As, or arsenic, is a highly toxic element that supports a surprising range of biogeochemical transformations. The biochemical basis of these microbial interactions is described, with an emphasis on energy-yielding redox biotransformations that cycle between the As$^{5+}$ and As$^{3+}$ oxidation states. The subsequent impact of As$^{3+}$-oxidising and As$^{5+}$-reducing prokaryotes on the chemistry of selected environments is also described, focusing on soda lakes with naturally high concentrations of the metalloid and on Southeast Asian aquifer sediments, where the microbial reduction of sorbed As$^{5+}$ and subsequent mobilisation of As$^{3+}$ into water abstracted for drinking and irrigation threaten the lives of millions.

**INTRODUCTION TO THE BIOGEOCHEMICAL CYCLING OF ARSENIC**

Although arsenic is highly toxic to humans, and indeed most other forms of life, some microorganisms have evolved to tolerate relatively high concentrations of the metalloid, while specialist examples even thrive on the element, using it as a source of energy for growth. This surprising array of microbial processes, together with inorganic and physical processes, constitutes the global arsenic cycle. Even at the low concentrations found in seawater and freshwater (see Vaughan this issue), microorganisms can accumulate arsenic, often to concentrations many times those encountered in the environment they inhabit. For example, in the marine environment, arsenic is normally present as arsenate, and can be taken up by a range of organisms (including phytoplankton, algae, crustaceans and molluscs), methylated and further metabolised into organic compounds, some of which are passed up through the marine food chain. Indeed, the major organoarsenic compound in marine animals is arsenobetaine, but it can be degraded eventually by sediment microorganisms, returning arsenic to the seawater and closing the marine cycle for this element (reviewed in Mukhopadhyay et al. 2002). In addition to the trace concentrations of arsenic encountered in most environments, there are areas where arsenic can reach much higher concentrations, due to either anthropogenic pollution or a specific geological setting (e.g. evaporative, hydrothermal, sulphidic or Fe$^{3+}$-oxyhydroxide-dominated environments). In these environments, specialised bacteria that gain their energy requirements from redox transformations of arsenic have been discovered.

The key to generating energy from arsenic lies in its redox chemistry, which is characterised by at least four oxidation states: -3, 0, +3 and +5. However, the predominant forms of inorganic arsenic are +5 (arsenate: $\text{H}_3\text{AsO}_4^-$ and $\text{HAsO}_4^{2-}$) and +3 (arsenite: $\text{H}_2\text{AsO}_3^+$ and $\text{H}_2\text{AsO}_3^{-}$). Energy is made available to support microbial life from the oxidation of As$^{5+}$ to As$^{3+}$, with the electrons from this transformation transferred to a suitable electron acceptor such as nitrate or oxygen. Conversely, under anaerobic conditions, the microbial reduction of As$^{5+}$ to As$^{3+}$ can be coupled to the oxidation of organic matter or inorganic electron donors (e.g. hydrogen, sulphide) or both, which also yields energy for growth. A synopsis of these processes is illustrated in Figure 1, with the underlying biochemistry (discussed later) also shown.

Although these two oxidation states of arsenic dominate in most terrestrial environments, the biogeochemical cycle of arsenic is rather more complicated. This is because in many environments one must consider not only direct microbially catalysed redox transformations of arsenic (and in some cases methylation reactions, as mentioned above), but also other environmental factors that may exert critical controls on arsenic speciation. These include the geochemical (aqueous) matrix of the system and the underpinning mineralogy, especially if there are mineral phases that may potentially host arsenic. Indeed, arsenate is especially well known to sorb to mineral phases, and this can have an impact on its environmental mobility. Arsenite, however, seems to sorb less strongly to some key mineral phases, although the relative strength of sorption depends upon a range of factors, including As concentration and pH. Nevertheless, the reduction of the sorbed arsenate could potentially result in the mobilisation of the more mobile arsenite, which is also considered to be more toxic than arsenate. However, this is clearly an oversimplification of the natural biogeochemical processes that control the solubility of arsenic. For example, the mineral phases hosting arsenate can change during As$^{5+}$ reduction because Fe$^{3+}$ oxyhydroxides may also be reduced by anaerobic bacteria under conditions similar to those required for As$^{5+}$ reduction, potentially altering arsenic mobility.

In this article we focus on the biological components of the global arsenic cycle. We initially discuss the diversity of microorganisms known to interact with arsenic (both aqueous and sorbed), and the genes and enzymes that catalyse these biotransformations. We then put these metabolic processes into an environmental context, first by focusing...
on the arsenic cycle in water columns with relatively high arsenic concentrations, and second by considering more complicated subsurface systems, paying due consideration to the problems of distinguishing other linked biogeochemical cycles in the sediments.

**MICROBIAL INTERACTIONS WITH ARSENIC: DETOXIFICATION AND RESPIRATION**

The poisonous nature of arsenic has been known for many years (see reviews by Vaughan and by Hopenhayn in this issue), but the mode of toxicity depends very much on the chemical form of the metalloid (Frausto da Silva and Williams 1993). Arsenate mimics phosphate and can therefore enter the microbial cell via transporters meant for the uptake of this essential nutrient. Once within the cell it can interfere with phosphate-based energy-generating processes, and as a result inhibit oxidative phosphorylation, for example. Arsenite, on the other hand, enters via a different route (aqua-glycerolporins) and targets a broader range of cellular processes, binding to the thiol groups in important cellular proteins such as pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase.

Microorganisms have evolved multiple strategies to protect themselves from arsenic. For example, fungi can use methylation as a detoxification strategy, producing monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA). Prokaryotes (including representatives from both the Archaeal and Bacterial Domains) can also produce volatile methylated arsines, which remove arsenic from the local environment by forming it into a gaseous compound. An alternative strategy used by bacteria and yeast (e.g. Saccharomyces cerevisiae) is based upon the “ArsC” arsenate reductase protein. The gene for this enzyme, along with those encoding other proteins required for arsenic detoxification, are often encoded on mobile genetic units called plasmids, and this feature has made them highly amenable to genetic study. For this reason, we know a lot about microbial ArsC-based resistance determinants, including crystal structures of ArsC proteins from several bacteria (as reviewed in Mukhopadhyay et al. 2002). This type of resistance determinant is very common in the prokaryotic world; more than 100 Ars operons (see glossary of Table 1) had been sequenced by 2002 (Mukhopadhyay et al. 2002), and this number will be significantly higher now. ArsC is a small (13–16 kilodaltons) protein that is found in the cytoplasm of the microbial cell and mediates the reduction of arsenate to arsenite, with glutaredoxin, glutathione or thioredoxin supplying the reducing power for this transformation (Silver and Phung 2005). The toxic arsenite is then excreted by an energy-requiring (ATP-dependent) efflux pump, ArsB. (see Fig. 1).

The above detoxification system clearly requires energy in the form of ATP to function. However some specialist bacteria are able to obtain energy for growth through redox transformations of arsenic. For example, specialist “dissimilatory arsenate-reducing prokaryotes” (see glossary) are able to respire using arsenate as the electron acceptor, in place of oxygen, under anaerobic conditions. Although pH and concentration dependent, the oxidation/reduction potential of As$^{5+}$/As$^{3+}$ is in the region of +60 to +135 mV (Oremland and Stolz 2003), which is sufficient to support growth when organic matter is supplied as the electron donor. Two closely related representatives of the ε-Proteobacteria phylogenetic group (Sulfurospirillum arsenophilum and S. barnesi) were the first dissimilatory arsenate-reducing bacteria identified in the mid-1990s, and representatives have since been identified in other phylogenetic groupings, including the γ- and δ-Proteobacteria, the low GC “Gram-positive” bacteria, thermophilic Eubacteria and Crenarcheae (Oremland and Stolz 2003). Although sharing the common ability to obtain energy required for growth through the reduction of As$^{5+}$, the overall metabolic diversity of these organisms is wide. Certainly, given the low concentrations of As$^{5+}$ in most environments, compared to

**Table 1. GLOSSARY**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td><strong>ATP</strong></td>
<td>Adenosine triphosphate, a high-energy phosphate molecule used to store and release energy</td>
</tr>
<tr>
<td><strong>Chemolithothroph</strong></td>
<td>An organism that gains energy from the oxidation of inorganic compounds, with carbon taken up as CO$_2$, HCO$_3^-$ or CO$_3^{2-}$</td>
</tr>
<tr>
<td><strong>Dissimilatory arsenic-reducing prokaryote</strong></td>
<td>Simple unicellular organisms (including bacteria and archaea) which can conserve energy for growth via cellular electron transfer reactions resulting in the reduction of arsenate to arsenite</td>
</tr>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td>Prokaryotic organisms that stain pink with the Gram-staining process, due to a characteristic cell architecture that includes an outer membrane and cytoplasmic membrane separating a periplasmic space containing soluble enzymes and a cell wall</td>
</tr>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td>Prokaryotic organisms that retain the violet stain during the Gram-staining process, due to a thick cell wall. They lack the outer membrane of Gram-negative bacteria.</td>
</tr>
<tr>
<td><strong>PCR</strong></td>
<td>Polymerase Chain Reaction – a method to rapidly synthesize many copies of a specific segment of DNA</td>
</tr>
<tr>
<td><strong>Operon</strong></td>
<td>A cluster of functionally related genes regulated and transcribed as a unit</td>
</tr>
<tr>
<td><strong>Oxycline</strong></td>
<td>The oxygen gradient in a marine or freshwater environment</td>
</tr>
<tr>
<td><strong>16S rRNA gene</strong></td>
<td>A highly conserved gene required for protein synthesis in bacteria and archaea. When amplified and sequenced, it can be used to identify and classify a given organism.</td>
</tr>
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**Figure 1.** Mechanisms of microbial transformations of arsenic in the environment
other electron acceptors, a high level of respiratory diversity may be crucial to the survival of these organisms. Indeed, to date only one obligate arsenate-reducing prokaryote has been identified, namely strain MLMS-1 (see below). Other electron acceptors used by these organisms are strain specific but include selenate, nitrate, nitrite, fumarate, Fe(III), thiosulphate, elemental sulphur, dimethylsulphoxide and trimethylamine oxide. They can also use a range of electron donors, including hydrogen, sulphide, acetate, formate, pyruvate, butyrate, citrate, succinate, fumarate, glucose and aromatics such as benzoate.

The mechanism of As(V) reduction by dissimilatory arsenate-reducing bacteria has not been studied in the same depth as the ArsC detoxification system described above. The first “respiratory arsenate reductase” characterised was from an organism called Chrysiogenes arsenatis, isolated from gold mine wastewater. The protein was found in one of the outer compartments, called the periplasm, of this “Gram-negative” bacterium and consisted of 87 and 29 kDa subunits (Kraft and Macy 1998). It is related to the dimethylsulfoxide (DMSO) family of mononuclear molybdenum-containing enzymes. Similar proteins (and the corresponding genes designated arrA and arrB) have now been characterised in Bacillus selenitireducens (Afkar et al. 2003) and Shewanella strain ANA-3 (Saltikov and Newman 2003), and the gene sequences used to develop polymerase chain reaction (PCR) primers to target arrA genes (Malasarn et al. 2004). This advance may make it possible to retrieve arrA genes from species present in the environment, but not currently available in culture. Such molecular tools also offer the potential to quantify active As(V)-reducing bacteria through real-time quantitative PCR techniques, which may give a relatively rapid indication of the rates of arsenic reduction and mobilisation in situ.

Finally, closing the arsenic cycle are the arsenite-oxidising bacteria that couple the oxidation of As(III) to the reduction of molecular oxygen or nitrate, the latter under anaerobic conditions. As(III) oxidation is also catalysed by a wide range of microorganisms; over 30 strains from at least nine different genera have been identified (Oremland and Stolz 2003). These include heterotrophic arsenite-oxidising bacteria which detoxify As(III) in the periplasm through oxidation, thus preventing its uptake. However, there are also chemolithoautotrophic examples that conserve energy for growth via arsenite oxidation, using some of this energy to fix CO₂ for the synthesis of cellular material. A good example here is the aerobic strain NT-26, which was isolated from a gold mine in the Northern Territory of Australia and shown to be related to soil bacteria in the Agrobacterium/Rhizobium branch of the α-Proteobacteria (Santini et al. 2000). As with most areas of microbiology, we are learning a lot about the arsenic cycle from newly available genome sequences which represent the “genetic blueprints” of microorganisms. For example, a recent review has given an excellent update of the genetic basis of arsenite oxidation by Alcaligenes faecalis, based on the genome sequence for this soil bacterium (Silver and Phung 2005). From these results, it is apparent that there are surprising similarities between the respiratory arsenate reductases of dissimilatory arsenate-reducing prokaryotes and respiratory arsenite oxidases, both of which are molybdoproteins comprising two subunits. The sequence annotation of the A. faecalis genome is especially noteworthy, as it shows the asoA and asoB arsenite reductase genes, encoding a large molybdopterin-containing peptide and a smaller [2Fe-2S]-containing subunit, respectively, located within an “arsenic gene island” containing more than twenty other genes potentially involved in arsenic metabolism (Silver and Phung 2005).

**THE ARSENIC CYCLE IN MONO AND SEARLES LAKES, CALIFORNIA**

Our fundamental understanding of the biogeochemical cycle of arsenic can be facilitated by studying environments that are particularly rich in this element, such as the alkaline soda lakes (Fig. 2) that occur in the desert regions of eastern California, USA, notably Mono Lake, located on the western edge of the Great Basin (Oremland et al. 2004), and Searles Lake, located some 400 kilometres to the south-southeast, in the Mojave Desert (Oremland et al. 2005). Both lakes are equally alkaline (pH = 9.8) and highly buffered by their abundant dissolved carbonate and bicarbonate anions (e.g. in Mono Lake their combined concentration is 400 mM). The salinity of Mono Lake is ~90 g/L, while that of Searles Lake is at or near saturation (~350 g/L).

![Figure 2A](image1.png) Satellite photograph of Mono Lake and the Mono Basin, California. The lake is 25 km across.

![Figure 2B](image2.png) Collection of samples beneath the thick salt crust at Searles Lake. Searles Lake is an evaporated version of Mono Lake. USGS scientists Larry Miller (foreground) and Tom Kulp had to chip a hole through the thick (~6 cm) salt crust to access the underlying dense brine, about 5 cm deep, below which lay the anoxic sediments that they recovered by use of hand corers. PHOTO SHELLEY E. HOEFT, USGS

Their corresponding levels of dissolved inorganic arsenic oxyanions are 0.2 mM and 3.9 mM for Mono and Searles lakes, or about 15,000 ppb and 292,500 ppb, respectively. This arsenic comes from the inflow of hydrothermal waters. Over time, coupled with evaporation, this inflow elevates the lake’s arsenic concentration. Owing to the unique
The alkaline soda lakes have given microbiologists excellent environments to explore the impact of microbial redox transformations of arsenic away from competing abiotic and respiratory processes that can impact on arsenic speciation. However, it is in these more complicated environments, e.g. in aquifers in West Bengal and Bangladesh, that microbial processes may play a key role in mobilising sediment-bound arsenic into water that is abstracted for drinking and irrigation (see Charlet and Polya this issue).
The sediments of the West Bengal and Bangladesh aquifers are derived from weathered materials from the Himalayas. Arsenic typically occurs at concentrations of 2–100 ppm in these sediments, much of it sorbed onto a variety of mineralogical hosts, including hydrated ferric oxides, phyllosilicates and sulphides (Nickson et al. 2000; Smedley and Kinniburgh 2002). The mechanism of arsenic release from these sediments has been a topic of intense debate, and both microbial and chemical processes have been invoked (Das et al. 1996; Nickson et al. 1998; Chowdhury et al. 1999; Nickson et al. 2000; Oremland and Stolz 2003; Akai et al. 2004). The oxidation of arsenic-rich pyrite has been proposed as one possible mechanism (Chowdhury et al. 1999; Das et al. 1996). Other studies have suggested that reductive dissolution of arsenic-rich Fe$^{3+}$-oxyhydroxides deeper in the aquifer may lead to the release of arsenic into the groundwater (Harvey et al. 2002; Nickson et al. 1998; Nickson et al. 2000; Smedley and Kinniburgh 2002). Additional factors that may add further complication to potential arsenic-release mechanisms from sediments include the predicted mobilisation of sorbed arsenic by phosphate generated from the intensive use of fertilizers (Acharyya et al. 1999), by carbonate (Appelo et al. 2002) produced via microbial metabolism (Harvey et al. 2002) or by changes in the sorptive capacity of ferric oxyhydroxides (Smedley and Kinniburgh 2002).

Microbially mediated reduction of assemblages comprising arsenic sorbed to ferric oxyhydroxides is gaining consensus as the dominant mechanism for the mobilisation of arsenic into these groundwaters (Nickson et al. 2000; Smedley and Kinniburgh 2002; Akai et al. 2004; Islam et al. 2004; van Geen et al. 2004). For example, a recent microcosm-based study provided the first direct evidence for the role of indigenous metal-reducing bacteria in the formation of toxic移动 As$^{3+}$ in sediments from the Ganges Delta (Islam et al. 2004). This study showed that the addition of acetate to anaerobic sediments, as a proxy for organic matter and a potential electron donor for metal reduction, resulted in stimulation of microbial reduction of Fe$^{3+}$ followed by As$^{5+}$ reduction and release of As$^{3+}$ (Fig. 4). Microbial communities responsible for metal reduction and As$^{3+}$ mobilisation in the stimulated anaerobic sediment were analysed using molecular (PCR; see glossary) and cultivation-dependant techniques. Both approaches confirmed an increase in numbers of metal-reducing bacteria, principally Geobacter species. We have also recently obtained similar results from Cambodian sediments liberating arsenic into groundwater (Rowland et al. submitted). These data suggest a key role for anaerobic metal-reducing bacteria in mediating arsenic release from sediments.

The exact mechanism of arsenic release in these experiments remains far from clear. For example, Geobacter species reduce many metals, but none are known to reduce As$^{3+}$, arguing against these organisms playing a direct role in the reductive mobilisation of As$^{3+}$. Of course it is possible that As$^{5+}$-reducing Geobacter species existed in the sediments used in these experiments and await isolation and discovery. Another explanation could be that As$^{5+}$ is mobilised during the reductive dissolution of ferric oxyhydroxides, and the soluble As$^{3+}$ is in turn reduced via arsenate-respiring bacteria or other microorganisms carrying an ArsC-based resistance system, leading to the formation of As$^{3+}$ detected under anaerobic conditions. Alternatively, the reduction and release of As$^{5+}$ could be mediated by the Fe$^{2+}$ produced by Fe$^{3+}$-reducing bacteria, such as Geobacter species. However, recent studies have suggested that this is not the case, as Fe$^{2+}$ minerals that form under anaerobic metal-reducing conditions retain arsenic quite effectively. One last possible explanation is that there exists a subpopulation of As$^{5+}$-respiring bacteria that are able to utilise the sorbed immobile As$^{5+}$, once the bioavailable Fe$^{3+}$ has been used up in the sediments. Indeed, many As$^{5+}$-reducing bacteria are also able to respire using Fe$^{3+}$ as an electron acceptor for growth (Oremland and Stolz 2003; Zobrist et al. 2000). This seems a sensible strategy for survival, given the far higher loadings of Fe$^{3+}$ compared to As$^{5+}$ in sediments. So, although the precise mechanism of arsenic release remains obscure, significant advances have been made over the last few years to support the view that As mobilisation is mediated by anaerobic prokaryotes.

**FIGURE 4 Release of As$^{3+}$ from sediments from West Bengal mediated by anaerobic metal-reducing bacteria. FROM ISLAM ET AL. (2004)**

**FUTURE DIRECTIONS**

There remains an urgent need for a better understanding of the mechanisms of arsenic release into groundwaters, especially how the biochemical reactions within the microbial cell drive the local chemistry at the microbe-mineral interface to result in arsenic mobilisation. With the imminent availability of genomic sequences for several model arsenic-respiring organisms, we can expect major advances in the understanding of the biological side of these processes, while improvements in spectroscopy and imaging techniques will play an important part in elucidating the mineralogical side of this complex system. One could also envisage the development of new biological sensing devices for aquifers at risk, once the molecular mechanisms of these transformations are fully grasped. It is also important to define the environmental factors that drive the mobilisation of arsenic into groundwaters, and here a better understanding of the source and role of organic matter in the sediments is important. Of course, a major driver for gaining a better understanding of these processes is the desire to ensure that the world’s population has access to safe water supplies. Here there is considerable interest in developing methods to remediate arsenic-contaminated groundwater, and again, biotechnology could be employed to reverse the anaerobic, arsenic-mobilising, microbial activities that seem to be at the heart of this human catastrophe.
REFERENCES


Afkar E, Lisak J, Saltikov C, Basu P, Oremland RS, Stolz JF (2003) The respiratory arsenate reductase from Bacillus selenitireducens sp. nov: two haloalka-

REFERENCES


Switzer Blum J, Burns Bindi A, Buzzelli J, Stolz JF, Oremland RS (1998) Bacillus arsenicoselenatis, sp. nov., and Bacillus selenitireducens sp. nov: two haloalka-