minerals

Review

Arsenic-Microbe-Mineral Interactions in Mining-Affected Environments

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Abstract: The toxic element arsenic (As) occurs widely in solid and liquid mine wastes. Aqueous forms of arsenic are taken up in As-bearing sulfides, arsenides, sulfosalts, oxides, oxyhydroxides, Fe-oxides, -hydroxides, -oxyhydroxides and -sulfates, and Fe-, Ca-Fe- and other arsenates. Although a considerable body of research has demonstrated that microbes play a significant role in the precipitation and dissolution of these As-bearing minerals, and in the alteration of the redox state of As, in natural and simulated mining environments, the molecular-scale mechanisms of these interactions are still not well understood. Further research is required using traditional and novel mineralogical, spectroscopic and microbiological techniques to further advance this field, and to help design remediation schemes.

Keywords: arsenic; prokaryotes; mine; mineral; tailings; contamination; remediation

1. Introduction

Throughout the world, the weathering of solid mine wastes contaminates waters with potentially toxic components, such as arsenic (As), in concentrations that may pose serious hazards to human and ecosystem health. The stability and potential mobility of As in mine wastes is controlled by their uptake in minerals [1,2]. Many of the reactions that result in the dissolution of primary As-bearing minerals, and formation of secondary products, are mediated by prokaryotes (*i.e.*, Bacteria and/or

Archaea). This review describes important As-bearing minerals and As-oxidizing and -reducing prokaryotes in mining environments, discusses how these organisms interact with the minerals, and outlines avenues for further research in this area.

2. Arsenic-Bearing Minerals in Mining-Affected Environments

Arsenic occurs in more than 300 minerals. These include arsenates (*ca.* 60% of the 300 As-bearing minerals), sulfides and sulfosalts (*ca.* 20%), oxides (*ca.* 10%) and arsenates, arsenides, native elements and metal alloys (*ca.* 10%) [1,3]. Examples of As-bearing minerals that occur in mining-affected environments are summarized in Table 1. Native As and As-bearing sulfides are regarded as primary minerals in mining-affected environments, since they are produced by the hydrothermic, mesothermic or diagenetic processes that generate the ores. In addition, their weathering and alteration by ore processing generates secondary oxides and arsenates (Table 1). The most common As-bearing sulfides in mining-affected environments are pyrite and arsenopyrite (Figure 1a) [4–6]. Realgar and orpiment are less common, while enargite and tennantite occur in Cu-rich deposits [7,8], cobaltite occurs in Co-rich deposits [9] and gersdorffite occurs in Ni-rich deposits [10]. The dominant forms of As in these primary phases are As(−I), As(III) and As(V) (Table 1).

Table 1. Examples of As-bearing minerals in mining-affected environments. Where known, speciation is shown for redox-sensitive elements.

Mineral	Formula	Mineral Group
Native arsenic	As	Native metalloid
Arsenopyrite	$Fe^{3+}As^-S^{2-}$	Sulfide
Arsenical pyrite	$Fe^{2+}(S^-,As^-)_2$	Sulfide
Loellingite	$Fe^{2+}As^-_2$	Arsenide
Niccolite	$Ni^{3+}As^{3-}$	Arsenide
Realgar	$As^{3+,1-}S^{2-}$	Sulfide
Orpiment	$As^{3+}_2S^{2-}_3$	Sulfide
Enargite	$Cu_3AsS^{2-}_4$	Sulfosalt
Tennanite	$(Cu,Ag,Fe,Zn)_{12}As_4S^{2-}$	Sulfosalt
Cobaltite	$CoAsS^{2-}$	Sulfosalt
Gersdorffite	$NiAsS^{2-}$	Sulfosalt
Claudetite	$As^{3+}_2O_3$	Oxide
Manganarsite	$Mn_3As^{3+}_2O_4(OH)_4$	Oxyhydroxide
Arseniosiderite	$Ca_2Fe^{3+}_3O_2(As^{5+}O_4)_3 \cdot 3H_2O$	Fe-arsenate
Kankite	$Fe^{3+}As^{5+}O_4 \cdot 3.5H_2O$	Fe-arsenate
Parasymplesite	$Fe^{3+}_3(As^{5+}O_4)_2 \cdot 8H_2O$	Fe-arsenate
Pharmacosiderite	$K[Fe^{3+}_4(OH)_4(As^{5+}O_4)_3] \cdot 6.5H_2O$	Fe-arsenate
Scorodite	$Fe^{3+}As^{5+}O_4 \cdot 2H_2O$	Fe-arsenate
Symplesite	$Fe^{3+}_3(As^{5+}O_4)_2 \cdot 8H_2O$	Fe-arsenate
Tooeleite	$Fe^{3+}_6(As^{3+}O_3)_4(S^{2-}O_4)(OH)_4 \cdot 4H_2O$	Fe sulfoarsenites
Beudantite	$PbFe^{3+}_3(As^{5+}O_4)(S^{2-}O_4)(OH)_6$	Fe sulfoarsenates
Zýkaite	$Fe^{3+}_4(As^{5+}O_4)_3(S^{2-}O_4)(OH) \cdot 15H_2O$	Fe sulfoarsenates
Yukonite	$Ca_7Fe^{3+}_{12}(As^{5+}O_4)_{10}(OH)_{20} \cdot 15H_2O$	Ca-Fe-arsenates
Hörnesite	$Mg_3(As^{5+}O_4)_2 \cdot 8H_2O$	Ca-Mg-arsenates

Table 1. *Cont.*

Mineral	Formula	Mineral Group
Pharmacolite	$Ca(HAs^{5+}O_4)\cdot2H_2O$	Ca-Mg-arsenates
Annabergite	$Ni_3(As^{5+}O_4)_2\cdot8H_2O$	Other metal arsenates
Erythrite	$Co_3(As^{5+}O_4)_2\cdot8H_2O$	Other metal arsenates
Köttigite	$Zn_3(As^{5+}O_4)_2\cdot8H_2O$	Other metal arsenates
Mimetite	$Pb_5(As^{5+}O_4)_3Cl$	Other metal arsenates
As(V)-sorbed hydrous ferric oxide	$Fe^{3+}(OH)_3$	Fe oxides/hydroxides
As(V)-sorbed akaganeite	$\beta\text{-}Fe^{3+}OOH$	Fe oxides/hydroxides
As(V)-sorbed goethite	$\gamma\text{-}Fe^{3+}OOH$	Fe oxides/hydroxides
As(V)-sorbed lepidocrocite	$\alpha\text{-}Fe^{3+}OOH$	Fe oxides/hydroxides
As(III)- & As(V)-sorbed maghemite	$\gamma\text{-}Fe^{3+}_2O_3$	Fe oxides/hydroxides
As(III)- & As(V)-sorbed hematite	$\alpha\text{-}Fe^{3+}_2O_3$	Fe oxides/hydroxides
As(V)-sorbed jarosite, natrojarosite, hydronium jarosite	$(K,Na,H_3O)Fe^{3+}_3(SO_4)_2(OH)_6$	Fe oxyhydroxysulfates

Figure 1. Examples of As-bearing minerals in mine wastes. (**A**) Tailings from the 1998 Aznalcóllar (Spain) tailings dam spill that contain arsenopyrite, described in [5]. (**B**) Microbial biofilm sampled from Giant Mine, Yellowknife, NWT, Canada. The mineral yukonite, a Ca-Fe arsenate, is shown by the reddish-brown coloration. From [11]. And (**C**) Electron microprobe X-ray map of secondary Fe-As-Sb-O phase in solids from Bolivian tailings weathering column experiments. Reprinted with permission from [2], Mineralogical Society of Great Britain & Ireland.
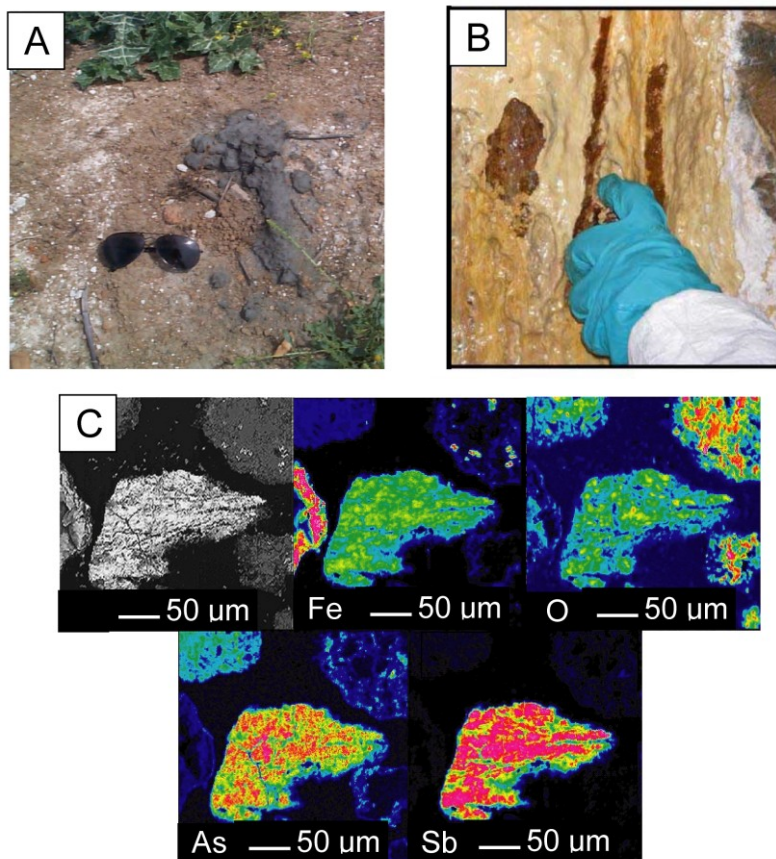
Secondary As-bearing minerals are abundant in mine wastes. These are produced by the natural weathering of primary sulfides, or by metal extraction processes (e.g., roasting, [12,13]). Secondary As-bearing minerals containing structural As include Fe arsenates such as scorodite [2] and kankite [14], Fe sulfoarsenates such as tooeleite [14,15], Ca-Fe arsenates such as yukonite (Figure 1b) and amorphous forms [14,16,17], and Ca-Mg arsenates such as pharmacolite [18]. Arsenic can also be incorporated in trace amounts in a variety of minerals such as Fe, Mn or Al oxides (Figure 1c), hydroxides and sulfates, other hydrous and anhydrous sulfates, phosphates and hydrous silicates [4,5,13,14,19–23]. In these cases, the As is taken up by sorption onto the mineral surface [24], or incorporated into the mineral structure by co-precipitation. The most common of these occurs in mine wastes include Fe oxides and hydroxides (Table 1) [2,5,14,25], and minerals of the jarosite family [4,5,19,25,26]. The dominant form of As in these secondary phases is As(V), but As(III) has been reported in roaster oxide phases [12] (Table 1), in secondary oxide and oxyhydroxide phases (claudetite, manganarsite, Table 1) in an underground gold mine [11] and in products of experiments involving bacteria and acid mine drainage water bacteria-acid mine drainage water experiments [27].

3. Prokaryotes in Arsenic-Bearing, Mining-Affected Environments

The two soluble inorganic forms of arsenic, arsenate (As(V)) and arsenite (As(III)), occur widely in mining-affected environments. Even though these forms of As are highly toxic, some prokaryotes can gain energy from their reduction and oxidation, respectively [28]. Prokaryotes that use As(V) as the terminal electron acceptor in anaerobic respiration are known as arsenate respirers, and they can couple As(V) reduction to the oxidation of both inorganic (e.g., sulfide) and organic (e.g., acetate) electron donors [29]. Other organisms can use As(III) as the electron donor coupled to the reduction of oxygen, nitrate or chlorate [30].

Most prokaryotes that oxidize As(III) or respire As(V) are able to survive and grow in As-contaminated mining environments and many have acquired additional As-resistance mechanisms. The most well studied resistance mechanism is the Ars (arsenic resistance system) [31]. This comprises, the arsenate reductase (ArsC) and the efflux pump (ArsB) which requires energy (as opposed to metabolism mentioned above) in the form of adenosine triphosphate (ATP) and contains an ATPase (ArsA) that hydrolyses the ATP to adenosine diphosphate (ADP). If As(V) enters the cells (most probably through the phosphate transport system) it is reduced to As(III) inside the cell (*i.e.*, in the cytoplasm) and the As(III) is pumped out of the cell through ArsB coupled to ArsA-mediated ATP hydrolysis (Figure 2). If, on the other hand, As(III) enters the cell it can be pumped straight out via ArsB. Some organisms also detoxify inorganic As by methylation. This produces monomethyl arsonate (MMA(V)), methylarsonite (MMA(III)), dimethylarsinate (DMA(V)), dimethylarsenite (DMA(III)) and trimethylarseine oxide (TMAO), as well as volatile arsines (As-III) [31]. Microbial oxidation of arsenite to arsenate is much more rapid than chemical oxidation of arsenite to arsenate [32]. Therefore, rapid arsenite to arsenate oxidation in the environment can be attributed to arsenite-oxidizing bacteria.

Table 2 shows a selection of aerobic arsenite oxidizers belonging to two different sub-phyla of the Bacteria (*Alpha* and *Betaproteobacteria*) isolated from mining environments. These organisms are all mesophiles (grow at moderate temperatures) except for GM1 which is psychrotolerant (grows ≤ 20 °C) and include chemolithoautotrophs (obtain carbon from the fixation of carbon dioxide and energy from

the oxidation of inorganic compounds) and heterotrophs (cannot fix carbon dioxide and instead use organic carbon for making cell material).

Figure 2. Conceptual diagram of the bacterial arsenic resistance mechanism. See text for explanation.
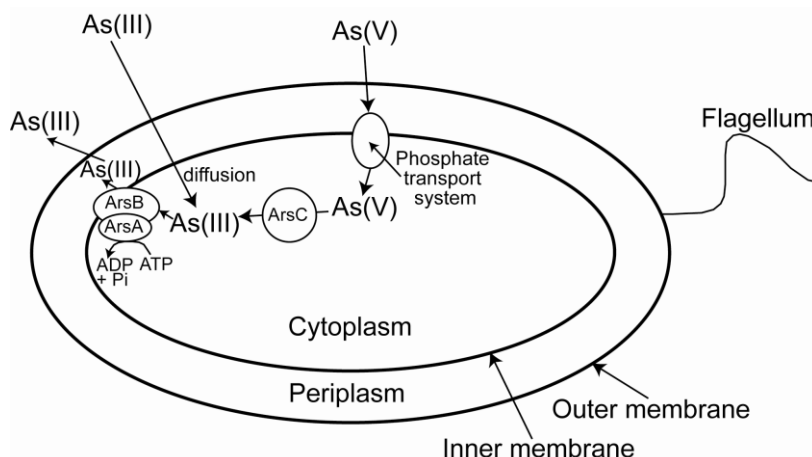

Table 2. Examples of isolated aerobic As(III)-oxidizing bacteria from mining-affected environments. Adapted and expanded from [30]. fac chem.: facultative chemolithoautotrophic; het: heterotrophic; α, β: Alpha-, Betaproteobacteria, respectively.

| Organism	Phylogenetic affiliation	Environment	Metabolism	Reference
Isolated bacteria				
BEN-4	β	As-contaminated mine water	het	[33]
BEN-5	α	As-contaminated mine water	fac chem	[33]
NT-4	α	arsenopyrite from gold mine	het	[33]
NT-10	β	arsenopyrite from gold mine	het	[33]
NT-14	β	arsenopyrite from gold mine	fac chem.	[33]
NT-26	α	arsenopyrite from gold mine	fac chem	[34]
GM1	β	As-contamined biofilm inside gold mine	het	[11]
Leptothrix sp. str. S1.1	β	mine drainage water	het	[35]
Ralstonia sp. str. 22	β	soil from mine site	het	[36]
Sinorhizobium sp. str. M14	α	gold mine waters	het	[37]
Thiomonas arsenivorans	β	gold mine	fac chem	[38]
Thiomonas sp. str. 4As	β	acid mine drainage	fac chem	[39]
Variovorax paradoxus	β	mine drainage water	het	[35]
Acinetobacter junni	γ	mining-affected sediment	het	[40]
Marinobacter sp.	γ	mining-affected sediment	het	[40]

Several anaerobic arsenate-respiring bacteria have also been isolated from arsenic-contaminated mining environments. For example, *Chrysiogenes arsenatis* str. BAL-1[T] was isolated from anoxic mud from a tailings pond in the Ballarat goldfields, Victoria, Australia [41]. *C. arsenatis* couples the reduction of As(V) to a variety of organic electron donors. Two As(V)-respiring bacteria were isolated from arsenic-contaminated anoxic muds from a tailings pond in Bendigo, Victoria, Australia. One of

these organisms, *Desulomicrobium* sp. Ben-RB could concomitantly reduce As(V) and sulfate, coupling the reduction of both to the oxidation of lactate forming As_2S_3 [42]. The second organism, *Bacillus macyae* str. JMM-4 uses As(V) as the terminal electron acceptor and acetate as the electron donor [43,44]. All three As(V) respirers were isolated in the presence of 10 mM arsenate and can probably tolerate much higher concentrations.

The organisms mentioned above gain energy from either the oxidation of arsenite coupled to the reduction of oxygen, nitrate or chlorate or the reduction of arsenate coupled to the oxidation of inorganic or organic electron donors. This means that they produce ATP which is then used for cell division. Two different enzymes (Aio and Arx) are responsible for the oxidation of arsenite to arsenate, depending on whether the organisms use oxygen or nitrate as electron acceptors [30,45,46]. Aio is the most well-characterized and it is located in the periplasm. The arsenite which enters the periplasm is oxidized to arsenate by the Aio and then the electrons are transferred to other proteins involved in the electron transport chain which results in the production of ATP and the reduction of oxygen to water (Figure 3). On the other hand, the respiratory arsenate reductase (Arr), which is located in the periplasm or is attached to the cytoplasmic membrane is the terminal reductase in an electron transport chain and receives the electrons for arsenate reduction from various electron donors which are oxidized in the cytoplasm [29] (Figure 3).

Several organisms found in mining-impacted environments which are involved in the metabolism of other constituents of the minerals (e.g., oxidation of Fe, S, *etc.*) are often resistant to As either via the Ars system or another unknown mechanism, but do not metabolize it. These include iron-oxidizing (e.g., *Acidithiobacillus ferrooxidans*), iron-reducing (e.g., *Shewanella putrefaciens*), and sulfur-oxidizing organisms (e.g., *Acidithiobacillus caldus*) [47–49]. Some species of *Shewanella* have, however, been shown to respire As(V) [50].

Figure 3. Conceptual diagram of aerobic arsenite oxidase. Arsenite enters the periplams and is oxidized to arsenate by Aio. See text for discussion.
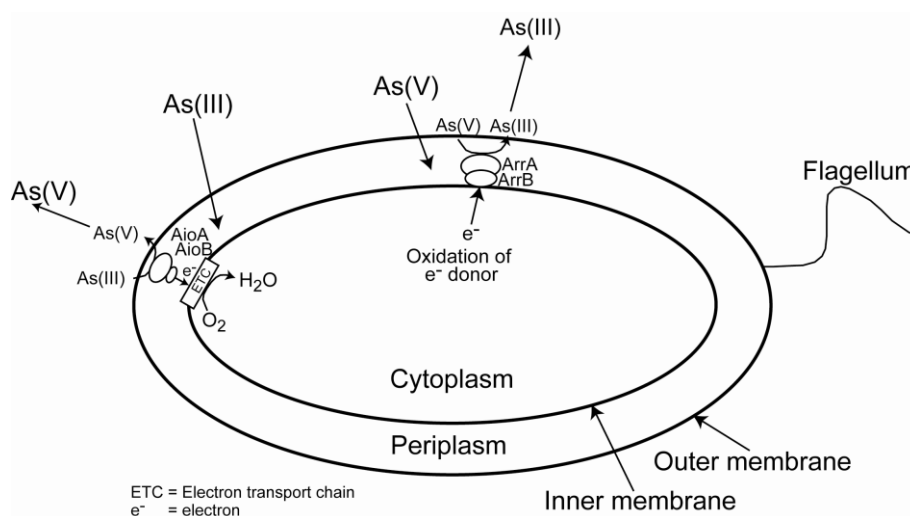
4. Arsenic-Microbe-Mineral Interactions in Mining-Affected Environments

Microbes play a significant role in the precipitation (*i.e.*, biomineralization) and dissolution of As-bearing minerals, and in the alteration of the redox state of As, in natural and simulated mining

environments. For example, the oxidation of Fe(II), contained in sterilized acid mine drainage water containing hundreds of mg of Fe(II) and tens of mg of As [mainly As(III)], by various *Acidithiobacillus ferrooxidans* strains results in the trapping of As(III) by newly precipitated Fe(III) minerals [27]. X-ray Diffraction (XRD) and Extended X-Ray Absorption Fine Structure (EXAFS) spectroscopic analysis showed that tooeleite and As(III)-sorbed schwertmannite are formed in the presence of the bacteria in the acid mine drainage (AMD) water. The nature, proportion and sequence of the biominerals formed were controlled by the type of bacterial strain used and the strain cell numbers. Arsenic-bearing schwertmannite was also found in a biomat formed 0.3 m downstream of discharging arsenic-bearing mine water of pH 4.7 (Figure 4a) [51]. The schwertmannite was characterized by scanning particle induced X-ray emission analysis, XRD and X-ray Absorption Near Edge Structure (XANES), which showed that it contained As(V). Another unnamed Fe-S phase contained As(III). Phylogenetic identification of the organisms by 16S recombinant DNA (rDNA) analysis of the discharged mine water showed that *Gallionella* species were the dominant bacteria present, but it was not shown whether these organisms were directly responsible for the biomineral formation. Benzerara *et al.* [52], using transmission electron microscopy and scanning transmission X-ray microscopy, showed that As(III)- bearing tooeleite and an As(V)-Fe(III) amorphous phase formed extracellularly or within the periplasm of microbial cells in the Carnoulès (France) AMD system (Figure 4b). *Acidithiobacillus ferrooxidans* strain CC1, isolated from acid mine drainage of the former Carnoulès mining site, was shown to oxidize Fe(II), resulting in the formation of poorly ordered schwertmannite that co-precipitated arsenite from the AMD [53]. An As-bearing Fe(III) phase was shown to form on the surfaces of cells of *Acidithiobacillus ferrooxidans* during the bioleaching of realgar [54], but the redox state of the As was not determined (Figure 4c). Various strains of *Acidithiobacillus ferrooxidans* also oxidized Fe(II) to Fe(III) in the presence of high concentrations of dissolved As(III), resulting in the formation of tooeleite [15].

Microbes solubilize minerals directly (through attachment) to secure essential nutrients, or indirectly (without attachment) by changing aqueous solution chemistry so that reactive solutes are produced. Attachment is particularly effective because soluble electron carriers can then diffuse through the periplasm (Figure 2). Mineral dissolution rates can then be increased or decreased due to the production of pH gradients at the mineral surface, or to the improvement of the kinetics of secondary mineral formation [55,56]. Experimental studies have demonstrated that bacteria are closely involved in the oxidative and reductive dissolution of As-bearing mine waste minerals. For example, As-bearing sulfide minerals such as arsenopyrite, enargite, orpiment and realgar can be oxidized by aerobic bacteria such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* (e.g., [47,57–59]). Such bacteria, which typically occur in acid mine drainage environments, are described in detailed reviews by Hallberg and Johnson [60], Baker and Banfield [61], González-Pastor and Mirete [62], Yelton *et al.* [63] and Drewniak and Sklodowska [64]. Islam *et al.* [65] have also demonstrated that native arsenic can be dissolved at alkaline pH (~8) with the assistance of microbes. The involvement of bacteria in the oxidation of arsenopyrite has been comprehensively reviewed by Corkhill and Vaughan [66]. The studies cited in their work demonstrated that oxidation of arsenopyrite by acidophilic Fe- and S-oxidizing bacteria is considerably more effective than abiotic oxidation. For example, the oxidative dissolution of arsenopyrite was shown to be much more rapid and extensive when it occurred in the presence of *Leptospirillum ferrooxidans* than abiotically [59]. This bacterium produced dissolution pits

in the mineral surface (Figure 5a), and As was oxidized more rapidly than S or Fe. The oxidation of arsenopyrite and As-bearing pyrite by *Acidithiobacillus ferrooxidans* can also result in pitting on the mineral surfaces, and of thin coatings of jarosite [67]. The oxidation of orpiment, with the concomitant release of arsenite, can also occur abiotically, but is much more rapid in the presence of *Ferrobacillus ferrooxidans* TM [58]. It should be noted, however, that in these cases the bacteria are oxidizing the Fe and/or S, making the As more accessible to abiotic oxidation.

Figure 4. Examples of mineralization of bacterial cells by As-bearing phases. (**A**) Transmission electron microscope (TEM) images of a stalk-like structure (A in a), a sheath-like structure (B in a), and a granular compound (C in b) with electron diffraction patterns of the sheath-like and granular ones in a biomat from an abandoned arsenic mine in the Gunma prefecture of Japan. The diffraction patterns and energy dispersive spectroscopy (EDS) analysis suggest that one of the minerals in the precipitates is schwertmannite, formed in the stalk-like and sheath-like compounds. Reprinted with permission from [51], Elsevier. (**B**) TEM image and analysis of ultramicrotomy thin sections in samples from Carnoulès (France). The TEM image shows a section of a microbial cell with a layered, concentric inner cell wall and massive outer cell wall encrustation by Fe- and As-bearing minerals, together with energy dispersive X-ray spectroscopy (EDXS) analysis obtained from the outer cell wall. Reprinted with permission from Figure 6 in [52], Elsevier. And (**C**) Arsenic-rich Fe(III) precipitates on the outer cells of A. ferrooxidans BY-3 after the experimental bioleaching of realgar. From [54].
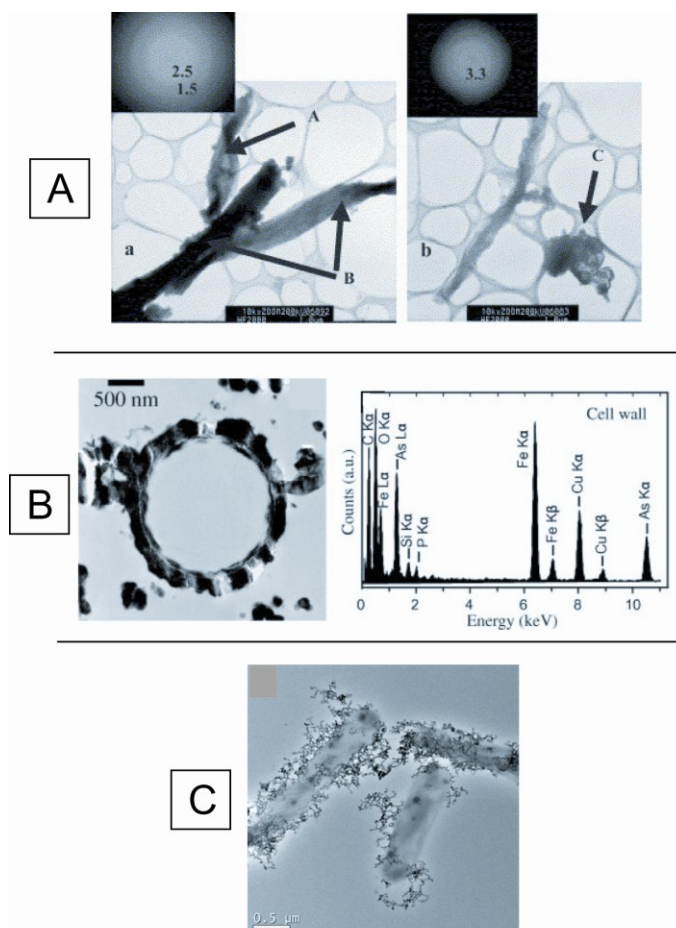
Figure 5. Microbial dissolution of As-bearing minerals found in mine wastes (**A**) Pits in arsenopyrite arising from the oxidative dissolution by *Leptospirillum ferrooxidans*. Reprinted with permission from Figure 7i in [59], Elsevier. And (**B**) Mechanism of simultaneous reduction of structural Fe(III) and aqueous As(V) during the dissolution of synthetic Pb-As jarosite by *Shewanella putrefaciens*. Solid Fe(II) phases are formed as a result of this process. Reprinted with permission from [48], Elsevier.
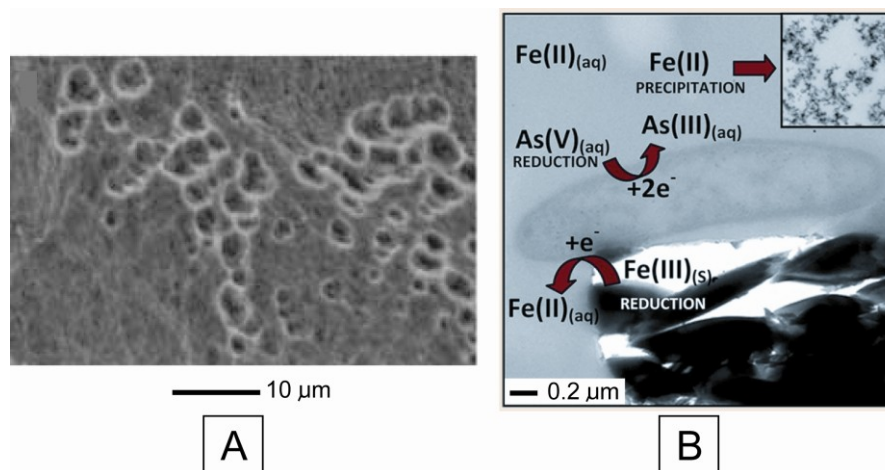
Several As-bearing minerals found in mine wastes have been shown to undergo microbially-assisted reductive dissolution. The dissimilatory arsenate reducers *Shewanella* sp. O23S and *Aeromonas* sp. O23A play a significant role in releasing As(V) from As-bearing minerals in the Zloty Stok gold mine [68]. The experimental anaerobic dissolution of Pb-As-jarosite ($PbFe_3(SO_4,AsO_4)_2(OH)_6$) by *Shewanella putrefaciens* at circumneutral pH resulted in immediate Fe(III) reduction followed by As(V) reduction after 72 h [48].

It was suggested that the Fe(III) reduction was thermodynamically driven, but the aqueous As(V) reduction was due to the Ars bacterial detoxification response (see Figure 2). Secondary As(III)- and Pb-bearing Fe(II)-O precipitates formed as a result of this process. A microbial community containing autotrophic dissimilative reducers of As and SO_4 was shown to promote the dissolution of As-bearing Fe(III) hydroxides, pharmacosiderite, arseniosiderite, and the precipitation of realgar in a natural Au-As geochemical anomaly at Mokrsko, Czech Republic [69]. Dissimilatory Fe(III)-reducing bacteria such as *Shewanella alga* and *Desulfuromonoas palmitatis* have also been shown to reduce scorodite, releasing dissolved arsenate [70,71] and in some cases, producing secondary symplesite [71].

5. Conclusions

This review outlines our present understanding of arsenic-microbe-mineral interactions in mining-affected environments. Although a considerable body of research exists on this topic, there are still several avenues for future research and discovery. The character of secondary minerals produced by microbial oxidation and particularly, reduction of As-bearing minerals in natural environments is still not well known. Although several As(III)-oxidizing and As(V)-reducing bacteria have been isolated and identified from mining-affected environments, these represent a very small proportion of those that likely exist. The specific role of bacteria in the dissolution of primary As-bearing minerals

and formation of secondary As-bearing minerals is still not well understood. Additional work involving the use of novel surface mineral characterization techniques such as low-vacuum scanning electron microscopy, atomic force microscopy, and synchrotron-based techniques such as scanning X-ray microscopy, is required to advance knowledge in this field.

Acknowledgments

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Drahota, P.; Filippi, M. Secondary arsenic minerals in the environment: A review. *Environ. Int.* **2009**, *35*, 1243–1255.
2. Kossoff, D.; Hudson-Edwards, K.A.; Dubbin, W.E.; Alfredsson, M.; Geraki, T. Cycling of As, P, Pb and Sb during weathering of mine tailings: Implications for fluvial environments. *Mineral. Mag.* **2012**, *76*, 1209–1228.
3. Bowell, R.; Parshley, J. Arsenic Cycling in the Mining Environment. In *U.S. EPA Workshop on Managing Arsenic Risks to the Environment: Characterization of Waste, Chemistry, and Treatment and Disposal Proceedings and Summary Report*; EPA/625/R-03/010; U.S. Environmental Protection Agency: Washington, DC, USA, 2003; pp. 10–11.
4. Foster, A.L.; Brown, G.E.; Tingle, T.N.; Parks, G.A. Quantitative arsenic speciation in mine tailings using X-ray absorption spectroscopy. *Am. Mineral.* **1998**, *83*, 553–568.
5. Hudson-Edwards, K.A.; Jamieson, H.E.; Charnock, J.M.; Macklin, M.G. Arsenic speciation in waters and sediments of ephemeral floodplain pools, Ríos Agrio—Guadiamar, Aznalcóllar, Spain. *Chem. Geol.* **2005**, *219*, 175–192.
6. Meunier, L.; Walker, S.R.; Wragg, J.; Parsons, M.B.; Koch, I.; Jamieson, H.E.; Reimer, K.J. Effects of soil composition and mineralogy on the bioaccessibility of arsenic from tailings and soil in gold mine districts of Nova Scotia. *Environ. Sci. Technol.* **2010**, *44*, 2667–2674.
7. Lattanzi, P.; da Pelo, S.; Musu, E.; Atzei, D.; Elsener, B.; Fantauzzi, M.; Rossi, A. Enargite oxidation: A review. *Earth Sci. Rev.* **2008**, *86*, 62–88.
8. Bruckard, W.J.; Davey, K.J.; Jorgensen, F.R.A.; Wright, S.; Brew, D.R.M.; Haque, N.; Vance, E.R. Development and evaluation of an early removal process for the beneficiation of arsenic-bearing copper ores. *Miner. Eng.* **2010**, *23*, 1167–1173.

9. Kwong, Y.T.J.; Beauchemin, S.; Hossain, M.F.; Gould, W.D. Transformation and mobilization of arsenic in the historic Cobalt mining camp, Ontario, Canada. *J. Geochem. Explor*. **2007**, *92*, 133–150.

10. Senior, G.D.; Smith, L.K.; Silvester, E.; Bruckard, W.J. The flotation of gersdorffite in sulfide nickel systems—A single mineral study. *Int. J. Miner. Process.* **2009**, *93*, 165–171.

11. Osborne, T.H.; Jamieson, H.E.; Hudson-Edwards, K.A.; Nordstrom, D.K.; Walker, S.R.; Ward, S.A.; Santini, J.M. Microbial oxidation of arsenite in a subarctic environment: Diversity of arsenite oxidase genes and identification of a psychrotolerant arsenite oxidiser. *BMC Microbiol.* **2010**, *10*, 205, doi:10.1186/1471-2180-10-205.

12. Walker, S.R.; Jamieson, H.E.; Lanzirotti, A.; Andrade, C.F.; Hall, G.E.M. The speciation of arsenic in iron oxides in mine wastes from the Giant gold mine, NWT: Application of synchrotron micro-XRD and micro-XANES at the grain scale. *Can. Mineral.* **2005**, *43*, 1205–1224.

13. Fawcett, S.E.; Jamieson, H.E. The distinction between ore processing and post-depositional transformation on the speciation of arsenic and antimony in mine waste and sediment. *Chem. Geol*. **2011**, *283*, 109–118.

14. Walker, S.R.; Parsons, M.B.; Jamieson, H.E.; Lanzirotti, A. Arsenic mineralogy of near-surface tailings and soils: Influences on arsenic mobility and bioaccessibility in the Nova Scotia gold mining districts. *Can. Mineral.* **2009**, *47*, 533–556.

15. Morin, G.; Juillot, F.; Casiot, C.; Bruneel, O.; Personne, J.C.; Elbaz-Poulichet, F.; Leblanc, M.; Ildefonse, P.; Calas, G. Bacterial formation of tooeleite and mixed arsenic (III) or arsenic (V)-iron (III) gels in the Carnoules acid mine drainage, France. A XANES, XRD, and SEM study. *Environ. Sci. Technol.* **2003**, *37*, 1705–1712.

16. Corriveau, M.C.; Jamieson, H.E.; Parsons, M.B.; Hall, G.E.M. Mineralogical characterization of arsenic in gold mine tailings from three sites in Nova Scotia. *Geochem. Explor. Environ. Anal.* **2011**, *11*, 179–192.

17. Weisener, C.G.; Guthrie, J.W.; Smeaton, C.M.; Paktunc, D.; Fryer, B.J. The effects of Ca-Fe-As coatings on microbial leaching of metals in arsenic bearing mine waste. *J. Geochem. Explor.* **2011**, *110*, 23–30.

18. Ashley, P.M.; Lottermoser, B.G. Arsenic contamination at the Mole River Mine, northern New South Wales. *Aust. J. Earth Sci.* **1999**, *46*, 861–874.

19. Hochella, M.F., Jr.; Moore, J.N.; Golla, U.; Putnis, A. A TEM study of samples from acid mine drainage systems: Metal-mineral association with implications for transport. *Geochim. Cosmochim. Acta* **1999**, *63*, 3395–3406.

20. Roussell, C.; Néel, C.; Bril, H. Minerals controlling arsenic and lead solubility in an abandoned gold mine tailings. *Sci. Total Environ.* **2000**, *263*, 209–219.

21. Savage, K.S.; Tingle, T.N.; O'Day, P.A.; Waychunas, G.A.; Bird, D.K. Arsenic speciation in pyrite and secondary weathering phases, Mother Lode Gold District, Tuolumne County, California. *Appl. Geochem.* **2000**, *15*, 1219–1244.

22. Mao, M.; Lin, J.; Pan, Y. Hemimorphite as a natural sink for arsenic in zinc deposits and related mine tailings: Evidence from single-crystal EPR spectroscopy and hydrothermal synthesis. *Geochim. Cosmochim. Acta* **2010**, *74*, 2943–2956.

23. Majzlan, J.; Lalinska, B.; Chovan, M.; Bläß, U.; Brecht, B.; Göttlicher, J.; Steininger, R.; Hug, K.; Ziegler, S.; Gescher, J. A mineralogical, geochemical, and microbiological assessment of the antimony- and arsenic-rich neutral mine drainage tailings near Pezinok, Slovakia. *Am. Mineral.* **2011**, *96*, 1–13.

24. Mohan, D.; Pittman, C.U., Jr. Arsenic removal from water/wastewater using adsorbents—A critical review. *J. Hazard. Mater.* **2007**, *142*, 1–53.

25. Hudson-Edwards, K.A.; Schell, C.; Macklin, M.G. Mineralogy and geochemistry of alluvium contaminated by metal mining in the Rio Tinto area, southwest Spain. *Appl. Geochem.* **1999**, *14*, 1015–1030.

26. Jamieson, H.E.; Robinson, C.; Alpers, C.N.; Nordstrom, D.K.; Poustovetov, A.; Lowers, H.A. The composition of coexisting jarosite-group minerals and water from the Richmond mine, Iron Mountain, California. *Can. Mineral.* **2005**, *43*, 1225–1242.

27. Egal, M.; Casiot, C.; Morin, G.; Parmentier, M.; Bruneel, O.; Lebrun, S.; Elbaz-Poulichet, F. Kinetic control on the formation of tooeleite, schwertmannite and jarosite by *Acidithiobacillus ferrooxidans* strains in an As(III)-rich acid mine water. *Chem. Geol.* **2009**, *265*, 432–441.

28. Rhine, E.D.; Garcia-Dominguez, E.; Phelps, C.D.; Young, L.Y. Environmental microbes can speciate and cycle arsenic. *Environ. Sci. Technol.* **2005**, *39*, 9569–9573.

29. Stolz, J.F. Overview of Microbial Arsenic Metabolism and Resistance. In *The Metabolism of Arsenite*; Arsenic in the Environment 5; Santini, J.M., Ward, S.A., Eds.; CRC Press: London, UK, 2012; pp. 55–60.

30. Osborne, T.H.; Santini, J.M. Prokaryotic Aerobic Oxidation of Arsenite. In *The Metabolism of Arsenite*; Arsenic in the Environment 5; Santini, J.M., Ward, S.A., Eds.; CRC Press: London, UK, 2012; pp. 61–72.

31. Stolz, J.F.; Basu, P.; Santini, J.M.; Oremland, R.S. Selenium and arsenic in microbial metabolism. *Annu. Rev. Microbiol.* **2006**, *60*, 107–130.

32. Tamaki, S.; Frankenberger, W.T., Jr. Environmental biochemistry of arsenic. *Rev. Environ. Contam. Toxicol.* **1992**, *124*, 79–110.

33. Santini, J.M.; Sly, L.I.; Wen, A.; Comrie, D.; De Wulf-Durand, P.; Macy, J.M. New arsenite-oxidizing bacteria isolated from Australian gold-mining environments—Phylogenetic relationships. *Geomicrobiol. J.* **2002**, *19*, 67–76.

34. Santini, J.M.; Sly, L.I.; Schnagl, R.D.; Macy, J.M. A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. *Appl. Environ. Microbiol.* **2000**, *66*, 92–97.

35. Battaglia-Brunet, F.; Itard, Y.; Garrido, F.; Delorme, F.; Crouzet, C.; Greffie, C.; Joulian, C. A simple biogeochemical process removing arsenic from mine drainage water. *Geomicrobiol. J.* **2006**, *23*, 201–211.

36. Lieutaud, A.; van Lis, R.; Duval, S.; Capowiez, L.; Muller, D.; Lebrun, R.; Lignon, S.; Fardue, M.L.; Lett, M.C.; Nitschke, W.; *et al.* Arsenite oxidase from Ralstonia sp. 22: Characterization of the enzyme and its interaction with soluble cytochromes. *J. Biol. Chem.* **2010**, *285*, 20433–20441.

37. Drewniak, L.; Matlakowska, R.; Sklodowsska, A. Arsenite and arsenate metabolism of *Sinorhizobium* sp. M14 living in the extreme environment of the Zloty Stok gold mine. *Geomicrobiol. J.* **2008**, *25*, 363–370.

38. Battaglia-Brunet, F.; Joulian, C.; Garrido, F.; Dictor, M.C.; Morin, D.; Coupland, K.; Johnson, D.B.; Hallberg, K.B.; Baranger, P. Oxidation of arsenite by *Thiomnonas* strains and characterization of *Thiomonas arsenivorans* sp. nov. *Antonie Leeuwenhoek* **2006**, *89*, 99–108.

39. Duquesne, K.; Lieautaud, A.; Retarouchniak, J.; Muller, D.; Lett, M.C.; Bonnefoy, V. Arsenite oxidation by a chemoautotrophic moderately acidophilic *Thiomonas* sp.: From the strain isolation to the gene study. *Environ. Microbiol.* **2008**, *10*, 228–237.

40. Chang, J.S.; Lee, J.H.; Kim, I.S. Bacterial *aox* genotype from arsenic contaminated mine to adjacent coastal sediment: Evidences for potential biogeochemical arsenic oxidation. *J. Hazard. Mater.* **2011**, *193*, 233–242.

41. Macy, J.M.; Nunan, K.; Hagen, K.D.; Dixon, D.R.; Harbour, P.J.; Cahill, M.; Sly, L.I. Chrysiogenes arsenatis gen. nov., sp. nov., a new arsenate-respiring bacterium isolated from gold mine wastewater. *Int. J. Syst. Bacteriol.* **1996**, *46*, 1153–1157.

42. Macy, J.M.; Santini, J.M.; Pauling, B.V.; O'Neill, A.H.; Sly, L.I. Two new arsenate/sulfate-reducing bacteria: Mechanisms of arsenate reduction. *Arch. Microbiol.* **2000**, *173*, 49–57.

43. Santini, J.M.; Stolz, J.F.; Macy, J.M. Isolation of a new arsenate-respiring bacterium—Physiological and phylogenetic studies. *Geomicrobiol. J.* **2002**, *19*, 41–52.

44. Santini, J.M.; Streimann, I.C.A.; van den Hoven, R.N. *Bacillus macyae* sp. nov., an arsenate-respiring bacterium isolated from an Australian gold mine. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 2241–2244.

45. Heath, M.D.; Schoepp-Cothenet, B.; Osborne, T.H.; Santini, J.M. Arsenite Oxidase. In *The Metabolism of Arsenite*; Arsenic in the Environment 5; Santini, J.M., Ward, S.A., Eds.; CRC Press: London, UK, 2012; pp. 81–97.

46. Oremland, R.S.; Stolz, J.F.; Saltikov, C.W. Anaerobic Oxidation of Arsenite by Autotrophic Bacteria: The View from Mono Lake, California. In *The Metabolism of Arsenite*; Arsenic in the Environment 5; Santini, J.M., Ward, S.A., Eds.; CRC Press: London, UK, 2012; pp. 73–80.

47. Jones, R.A.; Koval, S.F.; Nesbitt, H.W. Surface alteration of arsenopyrite (FeAsS) by *Thiobacillus ferrooxidans*. *Geochim. Cosmochim. Acta* **2003**, *67*, 955–965.

48. Smeaton, C.M.; Walshe, G.E.; Smith, A.M.L.; Hudson-Edwards, K.A.; Dubbin, W.E.; Wright, K.; Beale, A.M.; Fryer, B.J.; Weisener, C.G. Simultaneous release of Fe and As during the reductive dissolution of Pb-As jarosite by *Shewanella putrefaciens* CN32. *Environ. Sci. Technol.* **2012**, *46*, 12823–12831.

49. Edwards, K.J.; Hu, B.; Hamers, R.J.; Banfield, J.F. A new look at microbial leaching patterns on sulfide minerals. *FEMS Microbiol. Ecol.* **2001**, *34*, 197–206.

50. Saltikov, C.W.; Cifuentes, A.; Venkateswaran, K.; Newman, D.K. The ars detoxification system is advantageous but not required for As(V) respiration by the genetically tractable *Shewanella* species strain ANA-3. *Appl. Environ. Microbiol.* **2003**, *69*, 2800–2809.

51. Ohnuki, T.; Sakamoto, F.; Kozai, N.; Ozaki, T.; Yoshida, T.; Narumi, I.; Wakai, E.; Sakai, T.; Francis, A.J. Mechanisms of arsenic immobilization in a biomat from mine discharge water. *Chem. Geol.* **2004**, *212*, 279–290.

52. Benzerara, K.; Morin, G.; Yoon, T.H.; Miot, J.; Tyliszczak, T.; Casiot, C.; Bruneel, O.; Farges, F.; Brown, G.E., Jr. Nanoscale study of As biomineralization in an acid mine drainage system. *Geochim. Cosmochim. Acta* **2008**, *72*, 3949–3963.

53. Duquesne, K.; Leburn, S.; Casiot, C.; Bruneel, O.; Personne, J.C.; Leblanc, M.; Elbaz-Poulichet, F.; Morin, G.; Bonnefoy, V. Immobilization of arsenite and ferric iron by *Acidithiobacillus ferrooxidans* and its relevance to acid mine drainage. *Appl. Environ. Microbiol.* **2003**, *69*, 6165–6173.

54. Chen, P.; Yan, L.; Wang, Q.; Li, H. Arsenic precipitation in the bioleaching of realgar using *Acidithiobacillus ferrooxidans*. *J. Appl. Chem.* **2013**, 424253, doi:10.1155/2013/424253.

55. Barker, W.W.; Welch, S.A.; Chu, S.; Banfield, J.F. Experimental observations of the effects of bacteria on aluminosilicate weathering. *Am. Mineral.* **1998**, *83*, 1551–1563.

56. Welch, S.A.; Barker, W.W.; Banfield, J.F. Microbial extracellular polysaccharides and plagioclase dissolution. *Geochim. Cosmochim. Acta* **1999**, *63*, 1405–1419.

57. Sutton, J.A.; Corrick, J.D. *Bacteria in Mining and Metallurgy: Leaching Selected Ores and Minerals; Experiments with* Thiobacillus ferrooxidans; Rept. Invest. RI 5839; Bureau of Mines, U.S. Department of the Interior: Washington, DC, 1964.

58. Ehrlich, H.L. Bacterial action on orpiment. *Econ. Geol.* **1963**, *58*, 991–994.

59. Corkhill, C.L.; Wincott, P.L.; Lloyd, J.R.; Vaughan, D.J. The oxidative dissolution of arsenopyrite (FeAsS) and enargite (Cu_3AsS_4) by *Leptospirillum ferrooxidans*. *Geochim. Cosmochim. Acta* **2006**, *70*, 3593–3612.

60. Hallberg, K.B.; Johnson, D.B. Biodiversity of acidophilic prokaryotes. *Adv. Appl. Microbiol.* **2001**, *49*, 37–84.

61. Baker, B.J.; Banfield, J.F. Microbioal communities in acid mine drainage. *FEMS Microbiol. Ecol.* **2003**, *44*, 139–152.

62. González-Pastor, J.E.; Mirete, S. Novel metal resistance genes from microorganisms: A functional metagenomic approach. *Methods Mol. Biol.* **2010**, *668*, 273–285.

63. Yelton, A.P.; Comolli, L.R.; Justice, N.B.; Castelle, C.; Denef, V.J.; Thomas, B.C.; Banfield, J.F. Comparative genomics in acid mine drainage biofilm communities reveals metabolic and structural differentiation of co-occurring archaea. *BMC Genomics* **2013**, *14*, 485, 10.1186/1471-2164-14-485.

64. Drewniak, L.; Sklodowska, A. Arsenic-transforming microbes and their role in biomining processes. *Environ. Sci. Pollut. Res.* **2013**, doi:10.1007/s11356-012-1449-0.

65. Islam, A.B.M.R.; Maity, J.P.; Bundschuh, J.; Chen, C.-Y.; Bhowmik, B.K.; Tazaki, K. Arsenic mineral dissolution and possible mobilization in mineral-microbe-groundwater environment. *J. Hazard. Mater.* **2012**, doi:10.1016/j.jhazmat.2012.07.022.

66. Corkhill, C.L.; Vaughan, D.J. Arsenopyrite oxidation—A review. *Appl. Geochem.* **2009**, *24*, 2342–2361.

67. Henao, D.M.O.; Godoy, M.A.M. Jarosite pseudomorph formation from arsenopyrite oxidation using *Acidithiobacillus ferrooxidans*. *Hydrometallurgy* **2010**, *104*, 162–168.

68. Drewniak, L.; Matlakowska, R.; Rewerski, B.; Sklodowska, A. Arsenic release from gold mine rocks mediated by the activity of indigenous bacteria. *Hydrometallurgy* **2010**, *104*, 437–442.

69. Drahota, P.; Falteisek, L.; Redlich, A.; Rohovec, J.; Matoušek, T.; Čepička, I. Microbial effects on the release and attenuation of arsenic in the shallow subsurface of a natural geochemical anomaly. *Environ. Pollut.* **2013**, *180*, 84–91.

70. Cummings, D.E.; Caccavo, F., Jr.; Fendorf, S.; Resonzweigh, R.F. Arsenic mobilization by the dissimilatory Fe(III)-reducing bacterium *Shewanella alga* BrY. *Environ. Sci. Technol*. **1999**, *33*, 723–729.

71. Papassiopi, N.; Vaxevanidou, K.; Paspaliaris, I. Investigating the use of iron reducing bacteria for the removal of arsenic from contaminated soils. *Water Air Soil Pollut*. **2003**, *3*, 81–90.