

ORIGINAL ARTICLE

Bioremediation of a polymetallic, arsenic-dominated reverse osmosis reject stream

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Significance and Impact of the Study: This study provides evidence of the medium-to-high removal of metals and metalloids present in real industrial effluents by using naturally sourced metal-resistant bacterial inocula. Apart from the applied research significance, the coupling of cysteine degradation to metal removal sheds light into the microbially driven natural attenuation of industrial pollution in specific geochemical settings. The results warrant the scaling up of the process to treat larger effluent volumes and potentially recover valuable metals in the form of metal sulphides.

Keywords

arsenic, bioremediation, cysteine, industrial pollution, reverse osmosis retentate, selenium, *Shewanella*.

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Abstract

The treatment of metal-laden industrial effluents by reverse osmosis is gaining in popularity worldwide due to its high performance. However, this process generates a polymetallic concentrate (retentate) stream in need of efficient post-treatment prior to environmental discharge. This paper presents results on the bioremediation (in batch mode) of a metal-laden, arsenic-dominated retentate using *Shewanella* sp. O23S as inoculum. The incubation of the retentate for 14 days under anoxic conditions resulted in the following removal yields: As (8%), Co (11%), Mo (3%), Se (62%), Sb (30%) and Zn (40%). The addition of 1 mmol l⁻¹ cysteine increased the removal rate as follows: As (27%), Co (80%), Mo (78%), Se (88%), Sb (83%) and Zn (90%). The contribution of cysteine as a source of H₂S to enhancing the removal yield was confirmed by its addition after 7 days of incubations initially lacking it. Additionally, the cysteine-sourced H₂S was confirmed by its capture onto headspace-mounted Pb-acetate test strips that were analysed by X-ray diffraction. We show that real metal-laden industrial effluents can be treated to medium-to-high efficiency using a biological system (naturally sourced inocula) and inexpensive reagents (yeast extract, lactate and cysteine).

Introduction

Industrial effluents containing metals (polymetallic) require on-site, localized treatment, because these matrices cannot be depolluted efficiently by municipal wastewater treatment plants. A number of methods including adsorption, membrane processes, chemical, and electrochemical systems have been tested at bench- and full-scale, and some are commercially available (Staicu *et al.* 2020a; Castaño *et al.* 2021; Qasem *et al.* 2021). Of these, reverse osmosis (RO), a membrane process, is gaining in

popularity due to its high efficiency (Li *et al.* 2021). RO is essentially a pressure-driven membrane diffusion process, whereby a large portion (95–99%) of the dissolved solutes (organic and inorganic) from the feed stream is built up in the *retentate* (concentrate) stream. From this perspective, RO can be classified as a concentration process, generating waste streams enriched in chemical elements and salinity. As a negative side, the retentate streams pose challenges for both environmental and process management, needing treatment before environmental disposal (Li *et al.* 2021).

Selenium (Se), arsenic (As) and antimony (Sb) are chemical elements of environmental and human health concern due to their toxicity and mobility (Stolz and Oremland 1999). Chronic Se poisoning (by generating reactive oxygen species and substituting for sulfur) was recorded in fish (Lemly 2004) and aquatic bird (Ohlendorf *et al.* 2020) populations throughout North America. Arsenic is a metabolic poison (forms reversible combinations with thiol groups and it substitutes for phosphorus), affecting vast regions of Asia and South America (Podgorski and Berg 2020). Large quantities of Sb are being released from mining and smelting processes, leading to environmental pollution and toxicity (Sb and its compounds combine with internal sulfhydryl in animal or human body, affecting the enzyme activity, ion balance and, in extreme cases, generating hypoxia) (Li *et al.* 2018).

Interestingly, phylogenetically diverse bacteria can use the oxyanions of Se, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), and of As, arsenate (AsO_4^{3-}), as terminal electron acceptors in the process of anaerobic respiration (Staicu and Barton 2021). In this electrochemical process, the electrons released from an electron donor (e-donor) are routed via a protein-complex circuit to an electron acceptor (e-acceptor), generating cellular energy (ATP) (Canfield *et al.* 2005). The respiration of Se oxyanions leads to red, amorphous Se^0 biominerals (Stolz and

Oremland 1999), while the respiration of arsenate to arsenite (AsO_3^{3-}) can be accompanied by the formation of yellow orpiment, As_2S_3 (Newman *et al.* 1997).

This paper presents a detailed study on the bioremediation of a real industrial polymetallic (As- and Se-laden) stream (retentate) produced by a full-scale RO unit treating metal mining effluents. The aim of this study was to evaluate the bioremediation potential of *Shewanella* sp. O23S, a metal-resistant bacterial strain isolated previously from a metalliferous environment. The specific objectives were (i) to assess the impact of indigenous NO_3^- and SO_4^{2-} on As and Se oxyanions removal, (ii) establish the preferential removal of As or Se oxyanions based on thermodynamics predictions, and (iii) determine the contribution of cysteine to enhancing the removal yield of As, Se, Sb and various metals present in the effluent.

Results and discussion

Industrial effluent

The industrial effluent has circumneutral pH (~ 7.8) and a high electrical conductivity ($\sim 7.5 \text{ mS cm}^{-1}$). This high conductivity is generated by the ionic makeup of the system (Tables 1 and 2). Of these, sulphate is present in high concentration ($\sim 1.6 \text{ g l}^{-1}$). Bicarbonate (0.48 g l^{-1}) and other

Table 1 Characteristics and chemical composition (major anions) of the industrial effluent (pre- and post-incubation with *Shewanella* sp. O23S)

Parameter	Effluent	Day 14	Change (%)	Day 14*	Change (%)
pH	7.86 (0.20)	7.11 (0.20)	-9.5	7.09 (0.20)	-9.8
Conductivity (mS cm^{-1})	7.48 (0.20)	8.04 (0.10)	+7.5	8.29 (0.10)	+10.8
TOC (mg l^{-1})	63.0 (0.9)	17.0 (0.3)	-73		
	397.1 (7.5)*			167.9 (0.8)*	-57.7
Major anions (mg l^{-1})					
Cl ⁻	111.8 (2.3)	112.3 (1.7)	<1	110.4 (2.5)	<1
F ⁻	29.2 (0.2)	15.5 (0.1)	-47	16.4 (0.2)	-43.7
NO ₃ ⁻	52.5 (0.3)	n/d	-100	n/d	-100
SO ₄ ²⁻	1585 (7)	1518 (3)	-4.3	1554 (5)	-4.28

*With the addition of 1 mmol l^{-1} cysteine at the beginning of the incubation. Br⁻, NO₂⁻, PO₄³⁻ <LOD (limit of detection). n/d, not detected.

Table 2 Characteristics and chemical composition (trace elements) of the industrial effluent (pre-, mid- and post-incubation with *Shewanella* sp. O23S)

Element ($\mu\text{g l}^{-1}$)	Effluent	Day 7	Day 14	Change Day 14 (%)	Day 7*	Day 14*	Change Day 14 (%)
As	2247.1	2091.9	2064.8	-8.1	1700.7	1635.1	-27.2
Co	16.5	15.7	14.7	-11.8	5.9	3.3	-80
Cu	78.1	34.2	14.8	-81	2.7	1.1	-98.5
Mo	226.9	220.9	220.2	-2.9	65.9	50.1	-77.9
Se	165.2	79.7	63.4	-61.6	28.8	19.9	-87.9
Sb	171.9	160.4	158.6	-29.6	44.9	29.6	-82.8
Zn	97.8	65.1	58.7	-39.9	15.9	9.6	-90.2

*With the addition of 1 mmol l^{-1} cysteine at the beginning of the incubation.

anions listed in Table 1 such as nitrate and fluoride also contribute to its ionic strength. Of the microelements dominating the polymetallic effluent (Table 2), of particular relevance ($\mu\text{g l}^{-1}$) are As (2250), Mo (227), Se (165), Sb (172) and Zn (98). Based on chromatography analysis, As is present as AsO_4^{3-} . Other elements are present in various concentrations ($\mu\text{g l}^{-1}$): Ba (92), Fe (11), Ni (22), Mn (116) and U (21). However, these elements were not significantly impacted by the bioremediation process.

Growth of *Shewanella* sp. O23S

Shewanella sp. O23S was incubated anoxically in the effluent amended in various combinations with 1 mmol l^{-1} sodium lactate (electron donor and carbon source), 1 g l^{-1} yeast extract/YE (source of micronutrients) and 1 mmol l^{-1} cysteine/cys (reducing agent). The incubations were performed for 14 days and the results are presented in Fig. 1. The best growth was recorded when the strain was incubated with lactate and YE, reaching a maximum (~ 0.5) optical density (OD) at day 7 (D7). The addition of cys to lactate and YE also produced good growth, although lower than the previous system. Cysteine increased the TOC from 63 to 397 mg l^{-1} , however, this was not reflected in a better growth, suggesting cys was not used as a carbon source by the bacterial inoculum. Interestingly, the addition of YE without cys and lactate produced a significant growth. In contrast, the

incubations containing only cys or lactate did not sustain growth. The same situation was recorded when the bacterial culture was incubated in the effluent without amendments. The OD values indicate that the bacterial culture reached stationary phase after around 7 days of incubation. Overall, these results show the importance of adding external organic carbon and micronutrients to industrial effluents in the framework of bioremediation. The growth of bacteria under anoxic conditions cannot proceed to higher OD values (~ 0.5 in the current study); however, the oxygen limitation is essential for stimulating respiratory processes aiming to selectively target various electron acceptors such as the oxyanions of As and Se.

Removal of anions and trace elements

Of the growth conditions tested, two were chosen for the bioremediation study: (i) the addition of lactate and YE and (ii) the addition of lactate, YE and cys. In separate incubations, cys was added after 1 week to validate the contribution of cys to increasing the removal yield of various elements present in the effluent.

Both treatments (with and without cys) had similar impact on the removal of anions (Table 1). Fluoride showed a 44–47% removal from 29 mg l^{-1} , nitrate was totally removed from 52 mg l^{-1} , while limited decrease in sulphate was recorded ($\sim 4\%$), from an initial $\sim 1.5 \text{ g l}^{-1}$. The use of nitrate as an e-acceptor and the incapacity of the strain to metabolize sulphate are in line with a previous reports on *Shewanella* sp. O23S (Drewniak *et al.* 2015; Uhrynowski *et al.* 2019).

The incubation with *Shewanella* sp. O23S led to the removal of several elements (As, Se, Sb), and the addition of cys had a significant impact. The removal rate matches the exponential growth phase of the culture, reaching a maximum around D5/7 (the onset of the stationary phase). The abiotic controls indicate the bacterial culture is the causative agent leading to the removal of the elements. In the case of Se (Fig. 2a), its concentration decreased sharply during the first 7 days (52%), while for the next 7 the removal proceeded at a much slower rate (61% for D14). The addition of cys showed the same trend, characterized by a better removal yield for all time points, reaching 78% (D7) and 88% (D14). Another element displaying high removal was Sb (Fig. 2b), but only when cysteine was added (55% removal at D5 and 83%, D14). The incubation without cys had a minor contribution to Sb removal (8%, D14). The removal of As (Fig. 2c) was limited without cys (7–8%, D14), increasing in the presence of cys (23% at D3 and 27%, D14). Of the three elements, As had the lower relative removal rate, even in the presence of cys; however, if we consider the amount removed (absolute values), As had the highest

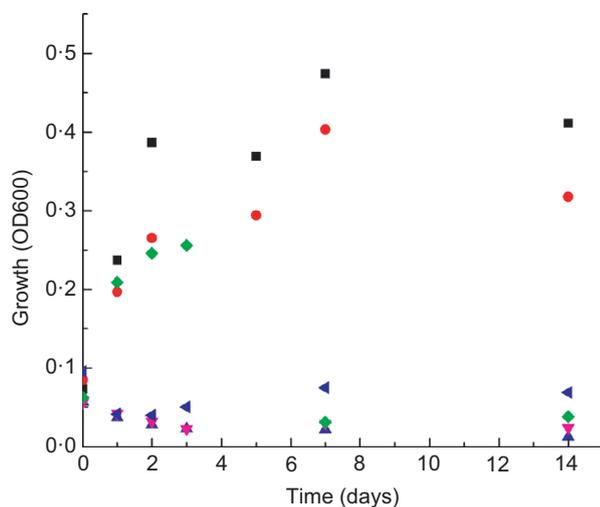


Figure 1 Growth of *Shewanella* sp. O23S in industrial effluent under various treatments. (■) + lactate + YE; (●) + lactate + YE + cys; (▲) no amendments; (▼) + cys; (◆) + YE; (◀) + lactate. Incubation conditions: anoxic, 30°C , pH ~ 7.5 , dark, static. Legend: YE, yeast extract (1 g l^{-1}); cys, cysteine (1 mmol l^{-1}); and sodium lactate (1 mmol l^{-1}). Standard deviation values were $<5\%$ and were not plotted.

removal: 615 $\mu\text{g l}^{-1}$ (As) vs 143 $\mu\text{g l}^{-1}$ (Se) vs 142 $\mu\text{g l}^{-1}$ (Sb), after 14 days of incubation. In a separate experiment, cysteine was added at D7 of incubation to further validate its impact on the removal yield and the concentration of elements was measured at D9, 12 and 14. For all three elements (As, Se and Sb), the addition of cys led to an improvement of the removal yield, leading to results comparable to the experiments with cys added at t_0 .

Cysteine degradation to H_2S was recorded by the darkening of the lead-acetate strips introduced in the head-space of the incubations (Figs S1 and S2). The H_2S released from cys reacts with the Pb(II) from the test

paper forming dark PbS (galena) (Fig. S3). The test paper used in the abiotic control did not change color, indicative of the absence of H_2S release (Fig. S2). Several bacterial enzymes could be involved in cysteine degradation to H_2S such as cysteine desulfurase, cystathionine β -synthase and cysteine desulfhydrase (reviewed by Mihara and Esaki 2002; Staicu *et al.* 2020b). In a follow up study, the enzymatic degradation of cysteine will be investigated, alongside the repertoire of carbon source utilization, other than lactate. Lactate is converted regularly by bacteria into acetate, propionate and butyrate (Chen *et al.* 2019); and it will be valuable to check the breakdown products of various carbon sources fed in the continuous flow mode.

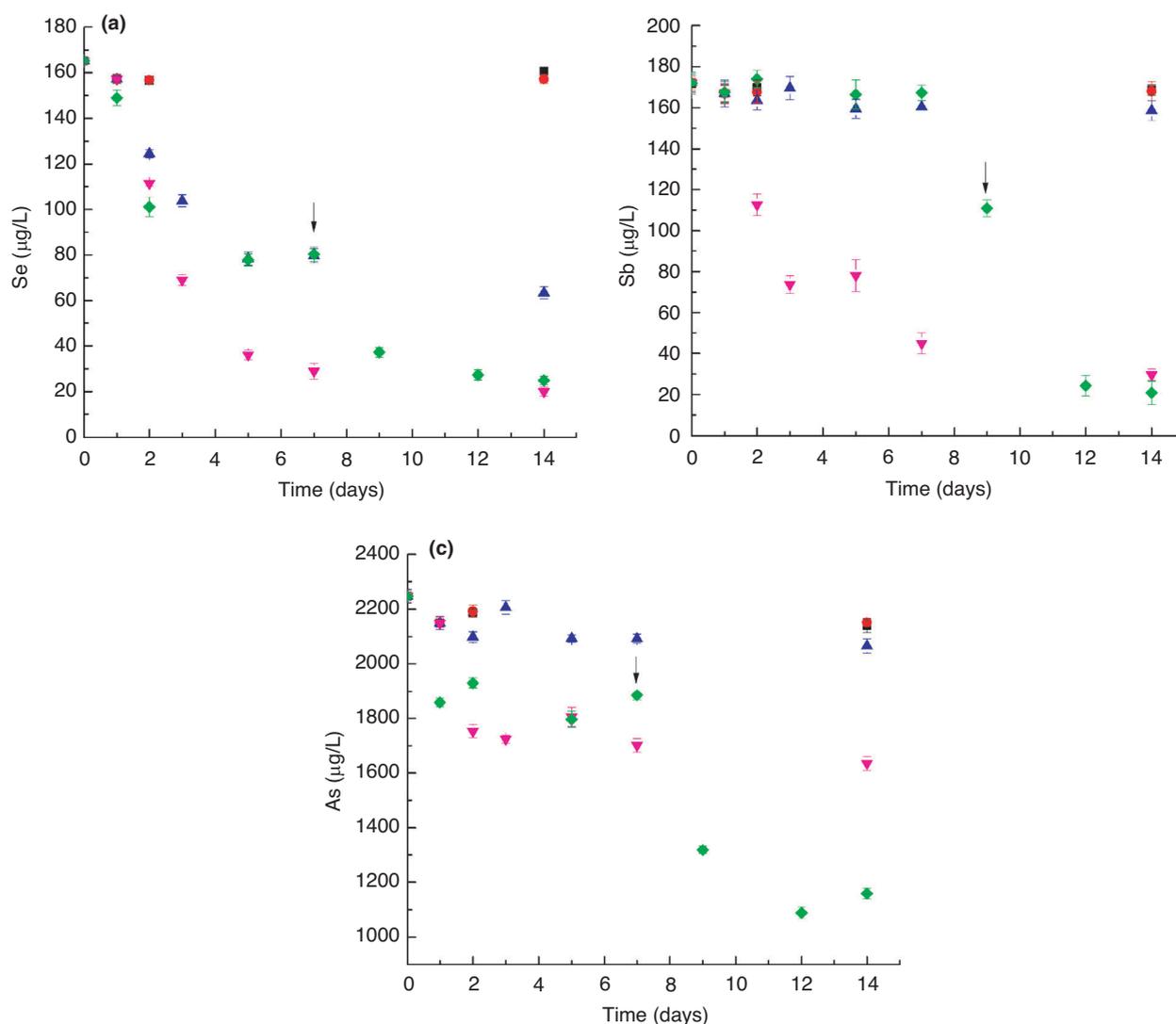


Figure 2 Bioremediation performance of the effluent using *Shewanella* sp. O23S. (a) Se, (b) Sb and (c) As. Key: (■) abiotic control; (●) abiotic control + cysteine; (▲) biotic (*Shewanella* sp. O23S) incubation without cysteine; (▼) *Shewanella* sp. O23S + cysteine; (◆) *Shewanella* sp. O23S + cysteine added at day 7 (D7). The arrow indicates the time point (D7) when 1 mmol l^{-1} of cysteine was added.

Mineralogy

Figure 3a presents the diffractogram of the pellets from incubations with *Shewanella* sp. O23S in industrial effluent with and without cysteine. The bacterial pellets were amorphous or possibly nanocrystalline in the case of the incubations with cysteine, since no diffraction peaks were detected and only broad bands were observed. X-ray diffraction (XRD) patterns of lead acetate strips corresponding to these experiments are shown in Fig. S3. Diffraction peaks corresponding to the unreacted lead acetate (ICDD code 00-054-0326) were observed in both diffractograms. Only the XRD pattern of the lead acetate strip from the biotic incubation amended with cysteine showed peaks matching those of lead sulfide (ICDD code 01-078-1058). Additionally, diffraction peaks matching those of lead carbonate (ICDD code 00-001-0687) were detected in strips from incubation without cysteine.

Both Raman spectra (Fig. 3b) show four bands that can be ascribed to the presence of As oxides. Two sharp bands centered near ~ 480 and ~ 750 cm^{-1} and two

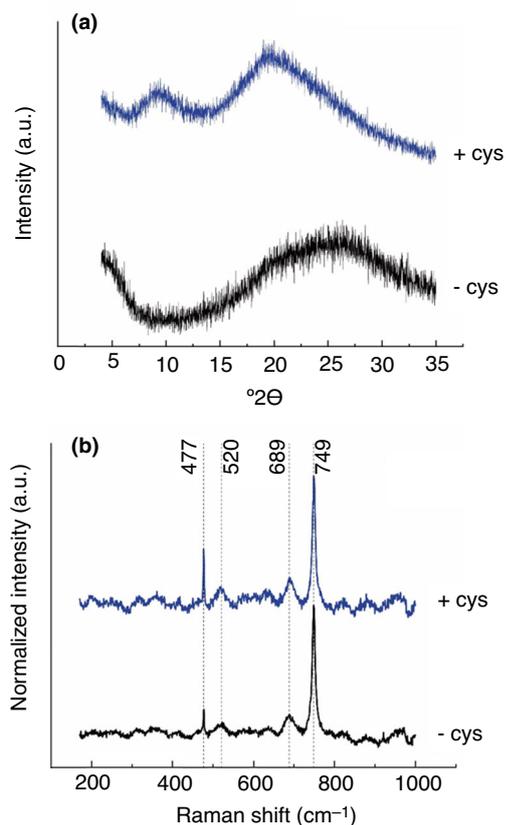


Figure 3 (a) XRD patterns and (b) Raman spectra of bacterial pellets from industrial effluent incubated with *Shewanella* sp. O23S with and without cysteine (— *Shewanella*; — *Shewanella* + cysteine).

broader, less intense bands at 525 and 696 cm^{-1} were systematically observed in the different spectra obtained. Several studies have reported Raman spectra of As oxides in its different solid, liquid and vapor phases (e.g. Papatheodorou and Solin 1975; Mercier and Sourisseau 1978). In the solid state, As oxide may be amorphous or take the crystalline forms of arsenolite (cubic As_4O_6) or claudetite (monoclinic As_2O_3). Band assignments differ between authors, but in general, bands in the region 600–900 cm^{-1} have been assigned to the antisymmetric stretches of AsO_3 , those in the region 500–600 to symmetric stretches and those in the region 300–500 cm^{-1} to symmetric deformations (Mercier and Sourisseau 1978). The 480 cm^{-1} band can be assigned to the ν_1 (A_1) AsO_3 symmetric vibration (it ranges from 464 to 496 cm^{-1} in the different As oxide phases) and the 525 cm^{-1} band to the ν_3 (E) asymmetric AsO_3 vibration (it ranges from 504–525 cm^{-1} in the different As oxide phases, Papatheodorou and Solin 1975). The bands at 750 and 690 cm^{-1} are more difficult to assign. Although it is slightly shifted compared to published values, the first band could be related to the vibration of As_4 tetrahedral linkages that results from intermolecular coupling (777–800 cm^{-1}), while the latter could be assigned to the ν_3' (B_2) As-O-As antisymmetric vibration (625–636 cm^{-1} , Papatheodorou and Solin 1975). Interestingly, these two bands match ν_3 (E) asymmetric (680 cm^{-1}) and ν_1 (A_1) symmetric (752 cm^{-1}) stretching of arsenite AsO_3^- (Loehr and Plane 1968). The poorly defined broad bands observed in the 300–400 cm^{-1} range could correspond to As-S phases or, in general, metal sulfides, although their low intensity precludes their unambiguous assignment to these phases. Characterization of the precipitates by XRD and Raman is made difficult by the low concentration of the precipitates in the bulk organic matter (bacteria, bacterial debris). In a recent publication, Battaglia-Brunet *et al.* (2021) investigated the removal of As from an acid mine drainage stream using a mixed-microbial community. Although the solution contained a much higher As load (167 mg l^{-1}) and the incubation was done in a continuous column experiment, allowing for more biominerals to accumulate in the system, the mineralogy was not resolved by XRD. Amorphous As_2S_3 was confirmed through advanced XANES and EXAFS spectroscopy, however, the mineralogy of the precipitate was not unambiguously clarified. A minor amount of oxygen-bound As(III) (0–13%) was also identified in the yellow mineral precipitate.

Proposed removal mechanisms

The removal of some of the chemical elements presented in this study could be explained by several mechanisms:

i As and Se could have been removed via respiratory processes. The capacity of *Shewanella* sp. O23S to respire AsO_4^{3-} to AsO_3^{3-} was reported previously (Drewniak *et al.* 2015). The production of H_2S from cysteine accelerates the removal of As, by potentially forming As_2S_3 biominerals (Fig. 2c). In this fashion, the soluble product of arsenate respiration is turned into a solid precipitate. The strain has the capacity to co-respire Se, alone and in the presence of As (Staicu *et al.* unpublished data). In the case of Se, the higher relative removal (compared to As) could be explained by the direct formation of a solid biomineral, Se^0 , as an end product of Se respiration, while the additional removal of Se in the presence of cys remains an open question. A possible explanation might be its contribution as a reducing agent to creating a lower redox environment (oxidation-reduction potential, ORP) (Wagner *et al.* 2019). Pickett *et al.* (2008) have proposed an optimal ORP for Se respiration ranging from -100 to -200 mV. Because the incubations were performed in serum bottles, analytical sensors for measuring ORP could not have been installed.

ii In this system, cysteine does not appear to function as a carbon and energy source (Fig. 1). In terms of energy production, the use of SeO_4^{2-} yields twice the amount of energy (Gibbs free energy/ ΔG^0) than arsenate, with lactate as e-donor ($343.1 \text{ kJ mol}^{-1}$ acetate *vs* 172 kJ mol^{-1} acetate), thus potentially explaining the higher relative removal of Se over As (Staicu and Barton 2021). Interestingly, a number of phylogenetically diverse bacteria (e.g. *Bacillus arseniciselenatis* E1H, *B. selenatarsenatis* SF-1, *Sulfospirillum barnesii* SES-3) have been reported to co-respire on both SeO_4^{2-} and arsenate, opening the discussion about the evolution of the respiratory repertoire to include scarcely available chemical elements in natural settings (Stolz and Orem-land 1999; Staicu and Stolz 2021). Antimony has also been documented as an e-acceptor in anaerobic respiration (Staicu and Barton 2021).

iii Cysteine is a component of glutathione (GSH), molecule capable of preventing damage (via H_2S -metal complexes) induced by numerous stressors including toxic metals (Helbig *et al.* 2020). Thus, the addition of cys might have increased the GSH concentration, leading to a superior removal yield. The removal of metal cations in the form of sulphides has been reported in numerous publications (Esposito *et al.* 2006; Sampaio *et al.* 2009; Staicu *et al.* 2019, 2020b). Metal sulphides are sparingly soluble and have high stability (Rumble 2018), therefore precipitate out from solution as non-toxic biominerals. The addition of cys and its degradation to H_2S led to a significant increase in metal

removal (Table 2): Co (from 12 to 80%), Mo (from 2 to 78%), Zn (from 40 to 90%) and Cu (from 81 to 99%). These elements are essential for numerous bacterial groups, therefore some of them are used as enzyme co-factors, thus explaining their removal in the absence of cys (Staicu *et al.* 2019). In order to optimize the costs related to cys addition, an alternative solution might be the use of yeast extract of various suppliers and in varying concentrations. Some publications indicate yeast extract can contain around 2.5 g kg^{-1} free cysteine (Podpora *et al.* 2016). Yeast extract is known to be an important source of aminoacids and micronutrients for bacterial growth and is considered a low cost and high-density nutrient (Jaeger *et al.* 2020).

The follow up study will attempt to increase the removal yield of the metals and metalloids present in this effluent by testing the following:

- Denitrification prior to incubation with *Shewanella* sp. O23S, since nitrate might act as a competitor (it has higher Gibbs free energy than arsenate and SeO_4^{2-} , as per Staicu and Barton 2021) for the electrons released from lactate.
- Particular attention will be devoted to measuring and controlling the redox potential at various ranges (from -50 to -300 mV) in order to assess the respiratory yield of the *Shewanella* strain against arsenate, SeO_4^{2-} , nitrate and potentially sulphate. This will be achieved using a chemostat equipped with redox potential electrode and by dosing various concentrations of cysteine.
- The treatment of the effluent in a continuous flow mode (constant addition and optimization of nutrients and cysteine input) could result in higher removal yields and faster removal kinetics.

In order to better understand the molecular mechanisms involved in the removal of metals/metalloids and the degradation of cys and lactate, a future study will employ a transcriptomic analysis. The genes that are up-regulated and down-regulated as a function of various growth stages and input of reagents (cys, lactate and other C-sources, yeast extract) will contribute to the better understanding of the removal mechanisms and help improve the bioremediation efficiency.

Materials and methods

Bacterial inoculum

Shewanella sp. O23S used during this study was previously isolated from an ancient gold mine in Zloty Stok

(SW Poland) (Drewniak *et al.* 2015). 1% bacterial culture (starter) was used to inoculate all incubations.

Reagents

Sodium lactate (sodium L-lactate syrup, 60%) was purchased from Sigma. The choice for lactate as a carbon source and e-donor was based on the initial report on this bacterial strain (Drewniak *et al.* 2015). Yeast extract and L-cysteine-HCl monohydrate were from Sigma. Lead-acetate test paper (Ref. 90744) was purchased from Macherey-Nagel (Düren, Germany). All other reagents, unless otherwise stated, were of analytical grade.

Industrial effluent

Industrial effluent (retentate) was obtained from a full-scale RO unit, which treats wastewater produced in mining and mineral processing activities (Southern Europe). The effluent was stored at 4°C and, prior to each experiment, was allowed to reach room temperature (22°C), then the pH and electrical conductivity were recorded.

Incubations

The industrial effluent was amended with 1 g l⁻¹ yeast extract and 1 mmol l⁻¹ sodium lactate. The final solution was sterilized by vacuum filtration using (Acrodisc®, pore size 0.2 µm) filters.

Incubations, including the abiotic controls, were performed anoxically in the industrial effluent using 1% *Shewanella* sp. O23S fresh starters at 30°C, pH 7.0, in the dark and under static conditions. The 500 ml serum bottles were sealed with sterile butyl rubber septa and closed with screw caps. The headspace was flushed with N₂ gas for 5 min through a 0.22 µm fresh filter to ensure sterility. Three centimetre Pb-acetate sterile paper strips were added to the headspace to record the formation of H₂S during incubation. In order to collect samples without wetting the paper strips, long needles (80 mm) were used. Abiotic incubations were performed in parallel in the same conditions.

Analytcs

Metal/metalloid analysis

Filter-sterilized samples were stored in sterile glass serum bottles in darkness at 4°C, and before analysis each sample was passed through a syringe-driven 0.45-µm pore size filter. Analysis was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7700; Agilent Technologies, Santa Clara, CA) using IDA (Isotopic Dilution Analysis) with a spike solution from ISC Science, Oviedo, Spain (Box S1).

Ion analysis

Cation and anion analyses were conducted by means of ion chromatography (883 Basic IP plus; Metrohm, Herisau, Switzerland). For the calibrations, commercial patterns from Sigma Aldrich were used (Box S2).

For determining As speciation, As species were separated in a 4.6 mm × 150 mm As Separation Column (Agilent Technologies) fitted to a 1260 Infinity HPLC coupled to the ICP-MS referred above using a mobile phase of 2 mol l⁻¹ PBS (phosphate buffered saline)/0.2 mol l⁻¹ EDTA (pH = 6.0) at a flow of 1 ml min⁻¹.

Total organic carbon (TOC) was measured using a TOC-VCSH (Shimadzu, Kyoto, Japan).

Electrical conductivity and pH were measured using a multiparameter instrument (Prolab 4000; Schott, Jena, Germany).

Mineralogy

Samples were analysed by XRD using an X'Pert PRO diffractometer (PANalytical) with the following instrumental parameters: Cu Kα-radiation ($\lambda = 1.5405 \text{ \AA}$), 40 mA current, 45 kV tension, 3–70°2θ measurement range, 4 s per step and 0.04°2θ step size. Diffraction patterns were analysed using the computer code HighScore Plus 2.2.4 (PANalytical) and minerals were identified matching the experimental diffraction peaks with those included in the Joint Committee for Powder Diffraction Standards (JCPDS) PDF-2 database. Lead acetate strips were directly stuck to the sample holder using C tubs, while precipitates were crushed for XRD analysis.

Micro-Raman spectroscopy spectra were acquired with a HORIBA XploRa Plus microscope with a Peltier-cooled (213 K) CCD detector (1064 × 256 pixels) using a high power 532-nm diode laser in combination with a 2400 l mm⁻¹ grating. The instrument was calibrated using a silicon wafer. Samples were analysed using a 50× objective in a direct-coupled microscope with enclosure. To avoid degradation or heat-induced physical changes, the power on the samples was reduced using a 10% neutral density filter. A sample exposure time of 10 s (five accumulations) was employed, resulting in an adequate signal-to-noise ratio. Spectra were acquired using the Lab-SPEC6 Raman software and were baseline corrected using the OriginPro 8 software.

The results are presented as average values and standard deviation of three independent experiments ($n = 3$) unless otherwise stated.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' contribution

LCS designed the experimental part and wrote the manuscript (ms). PJW performed the experimental part and revised the ms. DB and JLRG performed chemical analyses and revised the ms. ZM and MP designed the experimental part and revised the ms. ERA performed the mineralogical analysis and revised the ms.

Data Availability Statement

Data presented in the current manuscript data have been made available.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Box S1. ICP-MS.

Box S2. Ion chromatography.

Figure S1. Incubations using *Shewanella* sp. O23S: (a) t_0 (–cys, +cys), (b) day 14 (–cys, +cys); and abiotic controls: (c) t_0 (–cys, +cys), (d) day 14 (–cys, +cys). Incubation conditions: anoxic, 30°C, pH 7.0, dark, static. cys, cysteine (1 mmol l⁻¹).

Figure S2. Pb-acetate strips from Fig. S1 (incubation with *Shewanella* sp. O23S).

Figure S3. XRD of Pb-acetate strips from Fig. S3.

Supplementary file

Bioremediation of a polymetallic, arsenic-dominated industrial effluent

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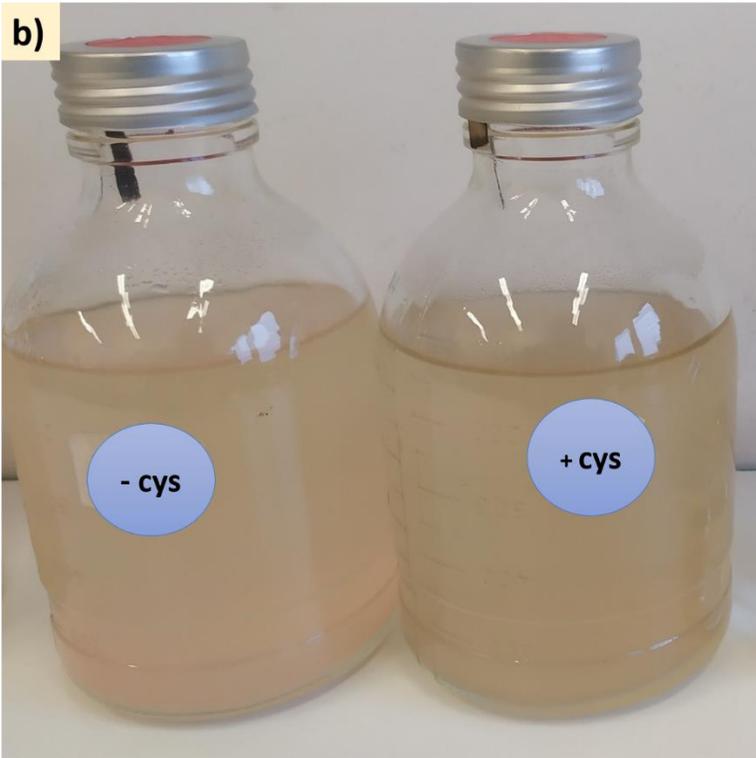
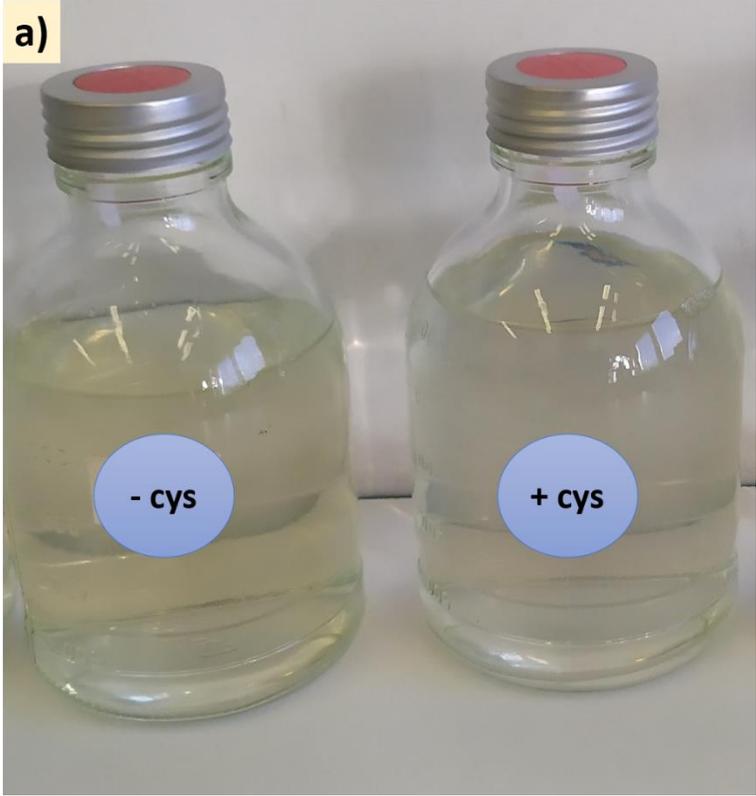
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Box S1. ICP-MS

Analysis of twenty-eight elements (B, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb, U) was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7700, Agilent Technologies, California, USA) using IDA (Isotopic Dilution Analysis) with a spike solution from ISC Science, Spain. High-purity standards (Charleston, SC, USA) were used for calibration. Detection limit for all elements were $0.2 \mu\text{g L}^{-1}$, except for Hg and U ($0.1 \mu\text{g L}^{-1}$); B, Al, and Fe ($20 \mu\text{g L}^{-1}$); Na, Mg, K, and Ca ($200 \mu\text{g L}^{-1}$).

Box S2. Ion Chromatography

The concentrations of cations and anions patterns were: $1000 \pm 4 \text{ mg/kg}$ for Na^+ , NH_4^+ , Ca^{2+} , K^+ , F^- , Cl^- , NO_3^- , PO_4^{3-} and SO_4^{2-} ; and $1000 \pm 5 \text{ mg/kg}$ for Mg^{2+} . As quality control (QC), mixtures of analytes of 10 mg/kg were prepared for all analytes, except for NH_4^+ that is 1 ppm , considering suitable results those of $10 \pm 1 \text{ ppm}$ and $1 \pm 0.1 \text{ mg/kg}$ respectively. The quantification limits are: $10 \mu\text{g/kg}$ for NH_4^+ ; $20 \mu\text{g/kg}$ for F^- ; $100 \mu\text{g/kg}$ for K^+ ; $200 \mu\text{g/kg}$ for Cl^- , NO_3^- , PO_4^{3-} and SO_4^{2-} ; and $500 \mu\text{g/kg}$ for Na^+ , Ca^{2+} and Mg^{2+} .



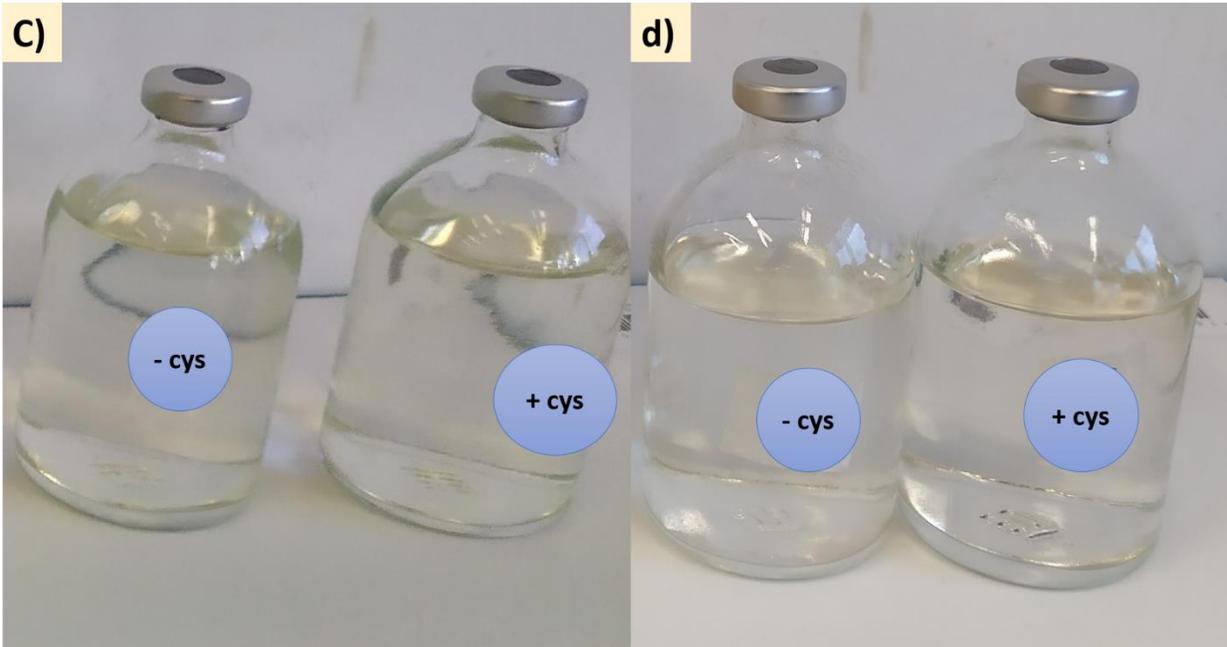


Figure S1. Incubations using *Shewanella* sp. O23S: a) t₀ (-cys, +cys), b) Day 14 (-cys, +cys); and abiotic controls: c) t₀ (-cys, +cys), d) Day 14 (-cys, +cys). Incubation conditions: anoxic, 30 °C, pH 7.0, dark, static. cys, cysteine (1 mM).



Fig. S2. Pb-acetate strips from Fig. S1 (incubation with *Shewanella* sp. O23S). Legend: X-14 (+cys, collected at day 14), Y-14 (-cys, collected at day 14), Z-14 (abiotic control + cys, collected at day 14).

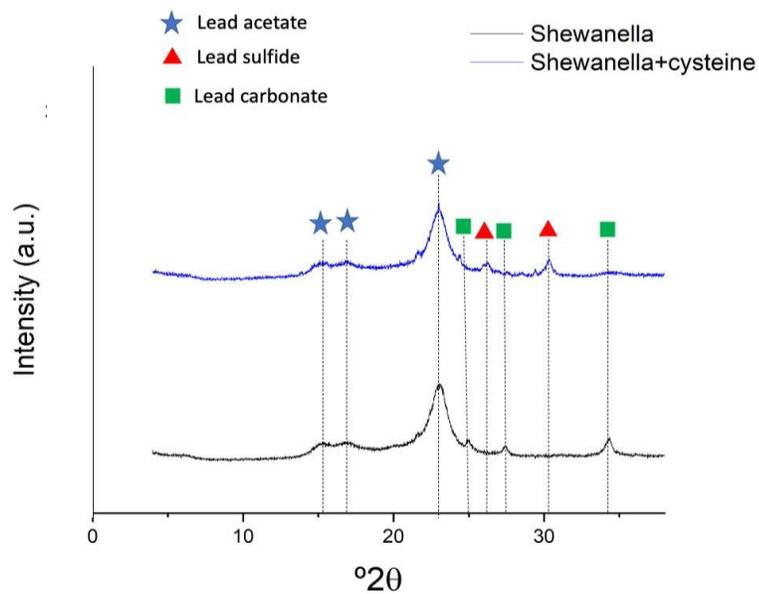


Fig. S3. XRD of Pb-acetate strips from Fig. S2. Legend: *Shewanella* + cys represents X-14, *Shewanella* (-cys) represents Y-14.