ore was previously mined. A particle size of 0.2-0.5 mm was used in the study. The selected fraction contained mg/kg: 19581 As, 56465 Fe, 211.8 Pb, and 74.71 Zn.

A consortium of acidophilic microorganisms dominated by the genera *Acidithiobacillus* and *Acidiphilium* was used. Bacteria prior to leaching were grown on a modified Silverman-Lundgren mineral medium composed of 44.8 g/L $FeSO_4 \cdot 7H_2O$, 3.0 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$ g/L, 0.5 g/L K_2HPO_4, 0.1 g KCl. Inoculum content was 10% (v/v), pH 2.0. Microorganisms were cultivated in shaken flasks at 35 °C for 24 h. Leaching was conducted in glass columns, with a solid content of 50 g and 500 mL of medium. The control sample was deionized water of pH 2.0. Ferrous and ferric iron concentration was determined, as reported previously (Pawlowska *et al.* 2023).

In order to obtain a pure cell suspension for adsorption studies (without culture medium), the cultures were separated on a Whatmann no. 1 paper filter. Then, the solution was centrifuged for 10 minutes at 10°C, 9000 rpm. The supernatant was poured off, and bacterial cells were re-suspended in a water solution of pH 2.5 and centrifuged again. This procedure was repeated until high-purity microorganisms were obtained, which were finally suspended in 2 mL of water with a pH of 3 (Jin *et al.* 2019). The cell concentration was determined by measuring the absorbance at 500 nm.

For surface modification, four surface-active agents were used: i) cetyltrimethylammonium bromide (CTAB), ii) sodium dodecyl sulfate, iii) polyoxyethylenesorbitan monooleate (Tween 80) and iv) rhamnolipids. CTAB was purchased from Thermo Fisher Scientific (USA), SDS and Tween 80 from Sigma-Aldrich (USA), and biosurfactant 90% pure from AGAE Technologies, LLC (USA). Mineral surface modification was done by suspending 25 g of mineral sample in 100 mL of 0.001 M surfactant solution and placed in a rotary shaker for 3 h at 155 rpm. The surfactant solution was decanted, solid dried at 35 °C for 24 hours, and placed in glass columns. Migration through the bed was carried out with 70 mL of bacterial suspension (IS 10^{-3} KCl) and an initial 2 mL/min flow. After the experiment was completed, the solid was left to dry at 30 °C for about 48 hours.

The pH and redox potential were measured using a pH/Eh metre Elmetron CX-401. Element content in liquid samples was determined using the Agilent 5110 ICP-OES (United States) instrument. The solid was analyzed after mineralization with nitric acid. Zeta potential was determined using Zetasizer 2000 (Malvern, United Kingdom) at a constant ionic strength of 10^{-3} M KCl and 20 °C. The final result was the average of five measurements.

Results

Bioleaching experiments

Results of arsenic extraction are presented in Fig. 1a. The final solutions were also tested for zinc and lead content. Waste bioleaching yielded 586.8 mg/L As, 6.380 mg/L Pb and 3.479 mg/L Zn in 21 days. In the control, 50.44 mg/L As, 1.345 mg/L Zn, and 0.379 mg/L Pb were leached. There was a rapid drop of ferrous iron with a simultaneous increase in ferric iron due to bacterial activity (Fig. 1b). After the fifth day of the process, a slight decrease in Fe(III) concentration was observed, most likely due to iron precipitation. In bioleaching, the pH remained constant at an average of 2.16 (±0.12). A continued rise in pH was observed for control sample with a final pH of 4.4, which might be due to the neutralizing capacity of mineral waste or acid leaching (Fig. 1c). The Eh values for abiotic control was within range of 238 – 365 mV (Fig. 1d). Redox potential during bioleaching rapidly increased within the first two days and remained constant until the end of the process, within a range of 552-584 mV.

Zeta potential and migration experiments

Measurements of the zeta potential allowed us to indirectly determine the surface charges of microorganisms and mineral material at the conditions of the experiments (Fig. 2). The mineral suspension had a negative electrical potential within the tested pH range. At pH 6.5, the zeta potential of mineral waste was -29.1. The suspension of microorganisms exhibited slightly positive values at highly acidic pH. The isoelectric point was identified at pH 2.6, and above this point, bacterial cells had negative zeta potential, reaching a maximum of -23.1 mV at pH 11.4.

Migration experiments were conducted on an unmodified bed with adsorbed surfactants

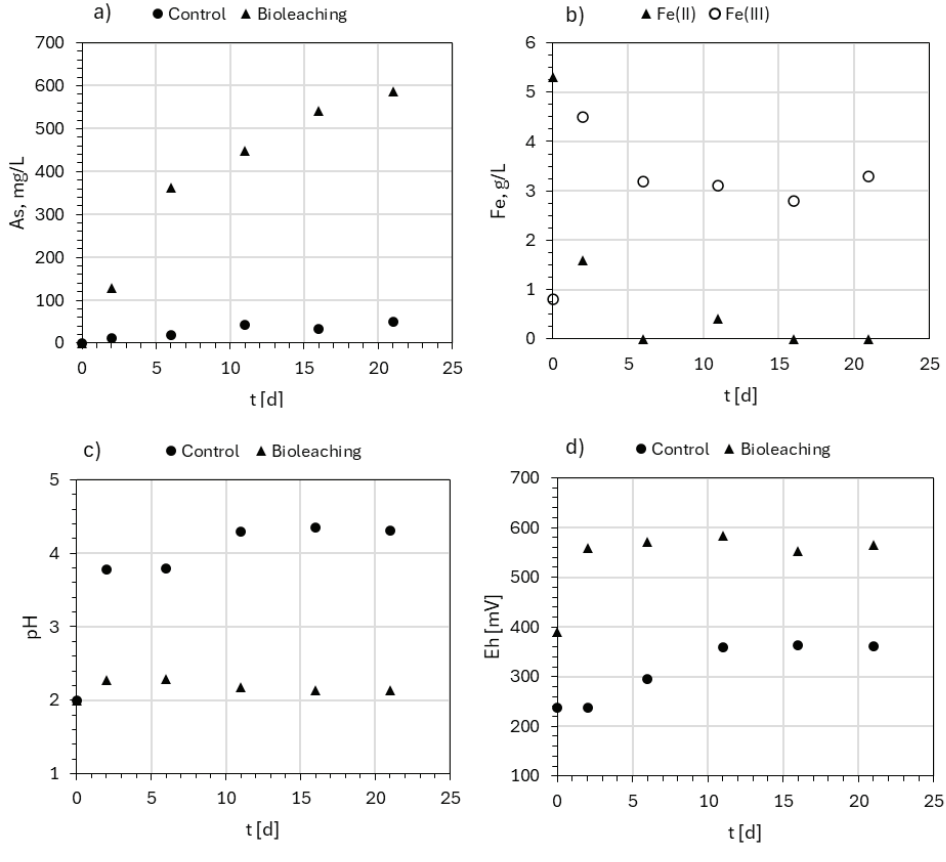

Figure 1 *Results of mineral waste leaching: a) arsenic concentration, b) ferrous and ferric changes during bioleaching, c) pH, and d) Eh versus time*

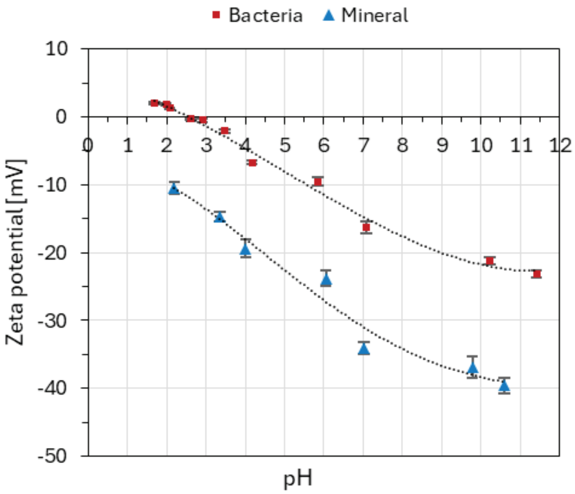

Figure 2 *Zeta potential of bacteria and mineral waste depending on pH. Ionic strength 10^{-3} M KCl*

and a constant ionic strength of 10^{-3} KCl. The results are presented as plots of the relative concentration of cells passing through the bed (*C*) against their initial concentration (C_0). Changes are shown as a function of time and depicted in Fig. 3. As can be seen, bacterial cells migrate best through the bed modified with SDS. In the initial phase, the cell concentration in the filtrate increases; after 10 minutes, it reaches a fixed level. Only 7.06% of cells were attached, the lowest value obtained (Tab. 1). This effect was attributed to increased electrostatic repulsion between the negatively charged mineral surface and bacterial cells. The use of SDS decreased particle zeta potential to -39.3 mV (Tab. 2) due to negatively charged sulfate polar heads of the anionic surfactant. The facilitated migration led to a decrease in total flow time compared to other experiments.

The highest percentage, 17.4%, of cells was adsorbed when the mineral was previously conditioned with CTAB (Tab. 1). It was due to the negatively charged surface of bacterial

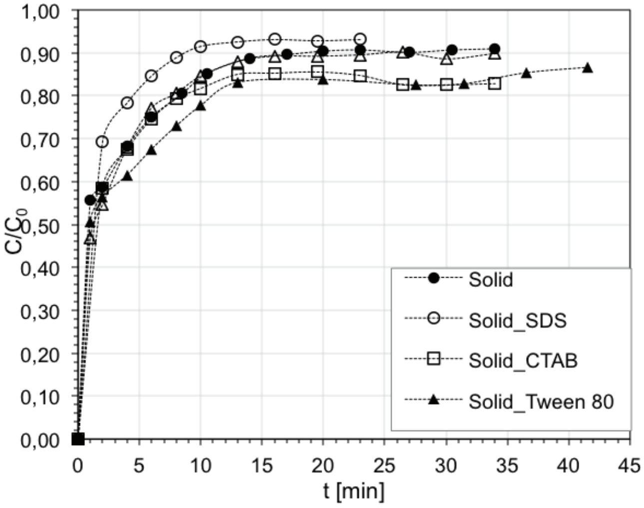

Figure 3 *A normalized concentration C/C_0 versus reaction time for bacterial adsorption in various mineral beds, pH 6.5; ionic strength 10^{-3} KCl*

Table 1 Summary of transport experiments

Surfactant	Total time min	Cells adsorbed %	Molar mass g/mol
-	34	9.42	-
CTAB	34	17.4	364.4
SDS	23	7.06	288.4
Tween 80	42	16.0	1310
Rhamnolipids	34	10.6	650.8

Table 2 Zeta potential of selected samples at pH 6.5 and ionic strength 10^{-3} KCl

Sample	Zeta potential mV
Bacterial cells	-17.7
Pure mineral waste	-29.1
Mineral waste with CTAB	27.2
Mineral waste with SDS	-39.3
Mineral waste with Tween 80	-20.5
Mineral waste with rhamnolipids	-45.9

molecules at pH 6.5, which has a zeta potential of -17.7 mV and remains retained on the bed due to electrostatic interactions with the positively charged mineral surface (27.2 mV). The negative zeta potential of bacterial cells was due to anionic surface groups, such as carboxyl and phosphate (Zhong *et al.* 2017). In the case of cationic surfactant, the CTAB-charged cation head group ($N^+(CH_3)_3$) was responsible for a positive electrical potential of modified particles.

Tween 80, a non-ionic surfactant, increased bacterial retention. The percentage of adsorbed cells was 16.0%. The mineral surface showed a negative charge, as did the bacteria, so the retention effect in the bed may have been caused by pore-clogging by surfactant molecules, as Tween 80 is characterized by a much larger molar mass than other tested surface active agents. Confirmation of this phenomenon requires further research.

Biosurfactants are gaining popularity as eco-friendly alternatives for their chemical counterparts. Therefore, except for chemical surfactants, rhamnolipids were also tested. Surprisingly, using rhamnolipids showed no major changes in cell migration through the bed. The retention time was comparable to an unmodified bed. The zeta potential of the mineral with biosurfactant adsorbed decreased to -45.9 mV due to negatively charged carboxyl groups in the biomolecule. Nevertheless, the effect of its presence differed from that of SDS or Tween 80. It suggests that other interactions occur when biosurfactants are present, which will be the subject of further research.

Conclusions

The preliminary tests showed that mineral waste might be a secondary source of arsenic, one of the critical raw materials identified by the European Union. The material to be bioleached is currently stored in heaps under atmospheric conditions, so it should be considered that toxic elements such as arsenic might, under favourable conditions, be released into the environment. Therefore, the ability to control these phenomena is very important. The use of surface-active compounds can help to control the above-mentioned processes. The retention of acidophilic microorganism cells favours bioleaching processes, and their leaching from the deposit will have the opposite effect.

Preliminary experiments on cell migration through a bed modified with surfactant compounds of different types have shown that electrostatic factors partially control the adhesion process. The zeta potential showed that the tested mineral waste maintains a constant negative charge across the pH range with no isoelectric point. The microbial suspension exhibited a slightly positive charge at highly acidic pH, with an isoelectric point at pH 2.6, after which the bacterial cells became negatively charged.

The study demonstrated that surfactants influence bacterial retention on mineral surfaces due to electrostatic interactions and physical effects. Modification with CTAB, a cationic surfactant, resulted in the highest bacterial adsorption due to electrostatic attraction between the positively charged surface and negatively charged bacterial cells at pH 6.5. In contrast, SDS, an anionic surfactant, reduced bacterial retention to the lowest level, probably due to increased electrostatic repulsion. The biosurfactant on the mineral surface did not affect cell retention, suggesting that electrostatic factors alone do not control adhesion. These findings highlight the potential of surfactant modification in controlling microbial behaviour in historical waste heaps and suggest the need for further research on bacterial interactions in heterogeneous mineral systems.

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References

Bai G, Brusseau ML, Miller RM (1997) Influence of a rhamnolipid biosurfactant on the transport of bacteria through a sandy soil. Appl Environ Microbiol 63:. https://doi.org/10.1128/aem.63.5.1866-1873.1997

Cai Z, Wang Y, Zhang Y, Tian H (2022) Effects of surfactant on Bacillus mucilaginosus adsorption characteristics during vanadium bioleaching process. J Environ Chem Eng 10:. https://doi.org/10.1016/j.jece.2022.108961

Chen G, Qiao M, Zhang H, Zhu H (2004) Bacterial desorption in water-saturated porous media in the presence of rhamnolipid biosurfactant. Res Microbiol 155:. https://doi.org/10.1016/j.resmic.2004.05.001

Ghadiri M, Harrison STL, Fagan-Endres MA (2019) Effect of surfactant on the growth and activity of microorganisms in a heap bioleaching system. Miner Eng 138:. https://doi.org/10.1016/j.mineng.2019.03.028

Gross MJ, Logan BE (1995) Influence of different chemical treatments on transport of Alcaligenes paradoxus in porous media. Appl Environ Microbiol 61:. https://doi.org/10.1128/aem.61.5.1750-1756.1995

Jin D, Liu L, Zheng G, *et al.* (2019) A rapid method to quantify the biomass of viable Acidithiobacillus ferrooxidans in iron-based bioleaching matrix of sewage sludge. Biochem Eng J 152:. https://doi.org/10.1016/j.bej.2019.107360

Katsikogianni M, Missirlis YF, Harris L, Douglas J (2004) Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater 8

Pawlowska A, Sadowski Z (2020) Effect of schwertmannite surface modification by surfactants on adhesion of acidophilic bacteria. Microorganisms 8:. https://doi.org/10.3390/microorganisms8111725

Pawlowska A, Sadowski Z, Winiarska K (2023) Microbial communities of acidic post-mining environments and their use in laterite bioreduction. Acta Montanistica Slovaca 28:918–928

Pawlowska A, Sadowski Z, Winiarska K (2021) Effect of rhamnolipids and lipopolysaccharides on the bioleaching of arsenic-bearing waste. Minerals 11:1303. https://doi.org/10.3390/min11121303

Watling HR (2006) The bioleaching of sulphide minerals with emphasis on copper sulphides – A review. Hydrometallurgy 84:. https://doi.org/10.1016/j.hydromet.2006.05.001

Zhong H, Liu G, Jiang Y, *et al.* (2017) Transport of bacteria in porous media and its enhancement by surfactants for bioaugmentation: A review. Biotechnol Adv 35