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Bioleaching of arsenic-rich cobalt mineral resources, and evidence for concurrent biomineralisation of scorodite during oxidative bio-processing of skutterudite

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ABSTRACT

Experiments were carried out to test the amenabilities of mineral deposits that contained cobalt deported in arseno-sulfide (cobaltite) and arsenide (skutterudite) minerals, to oxidative bioleaching at mesophilic temperatures and low pH. An ore sample from the Iron Mask deposit (Canada) and a mineral concentrate from a working mine (Bou Azzer, Morocco) were thoroughly characterised, both prior to and following bio-processing. A "top down" approach, using microbial consortia including (initially) 13 species of mineral-degrading acidophiles was used to bioleach the ore and concentrate in shake flasks and bioreactors. Cobalt was successfully liberated from both materials tested (up to 93% from the ore, and 49% from the concentrate), though the chemistries of the leach liquors were very different, with redox potentials being > 200 mV lower, and concentrations of soluble arsenic about 7-fold greater, with the concentrate. Addition of pyrite to the arsenide concentrate was found to promote the biomineralisation of scorodite (ferric arsenate), which was detected by both XRD and SEM-EDX, but was not found in bioleached residues of the arseno-sulfide ore. A model was proposed wherein pyrite had three critical roles in facilitating the genesis of scorodite: (i) providing the catalytic surface to promote the oxidation of As (III) to As (V); (ii) acting as a putative "seed" for scorodite crystallisation; (iii) being a secondary source of iron, since the molar ratios of iron:arsenic in the concentrate itself (0.19:1) was well below that required for effective removal of soluble arsenic as scorodite (1:1). This work provided proof of concept that cobalt arseno-sulfide and arsenide ores and concentrates are amenable to bio-processing, and also that it is possible to induce concurrent solubilisation of arsenic from primary minerals and immobilisation in a secondary mineral, scorodite.

1. Introduction

The global demand for cobalt has been increasing greatly in recent decades, chiefly due to its use in super-alloys, rechargeable batteries and catalysts, and cobalt is currently widely considered to be a "critical" metal (e.g. European Commission, 2017). Most of the cobalt currently produced is a by-product of copper or nickel mining, with the bulk (> 60%) originating from the Central African Copperbelt mines located in the Democratic Republic of Congo and Zambia (Roberts and Gunn, 2014). Cobalt is currently sourced as the major metal produced from primary ores in only one location (the Bou Azzer mines in Morocco; Petavratzi et al., 2019). In the light of political uncertainties and ethical issues of the means by which cobalt is produced in some areas, there are concerns about the future security of supply of this metal. New sources of cobalt are therefore being sought, together with identifying means by which the metal can be extracted from primary ores and waste materials while minimising how this impacts the environment.

Cobalt exhibits both chalcophilic and siderophilic characteristics, bonds preferentially with sulfur, and is associated with iron, copper and nickel in a variety of sulfide and arseno-sulfide phases (Roberts and Gunn, 2014). Sulfide minerals found in reduced ores include cobaltite (Co,Fe)AsS, carrollite ($CuCo_2S_4$), and linnaeite $(Co,Ni)_3S_4$, while skutterudite (chemical formula $(Co,Ni)As_{3-x}$; empirical formula $Co_{0.75}Ni_{0.25}As_{2.5}$) is the most commonly encountered cobalt arsenide

Abbreviation: ZVS, zero-valent sulfur

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mineral. Lateritic ores, which account for ~70% of nickel present in accessible reserves in the lithosphere, also contain significant amounts (generally between 0.025 and 0.18%) of cobalt (Berger et al., 2011). Within the limonitic zones of laterite deposits, cobalt is commonly associated with manganese (IV) minerals such as asbolane ($(Ni,Co)_xMn(O,OH)_4.nH_2O$) rather (as is the case with nickel) than iron (III) minerals.

Bioleaching has been successfully demonstrated in laboratory experiments and full-scale operations as a mechanism by which cobalt can be extracted from both reduced (sulfidic) ores and wastes, and oxidised (limonitic) mineral reserves. Nkulu et al. (2015) described the oxidative dissolution of high-grade carrollite by a mixed population of mesophilic bacteria (*Leptospirillum* (*L.*) *ferrooxidans, Acidithiobacillus* (*At.*) *ferrooxidans* and *At. thiooxidans*) in shake flasks (pH 1.8–2.0, and incubated at 33 °C). A full-scale tank bioleaching process, operated at Kasese (Uganda) between 1999 and 2013, recovered cobalt from biologically-active tailings produced and ultimately abandoned from a copper mine enterprise. Most of the cobalt in the tailings was disseminated in ionic form within the pyrite lattice (Morin and d'Hugues, 2007). The tanks were operated at 42 °C and between pH 1.4 and 1.7, and the microbial population was dominated by *L. ferrooxidans, At. caldus, Sulfobacillus* (*Sb.*) *benefaciens* and a *Ferroplasma*-like archaeon. Microbially-enhanced solubilisation of cobalt from limonitic ores is mediated by reductive dissolution of (principally) manganese (IV) minerals, which can be catalysed by ferrous iron reduced by the dissimilatory reduction of iron (III) minerals such as goethite (Johnson and du Plessis, 2015). Early experiments used *At. ferrooxidans* which, in the absence of oxygen can couple the oxidation of zero-valent sulfur (ZVS) to the reduction of iron (III), though more recently consortia of acidophiles have been used.

In contrast to the above, the ability of microorganisms to liberate cobalt from arsenic-rich deposits and mineral concentrates has not been widely reported, possibly due to the fact that such ore bodies are less widely distributed than sulfide and oxide deposits, and the potential toxicity of soluble arsenic to bioleaching microflora. Here we report the successful bio-processing of a mineral deposit where cobalt was chiefly deported in an arseno-sulfide (cobaltite), and a mineral concentrate where it was mainly present as cobalt arsenide (skutterudite). An approach was devised to facilitate the concurrent bioleaching of skutterudite with the biomineralisation of scorodite, in an attempt to minimise the accumulation of soluble arsenic in mineral leachates.

2. Materials and methods

2.1. Iron Mask

The Iron Mask deposit is located in the Hart Township in Ontario, Canada, some 45 km northwest of the city of Sudbury (approx. Centred on 46° 42′ north, 81° 38′ west, UTM). The deposit lies in part of the south-western geological continuation of the Cobalt district of Ontario, which itself produced more than 420 million ounces of silver, with cobalt as a by-product, from As-rich ores. In the Cobalt district, significant Co−Ag mineralisation is related to carbonate-bearing structures developed close to the margin of the regionally extensive Nipissing intrusive body (Andrews et al., 1986). Iron Mask is located at the geological contact between the Nipissing gabbro sill and calcareous Huronian sediments, extensively developed at the former mine site. The calcareous rocks host disseminated to massive magnetite mineralisation, (hence the name Iron Mask) with Co−Ag arsenide mineralisation restricted to carbonate-filled fractures developed in the magnetite-bearing, altered calc-silicate sediments. Diabase from abandoned shafts recorded grades of up to 15 wt% cobalt and 255 g t^{-1} silver (unpublished company reports), and limited production of silver-bearing ore has been recorded from the property. In September 2017, fresh samples of the host diabase were collected from the shaft area where 6 tons of Co−Ag ore had been recovered historically, as the bulk representative sample for bioleaching. The sample was crushed and dry-milled by ALS Laboratories (Loughrea, Ireland) with the final product recording more than 86% of particles < 75 μm.

2.2. Bou Azzer

The Bou Azzer district is in the Ourzazate Province of Morocco (roughly centred on 31° 3.5′ north and 7° 24′ west (UTM), and 120 km south of the town of Ourzazate) and forms a famous mining region where more than 60 arsenic-rich cobalt-bearing ore bodies are recorded and from where more than 2300 t of cobalt is produced annually. Mineralisation in the district is dominated by Co-Ni-Fe-arsenides with minor sulfoarsenides, sulfides and gold hosted in a quartz-carbonate gangue (Leblanc and Billaud, 1982). For the present study, a sample of run-of-mill concentrate blended from several ore bodies was provided by Managem in late 2017 for study. The concentrate provided was further milled by Grinding Solutions (Cornwall, UK) to pass a mesh size of < 100 μm prior to bioleaching tests.

2.3. Analysis of ore/concentrate and bioleached residues

Samples were analysed for a suite of 53 elements by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and inductively coupled plasma atomic mass spectrometry (ICP-MS) at the ALS Laboratories. For the following elements, samples were prepared by lithium borate fusion and analysed by ICP-MS: Ba, Ce, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hf, Ho, La, Lu, Nb, Nd, Pr, Rb, Sm, Sn, Sr, Ta, Tb, Th, Tm, U, V, W, Y, Yb and Zr. Samples were prepared by acid digestion and analysed with ICP-AES for the remaining elements: Si, Al, Fe, Ca, Mg, Na, K, Cr, Ti, Mn, P and Ba (major elements), and Ag, As, Cd, Co, Cu, Li, Mo, Ni, Pb, Sc, Tl, Zn (minor elements).

X-ray powder diffraction (XRD) patterns were collected with a PANalytical X'Pert Pro α1 MPD diffractometer equipped with an Xcelerator solid-state detector and using Co Kα radiation. Data were recorded in continuous mode over 4–90 degrees 2Θ with 0.017^0 step size and 175 s counting time per step. Samples were powdered using a mortar and pestle. The powders were packed into an aluminium sample deep well using a back loading method with portions of loose powder pressed firmly to achieve good packing density, a smooth surface and a random orientation of grains. Phase identifications were performed by pattern matching using the Powder Diffraction File (PDF) database of the International Centre for Diffraction Data (ICDD) and standard material from the mineral collection at the Natural History Museum. Manual pattern matching using the STOE WinXPOW software suite was supported by both manual identifications and automated pattern matching routines within the X'Pert HighScore Plus software suite. For scorodite identification reference pattern ICDD 037–0468 was used along with specimen BM1980,32.

Quantitative X-ray microanalysis was performed using the Oxford Instruments INCA XMax Energy Dispersive Spectrometer (EDS) on the Zeiss EVO 15LS scanning electron microscope (SEM). Objective lens to specimen working distance was kept constant at 10 mm (fixed focus). The electron beam accelerating voltage was 20 kV, and electron beam current 1.5 nA. Quant optimisation was performed on cobalt metal, typically every 3 h. The accuracy of EDX analysis was checked regularly at each session by collecting spectra and quantifying elemental concentrations in the reference sample of Kakanui augite (Jarosevich et al., 1980). Beam current was regularly checked throughout the analysis session. Point spectra and large area EDX maps were collected on the carbon coated polished block samples. Data were processed using the Oxford Instruments AzTech software. FTIR spectra were collected on a Perkin Elmer Spectrum One spectrophotometer. Scan conditions were set as follows: scan range: 200–4000 cm^{-1}, 32 scans per spectrum, scan resolution 4 cm^{-1}. Samples were prepared by mixing 190 mg of FTIR-grade KBr with a few mg of a sample and pressing into a pellet using hydraulic press.

2.4. Bioleaching tests: Iron Mask ore

Two shake flask experiments were carried out initially to test the feasibility of bioleaching the Iron Mask ore using a consortium of mesophilic and acidophilic bacteria and archaea (experiment 1). In the first of these, 1 g of non-sterile ore was added to 100 mL of acidophile basal salts/trace elements solution (Ñancucheo et al., 2016), inoculated with the microbial consortium described below, and incubated (shaken at 100 rpm) at 35 °C. Mineral leaching was evident within a short time by the change in colouration of the culture (from black to orange) and increased concentrations of soluble metals, but the increase in pH caused by the dissolution of basic minerals such as calcium carbonate in the ore caused the pH to increase to above 3. To obviate this problem, a second shake flask experiment was set up where 2% (w/v) ZVS was added to each shake flask, and the ore density increased to 5% (w/v). The objective here was to neutralise the alkalinity produced via the microbial oxidation of ZVS to sulfuric acid (an application of "sulfur-enhanced bioleaching"; Johnson, 2018). Flasks were incubated for up to 21 days, with samples being withdrawn regularly to determine pH, redox potentials (as E_h values) and concentrations of soluble cobalt, iron and arsenic. At the end of the experiment, the solid residue was removed, dried and analysed for residual minerals.

Following this, Iron Mask ore was leached in two 1.5 L (working volume) bioreactors (Electrolab, UK) operated under the same conditions, but for different lengths of time (experiment 2). The concentrations of the ore and ZVS were increased to 5% and 3% (*w/v*), respectively, and the bioreactors were stirred at 180 rpm and gassed with ~1 L sterile air min^{-1}. The pH of the leach liquor was allowed to decline to a minimum of 1.3 by automated addition of sterile 1 M NaOH, and the bioreactors were maintained at 35 °C. The first experiment lasted 18 days, and the second for 28 days. The bioleached solids from the second experiment were analysed to identify residual minerals.

2.5. Bioleaching tests: Bou Azzer concentrate

Tests to bioleach cobalt from the Bou Azzer concentrate were also first carried out in 250 mL shake flasks (with 1% ore, w/v) in 100 mL acidophile basal salts/trace elements solution, supplemented with 5 mM ferrous sulfate and 0.1% ZVS (experiment 3). Flasks were inoculated with the microbial consortium described below, and shaken at 30 °C. After 20 days, viable bacteria were present in the leach liquors (detected by plating onto selective solid media, described below) and concentrations of soluble cobalt and iron were both found to have increased. A leaching trial was then carried out in a 2 L bioreactor (1 L working volume), as described above but containing 1% (*w/v*) mineral ore, 0.5% ZVS and 5 mM ferrous sulfate (experiment 4). The initial pH of the bioreactor was set at 1.8; this was allowed to fall to 1.5 and maintained at this value by automated addition of 1 M NaOH. The bioreactor was operated at 35 °C, and was stirred and aerated as described above. The inoculum used was a mixture of both the original consortium and some of the leachate from the shake flask experiment. Samples were removed periodically to measure redox potentials and to determine concentrations of soluble cobalt, iron and arsenic, and the mineral residue at the end of the experiment removed for analysis. A second shake flask experiment, similar to the first but with the addition of 5% (w/v) sterile pyrite (Strem Chemicals, Cambridge, UK), was subsequently set up in an attempt to remove some of the arsenic liberated from the concentrate as a secondary mineral product, scorodite ($FeAsO_4.2H_2O$; experiment 5). In this case, an active bioleaching population was first established in culture containing acidophile basal salts/trace elements, 5% (w/v) sterile pyrite, 5 mM ferrous sulfate, and after 5 days non-sterile Bou Azzer concentrate was added to this (at 1%, w/v). The composition of the microbial community was determined several hours after the addition of the concentrate, and again 16 days later. Samples were removed periodically to measure redox potentials and to determine concentrations of soluble cobalt, iron and arsenic, and the solid material present at the end of the experiment was again analysed.

2.6. Microbiological consortium and analysis of microbial populations

A "top down" approach in terms of the design and application of the microbial consortium (Rawlings and Johnson, 2007) was used to bioleach the cobalt ore and concentrate. For this, a mixed population of iron- and sulfur-oxidising mesophilic and thermo-tolerant prokaryotes was pre-grown in acidophile basal salts/trace elements solution supplemented with 5 mM ferrous sulfate,1% (w/v) pyrite and 0.5% (w/v) ZVS. The species and strains used were: *At. ferrooxidans*^{T}, *At. ferridurans*^{T}, *At. ferriphilus*^{T}, *At. ferrivorans* strain CF27, *At. caldus*^{T}, *At. thiooxidans* DSM103717, *L. ferriphilum* strain MT63, *L. ferrooxidans*^{T}, *Sb. thermosulfidooxidans*^{T}, *Sb. acidophilus* strain BOR3, *Ferroplasma acidiphilium* strain BRGM4 and *Acidibacillus sulfuroxidans*^{T}, all of which were sourced from the *Acidophile Culture Collection* maintained at Bangor University.

Analysis of the microbial community present at the end of the experiment 2, where which Iron Mask concentrate was bioleached in bioreactors, was carried out by metagenomic sequencing of amplified 16S rRNA genes by Illumina MiSeq™. PCR amplification of 16S rRNA genes was performed using the following conditions: 30 cycles of denaturation at 95 °C for 45 s; primer annealing at 50 °C, 1 min; DNA synthesis at 72 °C, 30 s, followed by final incubation for 5 min at 72 °C. Purification of PCR products was carried out using Cleanup Mini kits (Qiagen, UK). The quality of the final libraries was assessed using the electrophoresis in agarose gel. PCR products were cleaned up, quantified and the 16S rRNA genes were sequenced using the Illumina MiSeq™ platform (Illumina, San Diego, USA) targeting the V4 hyper variable region for 2× 250-bp paired-end sequencing (Fadrosh et al., 2014), and analysed as described by Korzhenkov et al. (2019). In the case of Bou Azzer concentrate (shake flask and bioreactor experiments) leachate samples were inoculated onto overlay solid media designed to facilitate the growth of all of the acidophilic prokaryotes included in the initial consortium (Johnson and Hallberg, 2007). Colonies were differentiated in terms of their coloration (e.g. whether they were ferric iron-stained, or not) and morphologies, the ratios of different colony types enumerated and their identities confirmed by analysis of their 16S rRNA genes (Falagán and Johnson, 2014).

2.7. The effect of pH on redox potentials of sulfate-rich As (III)/As (V) liquors

Stock solutions of sodium arsenite and sodium arsenate were prepared separately and mixed to give 50 mM concentrations of each. The pH value and redox potential (E_h value) of the solution was recorded, and its pH sequentially lowered (ultimately to 1.37) by dropwise addition of 25% (v/v) sulfuric acid, each time again recording pH and E_h values.

2.8. Miscellaneous analyses

Filtered samples were analysed for pH, redox potential, concentrations of transition metals and arsenic. pH values were measured using a pHase combination glass electrode (VWR, UK) and redox potentials measurements using a platinum/silver-silver chloride electrode (Thermo Scientific, UK) and were adjusted to be relative to a standard hydrogen electrode (i.e. E_h values). Both electrodes were coupled to an Accumet 50 pH meter. Concentrations of soluble iron were determined using the Ferrozine assay (Stookey, 1970). Soluble transition metals and arsenic were measured using SpectrAA Duo atomic absorption spectrophotometer (Varian, UK) and induction coupled plasma atomic emission spectroscopy (ICP-AES; Thermo iCAP 6500 Dual view Plasma, operating with the following conditions: power: 1150 W, neb gas flow: 0.7 L min^{-1}, cool gas 14 L min^{-1}, aux gas 1.0 L min^{-1}; 3 repeats per

Table 1
Elemental compositions of the cobalt ore and concentrate used in experiments (all shown as g kg^{-1}).

	Iron Mask Ore	Bou Azzer Concentrate
Co	14.85	91.9
As	21.4	598
S	13.6	8.1
Si	208	23.6
Fe	133	84.3
Al	37.3	5.8
Ca	97.2	12.1
Mg	35.8	6.3
Mn	2.0	0.285
Ni	0.123	17.5
Mo	0.011	1.25
Cu	0.242	1.9
Zn	0.061	0.41
Cr	0.07	0.95

sample).

3. Results

3.1. Elemental and mineralogical compositions of the ore and concentrate used in experiments

The elemental compositions of the Iron Mask ore and Bou Azzer concentrate are shown in Table 1. As expected the relative amounts of both cobalt and arsenic were far higher in the concentrate. The Iron Mask ore contained greater amounts of calcium, magnesium, iron, silicon, aluminium and manganese than the Bou Azzer concentrate, though markedly less nickel, copper and zinc. Quartz, clinochlore, calcite, albite and calcic amphiboles were identified as the most abundant minerals from XRD analysis, reflecting the silica-rich nature of this sample confirmed by the chemical analysis (Table 1). Cobaltite was identified with both XRD and SEM-EDX as the major mineral within which cobalt and arsenic were deported (Supplementary Fig. S1). Although the Iron Mask ore contained ~13 wt% iron, no primary Fe-rich phase was identified with XRD, while SEM-EDX studies revealed that iron was associated with silicates and cobaltite. In addition, traces of pyrite and Fe oxyhydroxide grains were also identified by SEM-EDX that were below the detection limits of XRD.

Cobalt in the Bou Azzer concentrate was deported chiefly in skutterudite, and loellingite ($FeAs_2$) was identified as an additional arsenide mineral (Supplementary Figs. S1 and S2). XRD analysis identified skutterudite as the most abundant phase in this sample, and also minor silicate phases (in agreement with bulk chemical analysis which found a relatively small amount of silicon in this sample) consisting of clinochlore and quartz. Cobaltite and bismuth arsenide grains were detected by SEM-EDX analysis, but both in very low abundance. SEM-EDX also revealed that nickel in this sample was associated primarily with skutterudite, and some grains of nickel skutterudite were also identified but in very low abundance. In addition, some grains of calcite and occasionally within fractures in a few skutterudite grains, traces of a naturally occurring Co- and Ni-rich mixed-metal arsenate were also identified with SEM-EDX (Supplementary Fig. S5). Analysis of the mixed-metal (Ca,Co,Ni,Fe) arsenate showed it comprised 3.8 wt% Ca, 9.1 wt% Co, 5.6 wt% Ni and 3.1 wt% Fe with 27.3 wt% As, suggesting that it is a phase similar to cobaltlotharmeyerite. Calcite and the mixed metal arsenate were both present in abundances < < 1 wt% and below the detection limit of XRD. No scorodite was identified by SEM-EDX or XRD in the Bou Azzer concentrate.

3.2. Bioleaching of Iron Mask ore

Data from experiment 1, where Iron Mask ore was bioleached in shake flasks, are shown in Fig. 1. The replicate flasks showed some differences in pH values (possibly due to slight differences in amounts of calcite present) though these tended to become more similar with time, presumably as a consequence of biogenic sulfuric acid production. Corresponding differences were seen also with concentrations of iron and arsenic, both of which were lower in the flask that had higher pH, though redox potentials were more similar in both vessels, and rapidly became highly oxidising, reflecting the dominance of ferric over ferrous iron. Concentrations of soluble cobalt were also quite similar in the replicate flasks (as were those of copper and nickel; data not shown). The mean amounts of cobalt and iron solubilised in this experiment were 50% and 4%, respectively.

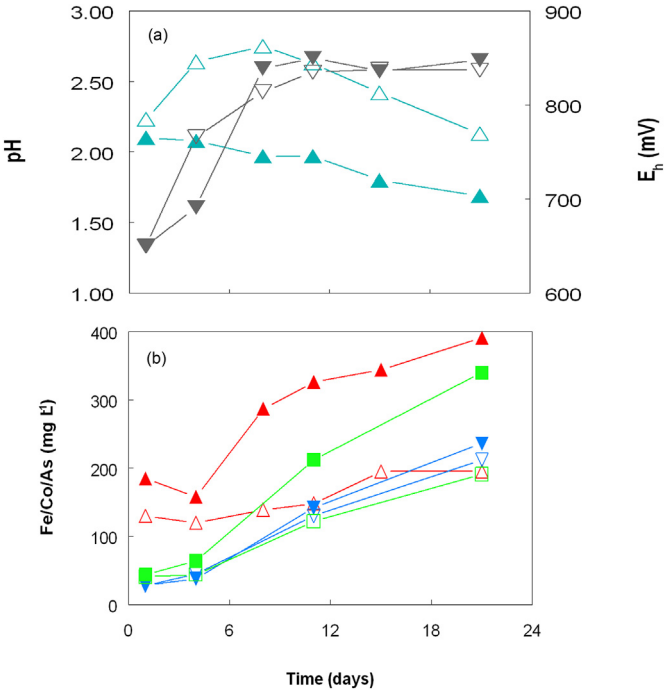


Fig. 1. Changes in (a) pH (▲, Δ) redox potentials (▼, ∇) and (b) concentrations of soluble cobalt (▼, ∇), iron (▲, Δ) and arsenic (■, □) during the bioleaching of Iron Mask ore in replicate shake flasks.

Data from experiment 2, where Iron Mask ore was bioleached in bioreactors, are shown in Fig. 2. Again, there were quite large differences between pH values and concentrations of iron and arsenic solubilised in the replicate reactors, though cobalt levels were more similar. Redox potentials again increased rapidly and remained above +800 mV for most of the experiment. The mean amounts of cobalt and iron solubilised by the end of 18 days of bio-processing were 58% and 8.3%, respectively. In experiment 2, 93% and 56% of the cobalt and 16% and 14% of the iron in Iron Mask ore had been solubilised in the replicate bioreactors after 45 days (Supplementary Fig. S3).

3.3. Bioleaching of Bou Azzer concentrate

Cobalt, iron and arsenic were also successfully bioleached from the Bou Azzer concentrate, in both bioreactor cultures and shake flask (Figs. 3 and 4). The maximum concentration of arsenic (2576 mg L^{-1}) found in the bioreactor leachate was ~7× greater than that found with the corresponding bioreactor containing Iron Mask ore, and the peak concentration of this metalloid (3876 mg L^{-1}) was even greater in shake flask experiment 5. Concentrations of both cobalt and iron were also greater in Bou Azzer leachates (and more so in the shake flask leachate) though differences were less pronounced in bioreactor leachates (Figs. 2b and 3b). When the Bou Azzer concentrate was bioleached in the bioreactor (experiment 4), 49% of the cobalt, 43%

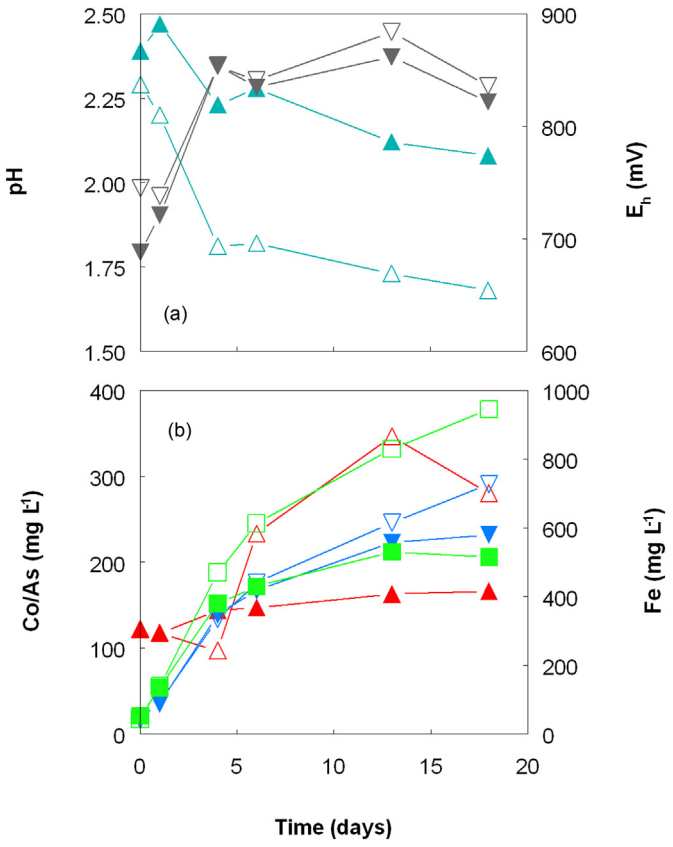


Fig. 2. Changes in (a) pH (▲, Δ) redox potentials (▼, ∇) and (b) concentrations of soluble cobalt (▼, ∇), iron (▲, Δ) and arsenic (■, □) during the bioleaching of Iron Mask ore in replicate bioreactors (experiment 1).

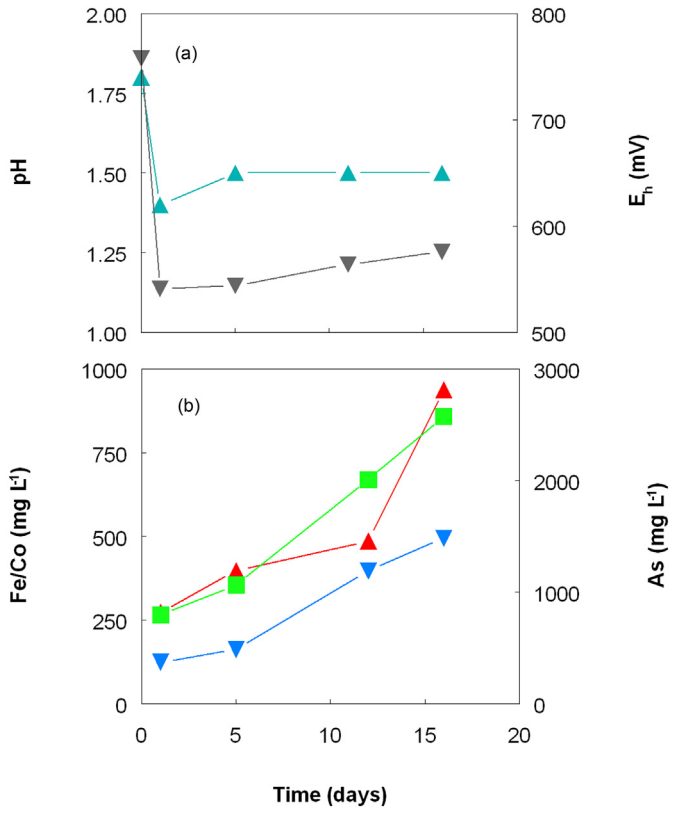


Fig. 3. Changes in (a) pH (▲) redox potentials (▼ and (b) concentrations of soluble cobalt (▼), iron (▲) and arsenic (■) during the bioleaching of Bou Azzer concentrate in a replicate bioreactors.

arsenic and 86% iron in the concentrate was solubilised after 16 days. Interestingly, 99% of the nickel, which was also mostly deported in skutterudite, was bioleached from the concentrate (data not shown). A greater amount of arsenic (> 61%) was bioleached in shake flasks, but since concentrations of both soluble arsenic and iron declined after day 13, it is not possible to evaluate accurately how much in total was bioleached from the concentrate (and in the case of iron, some would also have derived from pyrite dissolution). A notable difference between the chemistries of the Iron Mask and Bou Azzer leach liquors, in both flasks and bioreactors, was in their redox potentials. With the latter, these declined rapidly (to E_h values < 550 mV) following the onset of mineral dissolution and remained well below +600 mV for the duration of both bioreactor and shake flask experiments (Figs. 3a and 4a). Analysis of soluble iron speciation confirmed that this corresponded to the dominance of ferrous over ferric in both cases.

3.4. Mineralogical analysis of bioleached mineral residues

Data from XRD analysis of Iron Mask ore bioleached in both shake flasks and bioreactors are shown in Supplementary Fig. S1. The main difference observed between these and the non-processed ore was the presence of large amounts of gypsum ($CaSO_4.2H_2O$) that accumulated in the former as a consequence of acid dissolution of calcite and precipitation of calcium as gypsum due to the presence of elevated concentrations of soluble sulfate. Notably, there was no evidence from XRD, SEM-EDX or FTIR that scorodite or any other arsenates had formed as a secondary mineral in these experiments. SEM-EDX analysis revealed that cobaltite grains were solubilised during bioleaching as their abundance in the leached sample was visibly lower than in the bulk sample (Supplementary Fig. S4). Corresponding data for the Bou Azzer concentrate are shown in Supplementary Fig. S1, Fig. 5 and Fig. 6. Comparison of XRD profiles for this material showed that there were additional XRD peaks at 18.37, 20.53, 23.10, 27.24, 32.72, 34.02, 34.73, 33.23, 38.57, 43.37, 47.72, 55.69, 66.40 degrees 2Theta (Co Kα radiation) present in the residue leached in the second shake flask experiment which were not found in either the fresh concentrate or that leached in the bioreactor (Fig. 6). These corresponded to pyrite, identified with a reference file 042–1340 from the ICDD database (which was included only in the second shake flask experiment) and to the ferric arsenate mineral scorodite, identified with a reference file 037–0468 from the ICDD database. Neither pyrite nor scorodite were identified by XRD in the original Bou Azzer concentrate. The presence of scorodite in this sample was also supported by FTIR analysis. In particular, the following combined suite of absorption bands identified in the FTIR spectrum of the leached sample, can be ascribed to scorodite: sharp peak at 3519 due to O—H stretching, a broad peak centred at 3026 cm^{-1} due to H_2O molecules stretching, a sharp peak at 1621 cm^{-1} due to bending vibration of H_2O molecules, sharp peaks at 823 cm^{-1} 424 and 463 all due to the As—O stretching vibration. The corresponding bands observed in the reference scorodite sample analysed alongside the leached ore sample are shown in Fig. 5. The positions of scorodite characteristic absorption bands as found in this work compare very well with data reported in the literature for both natural (Ondrus et al., 1999) and biogenic scorodite (Gonzalez-Contreras et al., 2010).

To support the XRD and FTIR analyses and ensure that the newly formed scorodite was not linked to the traces of naturally occurring mixed-metal arsenate present in the ore concentrate, the bioleached residue was also examined with SEM-EDX. The presence of the newly precipitated scorodite in the bioleached Bou Azzer sample was confirmed by SEM-EDX studies where the morphology and chemistry of these precipitates was investigated. Examples of the biogenic scorodite precipitates are shown in Fig. 7 and Supplementary Fig. S5, where it

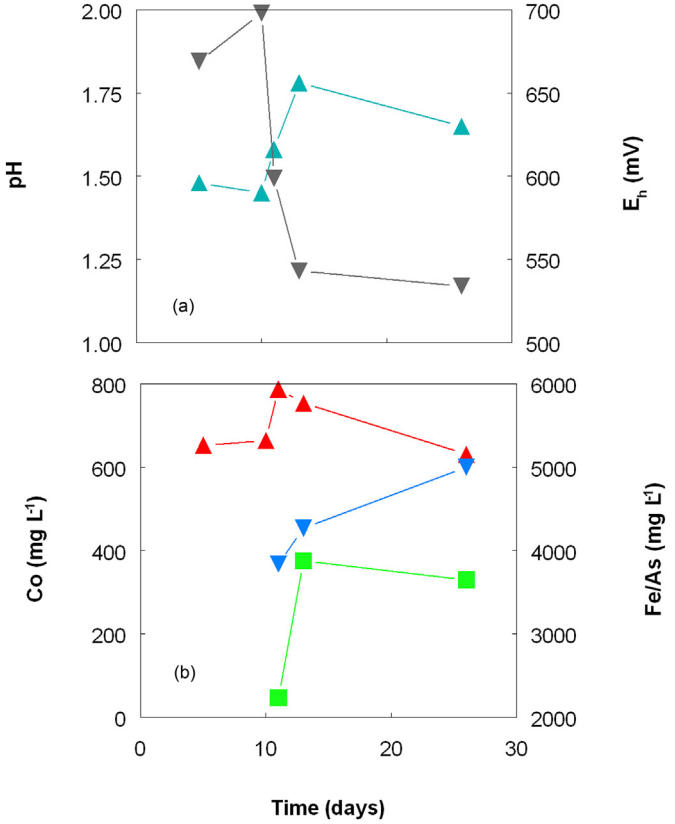


Fig. 4. Changes in (a) pH (▲) redox potentials (▼) and (b) concentrations of soluble cobalt (▼), iron (▲) and arsenic (■) during the bioleaching of Bou Azzer concentrate, supplemented with pyrite, in a shake flask culture.

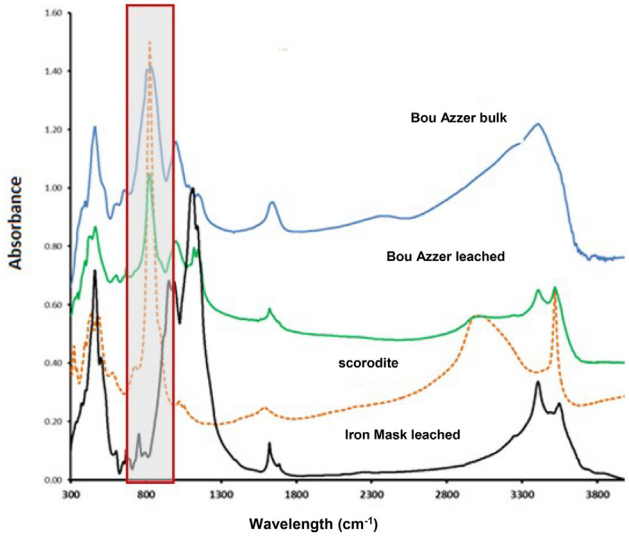
can be seen that the biogenic scorodite is very abundant and its morphology completely different from that of the traces of mixed-metal arsenate. SEM-EDX analysis of the precipitates found that the molar ratio of iron to arsenic varied from 0.70 to 1.04 (average of 0.93 based on 122 measurements) and an average iron content of 20 wt% and arsenic content of 28.94 wt%. This compares well with data previously reported on biogenic scorodite with Fe/As molar ratio varying 0.98–1.0 and natural scorodite 1.00 (Gonzales Contreras et al., 2010). The biogenic scorodite in this study also contained aluminium (1.20 wt%), silicon (0.94 wt%) and cobalt (0.61 wt%), and its chemistry was very different to that of the traces of mixed-metal arsenate in the Bou Azzer concentrate (Section 3.1) with no Ni or Ca detected and ~ 95% less Co.

3.5. Microbial communities identified in the bioleaching of Iron Mask ore and Bou Azzer concentrate

As is usually the case with a "top down" approach for bioleaching minerals, the microbial consortia that became established contained relatively few dominant microbial species with both the ore and concentrate samples. For the Iron Mask ore at the end of bioleaching (bioreactor experiment 2), biomolecular analysis indicated that the community was composed of 66% *Acidithiobacillus*, 13% *Ferroplasma*, 10% *Sulfobacillus*, 10% *Acidimicrobiaceae* and 1.5% *Thermogymnomonas*. The method used in this case was not able to identify microorganisms beyond the genus level. Analysis of viable prokaryotes in the case of Bou Azzer concentrate indicated that only two species (*At. thiooxidans* and *Sb. thermosulfidooxidans*) were present in significant numbers in the bioreactor culture after 16 days of bioleaching. In the second shake flask experiment, the microbial consortium analysed soon after addition of the concentrate contained, at this time, a more complex microbial community that included *At. ferridurans* and *L. ferriphilum* in addition to *At. thiooxidans* and *Sb. thermosulfidooxidans* (in the ratio 14 *Sb. thermosulfidooxidans*: 10 *At. thioxidans*: 5 *At. ferridurans*: 1 *L. ferriphilum*). Sixteen days after addition of the concentrate, only two acidophiles were again detected, *At. thiooxidans* and *Sb. thermosulfidooxidans*, in the ratio 5:1.

4. Discussion and conclusions

Cobalt arsenide (and arseno-sulfide) ores represent potentially significant reserves of this critical metal which, with the exception of the Bou Azzer mine, have been largely unexploited. Atmospheric pollution resulting from roasting As-rich ores and concentrates presents potentially serious health risks to people and animals living in the vicinity of a smelter. In contrast, (bio)hydrometallurgical processing allows the arsenic liberated from minerals to be retained in solution, facilitating its downstream removal in secondary relatively inert minerals such as scorodite. The primary aim of the current research was to demonstrate



Comparison of IR spectra of scorodite and Bou Azzer bioleached sample

Scorodite v [cm^{-1}]	Bou Azzer leached v [cm^{-1}]	Assignment
3518	3519	OH stretching
2993	3026	H_2O stretching
1588	1621	H_2O bending
1050	---	PO_4 3- stretching
1020	---	PO_4 3- stretching
824	823	AsO_4 stretching
578	---	Fe OH_2 vibration
470	463	AsO_4 stretching
438	424	AsO_4 stretching

Fig. 5. FTIR spectra collected from the Bou Azzer, Iron Mask and reference scorodite sample (NHM mineral collection BM 1980.32). Position for the diagnostic IR vibration for arsenate highlighted on the graph (a sharp peak at 824 cm^{-1} due to As—O stretching vibration). Other diagnostic peaks for scorodite are also present in the spectrum from the bioleached Bou Azzer concentrate.

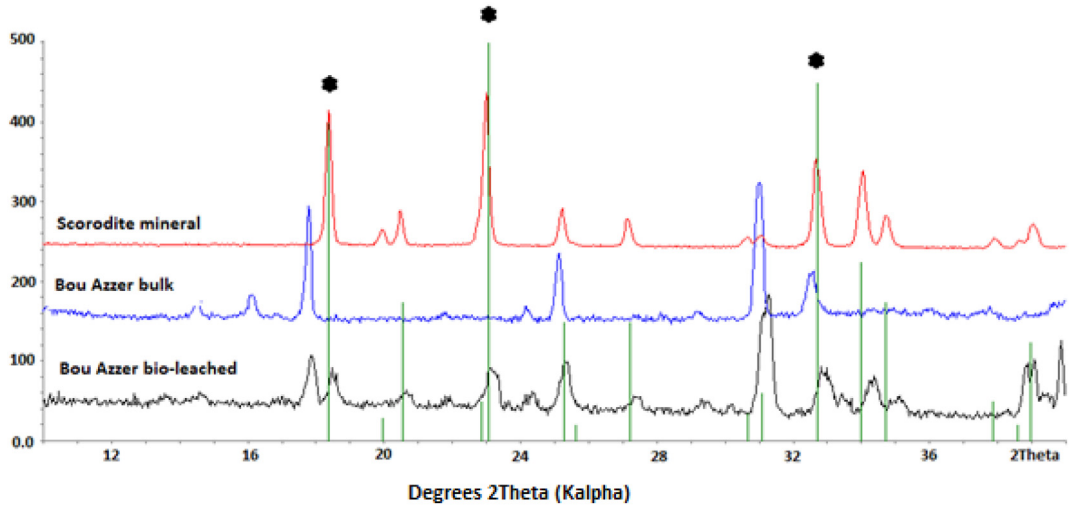


Fig. 6. XRD identification of scorodite in the Bou Azzer bioleached sample. The three most intense peaks of scorodite are labelled with asterisks. There are no scorodite XRD peaks in the pattern from the Bou Azzer bulk concentrate, but substantial amounts of scorodite is evident in the pattern from the bioleached Bou Azzer sample. The scorodite mineral standard is from the NHM mineral collection (BM 1980,32).

that a cobalt arseno-sulfide ore and a cobalt arsenide concentrate were both amenable to bio-processing, using known species of acidophilic microorganisms. Having accomplished this, an attempt was made to find whether biomineralisation and mineral dissolution could be induced to occur simultaneously during bio-processing of the arsenide concentrate, causing some, at least of the soluble arsenic generated to be re-precipitated as scorodite (Filippou and Demopoulos, 1997). Evidence was obtained from various analytical methods including XRD, FTIR and SEM-EDX that this was indeed the case.

Cobalt was more readily bioleached than iron from the Iron Mask ore, which is an important observation when considering the requirement for iron removal in downstream processing. The percentages of arsenic leached along with the cobalt in bioreactors (experiment 2) were 19.5% and 35.5%. The mechanism by which cobaltite was solubilised was not known, but is likely to be primarily due to oxidation by soluble ferric iron which would have been regenerated by iron-oxidising species in the microbial consortium in acidic liquors. Though acid dissolution alone may also have contributed to this, the situation is likely to be analogous to that of acid-soluble sulfide minerals which are also more rapidly leached in the presence of ferric iron (Vera et al., 2013).

The presence of significant amounts of calcite and other basic minerals in this ore lead to acid consumption during bioleaching. Biogenic sulfuric acid, formed from aerobic microbial oxidation of ZVS, was, however, able to satisfy this demand. The elevated redox potentials recorded during bioleaching of Iron Mask ore reflected the efficient microbial oxidation of ferrous iron, and the highly positive values suggest that arsenic (V) was likely to have been the dominant form in solution. The microbial population identified at the end of the second bioreactor experiment included some known iron-oxidisers from the initial microbial consortium (e.g. *Ferroplasma* and *Sulfobacillus*; it was not possible to infer whether the dominant *Acidithiobacillus* spp. were iron/sulfur- or just sulfur-oxidisers) but also others (*Thermogymnomonas* and *Acidimicrobiaceae*) that were not included in the original inoculum and which were possibly introduced in the non-sterile ore.

The Bou Azzer concentrate was very different to the Iron Mask ore, both on terms of its mineralogy (containing arsenides, rather than arseno-sulfides) and elemental composition, notably its content of about 60% (by weight) arsenic. However, this material was also successfully bioleached, though a much lower mineral ore concentration was used than with Iron Mask ore. The disparity between the percentage of nickel (~99%) and cobalt (~49%) solubilised suggests that skutterudite that was more enriched with nickel was also more amenable to bioleaching. Most of the iron in the concentrate was also solubilised in bioreactor cultures, which contrasted with the Iron Mask ore and seemingly reflected the greater susceptibility of the iron arsenide mineral loellingite to oxidative dissolution at low pH. Only the same two species of bacteria originally present in the initial microbial consortium were found as viable microorganisms by the end of the bioleaching tests of Bou Azzer concentrate in both shake flask and bioreactor tests. The fact that the iron-oxidising bacteria *L. ferriphilum* and *At. ferridurans* were able to survive for only a short period following addition of the concentrate to the shake flask, probably reflects the greater sensitivity of these prokaryotes to arsenic in general (and arsenic (III) in particular) than *At. thiooxidans* and *Sb. thermosulfidooxidans*.

Arsenic chemistry is clearly of great significance in both the mineralogies and leachate chemistries of the experiments carried out, as well as in its toxicity to bioleaching microflora. Arsenic can exist in four different oxidation states, −3, 0, +3 and + 5. Arsenic acid (oxidation state +5) has three dissociation constants (pK_a values of 2.2, 6.9 and 11.5) so that in the extremely acidic leach liquors in all experiments described, most As (V) would be present as non-dissociated arsenic acid (H_3AsO_4), which is a powerful oxidising agent, together with some dihydrogen arsenate ($H_2AsO_4^-$). Arsenous acid (oxidation state +3) also has three dissociation constants (pK_a values of 9.2, 12.1 and 13.4) so that neutral pH and acidic liquors As (III) exists exclusively as the non-dissociated weak acid, $As(OH)_3$. “Arsenite” has frequently, but erroneously, been referred to as being present in acidic leach liquors.

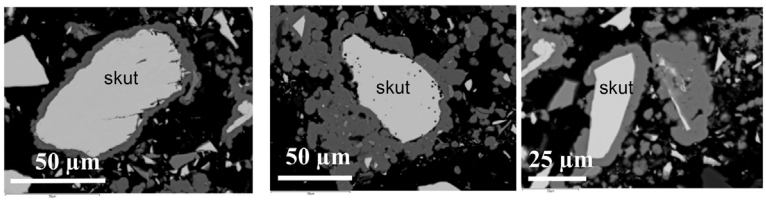


Fig. 7. High resolution BSE images of biogenically formed scorodite precipitated around the surfaces of skutterudite (skut) grains found in the Bou Azzer leached sample.

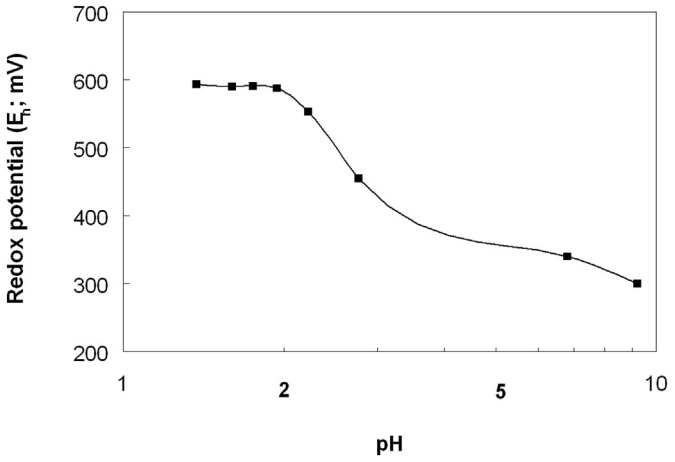


Fig. 8. Effect of pH on the measured standard redox potential (E^0) of a 50 mM arsenic (III)/50 mM arsenic (V) solution, acidified with sulfuric acid.

The redox potential of As (III)/As (V) aqueous system varies with pH because of its effect on speciation of both species, as shown in Pourbaix diagrams of this element. In the current work, the E_h values of As (III)/As (V) liquors, acidified with sulfuric acid, increased from +300 mV at pH 9.2 to +553 mV at pH 2.22. At pH values < 2, they remained fairly constant at ~ +590 mV (Fig. 8).

In both cobaltite and skutterudite, arsenic would have been present in its more reduced (−3 and 0) states, and its solubilisation would require it to be oxidised to either As (III) or As (V). In contrast to Iron Mask leachates, the low redox potential recorded in shake flask and bioreactor leachates of the Bou Azzer concentrate (+530 to +599 mV) imply that most was present as arsenous (As (III)) acid. Using the empirical formula of skutterudite ($Co_{0.75}Ni_{0.25}As_{2.5}$) as an approximate guide, about four ferric irons would be consumed in oxidising each As in the mineral to As (III), and a further two for its further oxidation to As (V). During the course of dissolution of Bou Azzer ore in the bioreactor, increasing concentrations of soluble iron (principally from dissolution of the ferrous iron mineral loellingite) were found to parallel those of cobalt and arsenic, and E_h values increased from +541 mV at day 1 to +576 mV by the end of the experiment. The inference is therefore that ferrous iron oxidation was both ongoing and dynamic, and that the low redox potentials recorded were a consequence of the rapid reduction of ferric iron, both from its attack on reduced minerals (skutterudite and loellingite) and its pyrite-catalysed oxidation of As (III) to As (V). Although concentrations of soluble As (III) and As (V) were not measured directly in these experiments, the ratio and relative amounts of As (III) and As (V) can be estimated from E_h potentials and concentrations of total soluble arsenic, using the Nernst equation. Using this approach, the leach liquor at the end of the bioreactor experiment was estimated to have contained ~1930 mg L^{-1} As (III) (75% of total As) and ~ 645 mg L^{-1} As (V) (25%), while in the second shake flask experiment the figures are ~3610 mg L^{-1} As (III) (99% of total As) and ~ 35 mg L^{-1} As (V) (1%).

The issue of arsenic toxicity is clearly a major consideration in any mineral bio-processing system in which this metalloid is solubilised. Arsenic (III) is known to be far more toxic to bioleaching microorganisms than As (V). Barrett et al. (1993) reported that acidophiles were inhibited by 750–2250 mg L^{-1} As (III), depending on the degree of adaptation, compared to > 3750 As (V), while the BIOX® culture, adapted over a long period to bio-oxidise arsenopyrite-rich refractory gold ore, can apparently tolerate up to 6 g L^{-1} As (III) and 15–20 g L^{-1} As (V) (Dew et al., 1997). Baker-Austin et al. (2007) reported that the iron-oxidising archaeon *Ferroplasma acidarmanus* FER1 grew readily in the presence of 10 g L^{-1} "arsenate", though growth yields were much lower when exposed to 5–10 g L^{-1} of "arsenite".There are relatively few published data of actual concentrations of arsenic in commercial biomining operations, due to issues of confidentiality and sensitivity, though Lee et al. (2011) reported concentrations of > 2000 mg L^{-1} As (V) and < 20–30 mg L^{-1} As (III) in column leachates of an enargite-containing sulfide ore.

Thermodynamic data suggest that As (III) should be oxidised by soluble iron (III) at low pH. However, this can be a relatively slow reaction, due to the activation energy barrier. Bacteria, such as some *Thiomonas* spp. are able to catalyse the oxidation of As (III) to As (V), using As (III) as an electron donor to sustain their growth (Battaglia-Brunet et al., 2006) though these are moderate rather than extreme acidophiles and generally not found in commercial biomining operations. Okibe and Fukano (2019) reported that carbon-fibre could function as an electron-mediator to catalyse abiotic oxidation of As (III) oxidation by Fe (III), generated by moderately thermophilic iron-oxidising bacteria, and thereby promote removal of As (V) as ferric arsenate. Earlier, Barrett et al. (1993) had shown that pyrite (though not arsenopyrite) could provide the catalytic surface for this reaction. Even though solubility product of ferric arsenate is small (log K_{sp} of −20.2 at 25 °C; Monhemius, 1977) scorodite may not crystallise spontaneously at moderately to low temperatures, as was found in the case of Iron Mask leachates, where both ferric iron and As (V) were present in relatively large concentrations. "Seeding" with, for example, hematite or magnetite, may induce its formation (Okibe et al., 2020) though the same authors noted that other mineral "seeds", such as goethite or ferrihydrite, promoted the formation of jarosites rather than scorodite. Jarosites were not detected in mineral residues in the current work either by XRD, SEM-EDX or FTIR. Gonzalez-Contreras et al. (2010) had previously reported that mineral "seeds" were not required to induce scorodite formation in cultures of the thermo-acidophile *Acidianus brierleyi*, when grown at 80 °C and pH 1.0.

The inclusion of pyrite in experiment 5, where Bou Azzer concentrate was again bioleached in shake flasks, was to fulfil three roles: (i) to provide the catalytic surface to promote the oxidation of As (III) to As (V); (ii) to act as a putative "seed" for scorodite crystallisation; (iii) to be a secondary source of iron, since the molar ratios of iron:arsenic in the concentrate itself (0.19:1) was well below that required for effective removal of soluble arsenic as scorodite (1:1). There was clear evidence for (ii) and implied evidence for both (i) and (iii). Concentrations of total soluble iron and arsenic declined greatly after day 11 of this experiment, in contrast to the previous experiments that did not include pyrite, where iron levels increased throughout. Redox potentials also decreased progressively after addition of the concentrate to the flasks, in contrast to the bioreactor experiment where, after an initial sharp fall, they increased somewhat. Both of these observations point to a sink for ferric iron and arsenic that was unique to this experiment. The confirmation that scorodite was present only in bioleached mineral residues from this experiment support the hypothesis that mineral (skutterudite) dissolution was occurring in parallel to biomineralisation (formation of scorodite) in these cultures. A schematic diagram, showing the proposed roles of ferric iron, pyrite and iron-oxidising microorganisms in the transformation of skutterudite to scorodite with concomitant release of soluble cobalt, is shown in Fig. 9.

This work has provided proof of concept that cobalt arseno-sulfide and arsenide ores and concentrates are amenable to bio-processing, using acidophilic prokaryotes, at relatively low temperatures, and also that it is possible to induce concurrent solubilisation of arsenic from primary minerals and immobilisation in a secondary mineral, scorodite. There are many avenues for following up these observations and optimising the process. The question of why scorodite did not appear to be formed during the bioleaching of the Iron Mask ore, even though the combined concentrations of ferric iron and arsenic (V) exceeded the published solubility product of ferric arsenate. This could be due to the absence of a suitable mineral "seed" in these cultures, and it would be interesting if this could be overcome by adding pyrite or hematite. The major bottleneck with the Bou Azzer concentrate would appear to be the oxidation of ferrous iron to ferric. Although one of the two bacteria

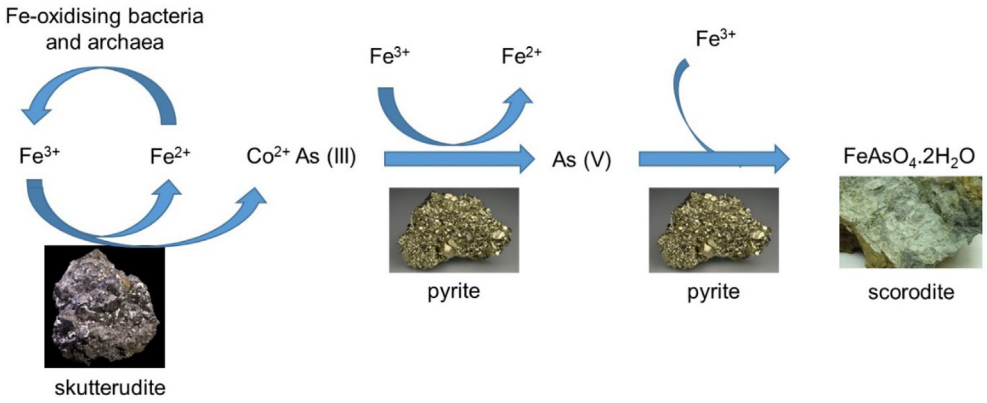


Fig. 9. Schematic representation of the combined bioleaching of skutterudite and biomineralisation of scorodite, showing the roles of ferric iron, pyrite and microorganisms.

that established in these cultures, *Sb. thermosulfidooxidans*, can oxidise ferrous iron, it is generally inferior in this to the more specialised *Leptospirillum* and iron-oxidising *Acidithiobacillus* spp. (Quatrini and Johnson, 2016). One of the challenges would therefore appear to be adapting more proficient iron-oxidising acidophiles to the elevated concentrations of soluble arsenic that they would be exposed to when bioleaching cobalt arseno-sulfides and arsenides. There is precedence for this, as reported for *L. ferriphilum* (Tuffin et al., 2006).

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hydromet.2020.105395.

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