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MiniReview

The microbial arsenic cycle in Mono Lake, California

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Abstract

Significant concentrations of dissolved inorganic arsenic can be found in the waters of a number of lakes located in the western USA and in other water bodies around the world. These lakes are often situated in arid, volcanic terrain. The highest concentrations of arsenic occur in hypersaline, closed basin soda lakes and their remnant brines. Although arsenic is a well-known toxicant to eukaryotes and prokaryotes alike, some prokaryotes have evolved biochemical mechanisms to exploit arsenic oxyanions (i.e., arsenate and arsenite); they can use them either as an electron acceptor for anaerobic respiration (arsenate), or as an electron donor (arsenite) to support chemoautotrophic fixation of CO_2 into cell carbon. Unlike in freshwater or marine ecosystems, these processes may assume quantitative significance with respect to the carbon cycle in arsenic-rich soda lakes. For the past several years our research has focused on the occurrence and biogeochemical manifestations of these processes in Mono Lake, a particularly arsenic-rich environment. Herein we review some of our findings concerning the biogeochemical arsenic cycle in this lake, with the hope that it may broaden the understanding of the influence of microorganisms upon the speciation of arsenic in more common, less ''extreme'' environments, such as drinking water aquifers.

Keywords: Anaerobic bacteria; Arsenic; Alkaline lakes; Dissimilatory reduction; Chemoautolithotrophs

1. Introduction

Certain arsenic compounds are recognized as potent poisons since antiquity [1]. In recent years, however, concern has arisen about prolonged human exposure to sub-acute levels of arsenic by ingestion of contaminated drinking water supplies. In the Ganges Delta, for example, groundwater in which naturally occurring arsenic has been mobilized into solution from the aquifer sediments has put millions of people at risk, while thousands have developed cases of severe arsenicosis (also called ''arseniasis'') [2,3]. It is not clear at this juncture what specific mechanisms are responsible for this mobilization, but they appear to include a combination of chemical, physical, and microbial factors [4]. These factors contribute to speciation changes in the arsenic itself, altering it back and forth from the more toxic and hydrologically mobile arsenite to the less toxic and less mobile arsenate. It should be borne in mind, however, that the dissolved arsenic concentrations in such aquifers are often quite low (~1 μM) from the point of view of experimental microbiology, although not from one of human health. These low concentrations make detection of in situ microbial arsenic metabolism or identification of the causative microbial reactions and agents somewhat problematic. A further experimental difficulty with aquifer systems is the presence of a solid phase in contact with an aqueous phase. Both arsenate and arsenite have different adsorptive affinities to various common mineral surfaces (e.g., ferrihydrite, alumina) which strongly affect their concentration in the aqueous phase [5] and hence will complicate the interpretation of microbial phenomena [6]. It is instructive, therefore, to study the microbial

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reactions that influence the speciation of arsenic in environments that are not only rich in this element, but are free of a sediment/aquifer solid phase. In these regards, the water column of Mono Lake, California is ideal.

Mono Lake is a closed basin lake in central California located on the eastern slope of the Sierra Nevada mountain range, lying within the western edge of the arid Great Basin (Fig. 1). It is only one of several alkaline lakes in this region that have naturally occurring high concentrations of dissolved inorganic arsenic, and hence this phenomenon is regionally widespread. These concentrations range from a freshwater end-member as represented by Crowley Lake [7] to the extreme case of salt-saturated Searles Lake. Crowley Lake receives As-laden hydrothermal waters from Hot Creek, an upstream tributary of the Owens River that drains through the lake [8]. This lake's dissolved arsenic concentration is seasonally variable, but nonetheless the concentration shown in Fig. 1 is about sixfold higher than that the recommended maximum concentration limit (MCL) of 10 ppb (~0.13 μM) for drinking water [9]. Because Crowley Lake is part of the water supply system for Los Angeles, this is a public health concern. For perspective, contrast Crowley's concentration with Pavin Lake (~0.14 μM) in France [10] or with Upper Mystic Lake (~0.02 μM) in Massachusetts, the latter system being contaminated with anthropogenic arsenic [11]. The arsenic concentrations in near-saturation, alkaline brines found in the western USA can be astonishingly high. For example, millimolar arsenic occurs in the dense brine of Searles Lake, which is located in the Mohave Desert. Similar levels of arsenic are present in the brines underneath the playa of Owens Lake, also located in eastern California [12,13]. The concentration of arsenic in Mono Lake lies between the two extremes of Crowley Lake and Searles Lake, but is certainly at the high end of what is typical of other closed basin soda lakes in this region and comparable to other volcanic/hydrothermal terrains around the world [14]. The ground waters of these regions are also rich in arsenic [15,16]. In Mono Lake, arsenic occurs primarily as either the oxyanion arsenate [$HAsO_4^{2-}$ or As(V)] or arsenite [$H_2AsO_3^-$ or As(III)]. Organic forms of arsenic (e.g., monomethylarsonic acid, dimethylarsenic acid) are not significant relative to these inorganic forms [17]. For this reason we have focused our research on inorganic arsenic species.

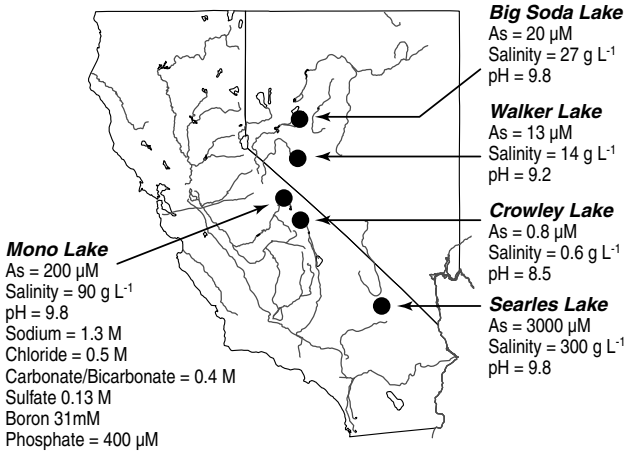

Fig. 1. Approximate locations and chemical features of five arsenic-rich lakes located in western Nevada and eastern California.

The biochemical reactions mediated by microbes with regard to reduction of arsenate or oxidation of arsenite can be divided into two basic categories. These consist of either detoxification reactions that confer As-resistance, or redox reactions that conserve the energy gained by the reaction to provide for cell growth. Arsenic detoxification (resistance) reactions have been known for some years, and were reviewed recently by Rosen [18] and Mukhopadhyay et al. [19]. However, they were not investigated in Mono Lake. Rather, the energy-conserving reactions that include respiratory arsenate reduction and chemoautotrophic arsenite oxidation, and which represent more recent discoveries, have become the focus of our efforts in Mono Lake. These processes have also been recently reviewed [20–24].

2. Limnological characteristics

Some of the salient facts concerning this lake's physical limnology, hydrochemistry, and biological properties are given in Fig. 1. The Mono Basin was formed about one million years ago by a local subsidence [25]. Volcanic activity in the region began with the eruption of the Long Valley Caldera, about 700,000 years ago [26]. The volcanism progressively migrated ~40 km northward, eventually forming the Mono Craters on the lake's south shore as well as several very recent volcanic features located in or around the lake itself [27] including discharge from arsenic-rich hot springs. The lake has expanded and contracted in size and volume over the long period of its existence in response to changes in climate. It is currently about fivefold smaller in area and contains 18-fold less water than it did during the Pleistocene, which has resulted in the evaporative concentration of a number of constituents, including sodium, chlorine, sulfur, boron, arsenic, and dissolved inorganic carbon (as HCO_3^- plus CO_3^{2-}). Mono Lake has exceptionally high concentrations of phosphate in its surface and bottom water. Although phosphate is a molecular analog of arsenate, it is not clear if this twofold difference in concentration between these elements ameliorates the toxic effects of the latter on various components of the lake's ecosystem. In contrast to phosphate, the inorganic nitrogen concentration of surface water is quite low, and provides a nutrient limitation on the extent of phytoplankton productivity. The lake's abundant dissolved carbonates

and organic ligands help to retain high concentrations of various fallout radionuclides in solution, whereas they would normally precipitate into the sediments in lakes with circumneutral pH [28–30]. Numerous natural gas seeps (mainly CH_4) are common in and around the lake [31], and both nitrification and methane oxidation occur in the water column [32].

Lake-wide vertical mixing is usually temperature-driven, occurring once per year in early winter (monomixis). However, strong El-Nino climatic events can cause episodic pulses of above average runoff into the lake from the Sierra Nevada, thereby diluting the salinity of the surface waters enough to offset the increased density caused by their normal cooling during winter. These events have resulted in periods without annual mixing (meromixis) that can last for several sequential years. The lake has been meromictic twice in recent decades, once in the 1980s [33] and again in the 1990s [34]. The effects of meromixis include decreased algal primary productivity [35,36], resulting from decreased upward flux of inorganic nitrogen [37] and the establishment of highly reducing conditions in the anoxic bottom waters (monimolimnion) [38]. During meromixis, the vertical distribution of arsenic species consists of predominantly arsenate in the oxic surface waters, followed by a change to mostly arsenite in the anoxic monimolimnion [39].

3. Microbial communities

Because of the unusual characteristics of alkaline, hypersaline environments in general and Mono Lake in particular, their bacterial communities have been studied for many years, in part in the quest for commercially valuable biotechnology [40,41]. These studies have been summarized recently by Oren [42], who includes a chapter reviewing the history and findings of microbiological studies in Mono Lake up to the date of publication. A paper published subsequent to Oren's review [43] reported the results of a phylogenetic characterization of Mono Lake bacteria. This study compared the distribution of ribotypes in libraries derived from samples collected from the mixolimnion (2 m, aerobic), the base of the oxycline (17.5 m, microaerophilic), the upper chemocline (23 m, seasonally anoxic) and the monimolimnion (35 m, perennially anoxic and highly reducing). The samples were collected during August 2001, when the lake was highly stratified and had been meromictic for six years. Because the analysis was based on PCR and cloning, the relative abundance of sequences in the libraries may deviate from the distribution in situ (e.g., see [44]).

Most of the 212 sequences retrieved (~60 clones were analyzed per sample) fell into five major lineages of the domain Bacteria: α- and γ-Proteobacteria (6% and 10%, respectively); Cytophaga-Flexibacter (CF, 19%); high G+C gram-positive (Actinobacteria, 25%); and low G+C gram-positive (*Bacillus Clostridium*, 19%). Twelve percent were identified as derived from chloroplasts. The remaining 9% represented β- and δ-Proteobacteria; Verrucomicrobiales, Planctomycetales and candidate divisions. The populations of sequences retrieved from mixolimnion and oxycline libraries were dominated by sequences related to Actinobacteria (49% and 63%, respectively) distributed into only three distinct ribotypes (defined as sequence similarities $\geqslant 97\%$, most were $\geqslant 99\%$) while the population of sequences retrieved from the monimolimnion library was dominated (52%) by low G+C gram-positive bacteria distributed in 12 distinct ribotypes. In most cases, the sequences that are most similar to those retrieved from Mono Lake were retrieved from other alkaline, hypersaline environments [43]. Archaea do not appear to be abundant in Mono Lake as we have not been able to retrieve archaeal ribotypes using published domain Archaea primers [45–47].

The Mono Lake Bacteria assemblage appears to have low diversity. A standard coverage estimate, $C = 1 - (n_1/N)$, where n_1 is the number of ribotypes that occurred only once in the clone library and N is the total number of clones analyzed [48] indicated that 59–98% of the diversity in these libraries was sampled by sequencing ~60 clones. Mixolimnion and oxycline libraries had the lowest sequence diversity with only 9 and 12 distinct ribotypes respectively; whereas chemocline and monimolimnion libraries were more diverse, containing 27 and 25 ribotypes, respectively. The composition of mixolimnion and oxycline libraries were not significantly different (LIBSHUFF analysis; [49]), but they were significantly different from chemocline and monimolimnion libraries ($p < 0.001$) and chemocline and monimolimnion libraries were not significantly different from each other ($p = 0.006/0.124$).

We used the distribution of ribotypes in our clone libraries to estimate the total number of ribotypes present in the samples using an analytical approach described by Curtis et al. [50]. This analysis indicated that the total number of different ribotypes per sample ranged from 200 (17.5 m sample) to 4700 (23 m sample). The number of species in oceanic plankton samples or soil samples estimated by the same [50] or other methods [51–53] is of the order of 10^4.

Our data indicate that Mono Lake libraries are strongly dominated by a few ribotypes. All of the clones from the 2 m sample could be assigned to just nine ribotypes, with the three most abundant ribotypes accounting for 63% of all sequences. Forty three percent of the clones in the 17.5 m library fell into one ribotype while the three most abundant ribotypes accounted for 73% of the sequence population. The most abundant ribotype in the most diverse library (23 m) accounted for 10% of the population while the three most abundant ribotypes in this library accounted for 27% of the sequence population.

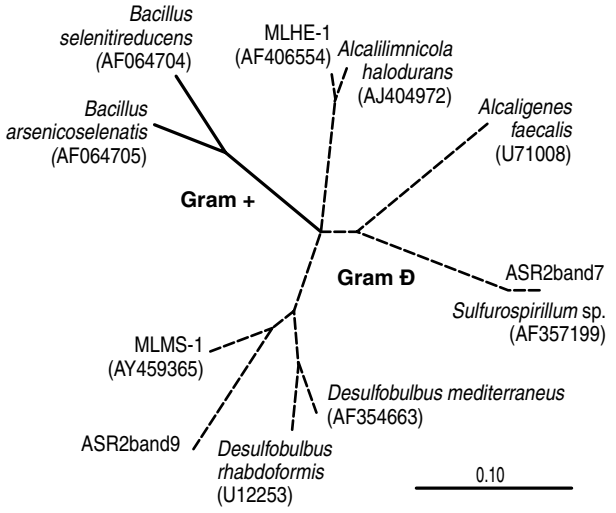

Fig. 2. Neighbor-joining tree of 16S rRNA sequences of bacteria thought to be involved in arsenic geochemistry of Mono Lake. Strain MLHE-1 is an anaerobic arsenite oxidizer, *B. arsenicoselenatis*, *B. selenitireducens* and strain MLMS-1 are arsenate reducers, and the rest are reference sequences. All sequences are about 1400 bp except the two obtained by DGGE bands: ARS2band7 (133 bp) and ARS2band9 (159 bp).

A broader survey using fingerprinting techniques similar to Hollibaugh et al. [54] indicated little seasonal variation in the richness and composition of the Mono Lake microbial community although there were seasonal shifts in apparent dominance, at least in the surface layer. Spatial variation in the relative abundance of ribotypes was related to mixing characteristics of the station sampled [55].

Sequences closely related (similarity >99%) to isolates known to be active in As cycling in Mono Lake have not been retrieved from clone libraries; however sequences of other members of clades containing these sequences (low G+C gram-positive bacteria, δ- and γ-Proteobacteria) are found regularly. An exception was the ε-Proteobacteria. Although they were not retrieved from the Mono Lake clone libraries, they were a dominant band in a lakewater-based arsenate-enrichment incubation experiment [56]. Fig. 2 shows a phylogenetic tree of arsenic-metabolizing bacterial species found to date in Mono Lake.

4. Water column arsenic speciation and in situ microbial activity

Vertical profiles of some physical, chemical, and biological constituents taken when the lake was meromictic in 1999 [57] are shown in Fig. 3. An oxycline was apparent from 10 m which descended to the disappearance of dissolved oxygen at 18 m, beneath which a chlorophyll *a* peak was evident (Fig. 3(a)). Unlike Big Soda Lake where photosynthetic bacteria are a prominent feature beneath the summer oxycline [58], this was not evident in Mono Lake. Rather, the chlorophyll maximum was mostly composed of *Picocystis* sp., an unusual eukaryotic alga [54,59] whose small diameter small (diameter, 1–3 μm) make it part of the "picoplankton."

The speciation of arsenic changed from arsenate ($HAsO_4^{2-}$) in the oxic mixolimnion to arsenite ($H_2AsO_3^-$) in the anoxic monimolimnion (Fig. 3(b)). The monimolimnion waters were highly reducing (Fig. 3(c)), denser than the mixolimnion (Fig. 3(d)), and contained abundant bacterial cells (Fig. 3(e)). The high concentrations of arsenate reported in the monimolimnion were an artifact of the initial analytical methodology employed, which determined it as the difference between total arsenic and arsenite. In subsequent analyses, arsenate was measured directly, which generated the profile shown in Fig. 4. Low, but significant quantities of arsenate (e.g., 5–10 μM) were consistently detected in the highly reducing monimolimnion.

Although the arsenite oxyanion was the main arsenic species in the lake's anoxic waters, a novel recent finding by the research team led by Hollibaugh was the detection of dissolved arsenic(III)/sulfide species with stoichiometries consistent with monothioarsenite ($H_2AsO_2S^-$), dithioarsenite ($H_2AsOS_2^-$) and trithioarsenite ($H_2AsS_3^-$) species. Collectively these three thioarsenites accounted for 25% and 67% of total arsenic at 25 and 38 m depth, respectively, during August 2002. Although sulfides can rapidly reduce arsenate to arsenite at low pH values, this does not occur at pH 9.8 [60]. Production of arsenic(III) trisulfide as a precipitate was noted during growth of *Desulfosporosinus auripigmentum*, an arsenate-respiring sulfate-reducer, when incubated at circumneutral pH [61]. In freshwater lakes any such arsenic sulfides formed in the anoxic hypolimnion accumulate as precipitates in the sediments and thereby represent a net sink for removal of arsenic from the water column [62]. In contrast, arsenic sulfides in the anoxic waters of Mono Lake are soluble and do not constitute a sediment sink and are oxidized back to arsenate when dissolved oxygen returns to the deeper waters during mixing events. The lack of such an arsenic sink contributes to the high dissolved arsenic content of Mono Lake's (and other alkaline lake's) waters. Rochette et al. [63] reported chemical formation of various $arsenic^{3+}$ sulfides under conditions of low pH. These reactions also take place at pH 9.8, but the thioarsenites remain in solution [64].

A radiotracer technique was devised to measure dissimilatory arsenate reduction in the lake's water column [57]. Water samples from the various depths were placed into sealed syringes, injected with ^{73}As-arsenate, and incubated in the dark at in situ temperatures. Subsamples were withdrawn periodically from the syringes to generate time progress curves. The ^{73}As-arsenate was separated from ^{73}As-arsenite using ion exchange resins.

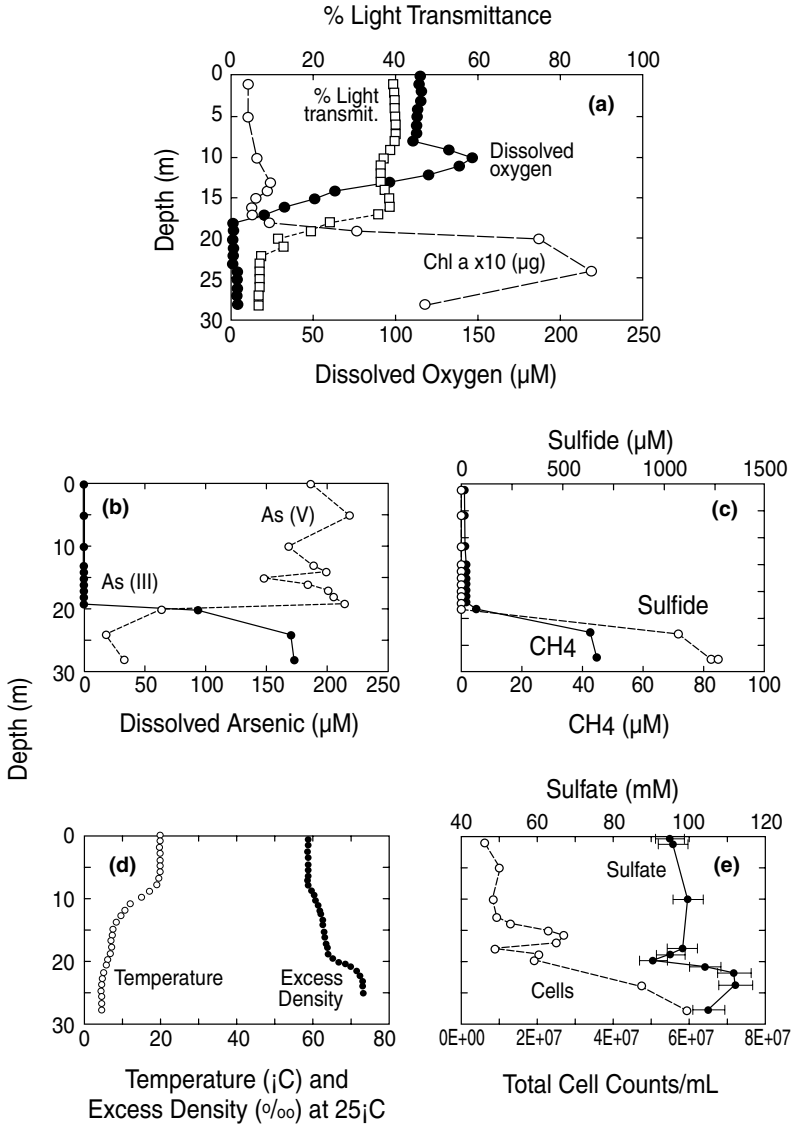

Fig. 3. Vertical profiles of physical, chemical, and biological parameters in Mono Lake obtained in July, 1999 when the lake was meromictic. Reproduced from Oremland et al. [57] with permission.

The ^{73}As-arsenate was not reduced in poisoned or filter-sterilized controls, thereby demonstrating its biological nature. The arsenate reduction assays were run concurrently with those for sulfate-reduction using ^{35}S-sulfate. A profile of activity for bacterial arsenate and sulfate reduction is shown in Fig. 4. Highest rates of arsenate reduction ($\sim$5.5 μmol l^{-1} d^{-1}) were evident at the bottom of the oxycline (18 m), then decreased to $\sim$0.5–1.0 μmol l^{-1} d^{-1} at greater depths. The highest sulfate reduction rates ($\sim$2.3 μmol l^{-1} d^{-1}) occurred at the deepest depths assayed, spatially separated by 10 m from the maximum for arsenate reduction.

The change in water column arsenic speciation over the course of a year as a response to vertical mixing was conducted in 2002 by the team led by Hollibaugh. This afforded an opportunity to determine the reliability of the arsenate reduction rates made with the radiotracer using an independent means of verification. Fig. 5 shows this change in speciation with time at a depth of 24 m as the upper water column underwent winter mixing, followed by the sinking of the late winter phytoplankton bloom, and the subsequent onset of anoxia. The rate of arsenate reduction between March and April corresponds to $\sim$6 μmol l^{-1} d^{-1}, after which it declines to 1.0 μmol l^{-1} d^{-1} from April to May. These calculations are in close agreement with rates determined experimentally during meromixis ($\sim$5.5 μmol l^{-1} d^{-1}; Fig. 4). Following summer stratification, temperature-associated mixing began in the fall and a trace of oxygen was detectable at 24 m depth by December. This created sub-oxic conditions as evidenced by a steep decline in sulfide and arsenite, and the reappearance of arsenate. The rate of arsenite oxidation from September to November extrapolates to $\sim$2.5 μmol l^{-1} d^{-1}, which is comparable to

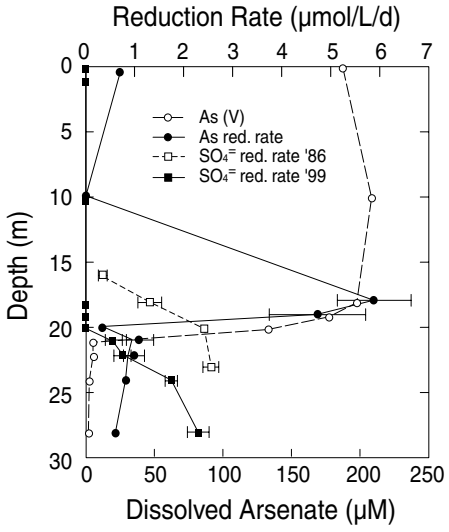

Fig. 4. Vertical profiles of As(V), arsenate reduction, and sulfate reduction in Mono Lake during October, 1999 when the lake was meromictic. The waters below 17 m depth were anoxic. An additional profile of sulfate reduction from the 1986 meromixis is included, when the lake was 4 m shallower. Reproduced from Oremland et al. [57] with permission.

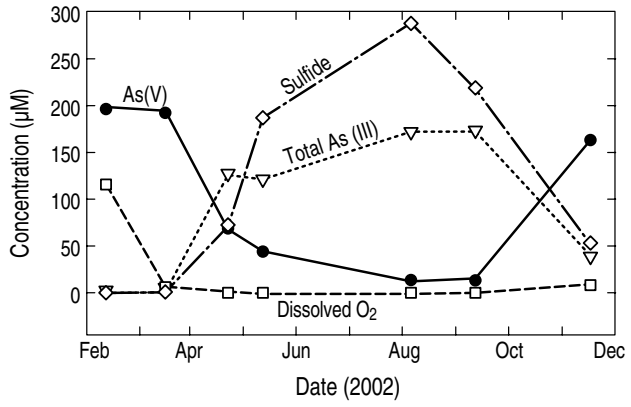

Fig. 5. Temporal variation of dissolved oxygen, arsenate, arsenite, and sulfide at 24 m depth in Mono Lake during 2002 obtained by Hollibaugh.

that observed for arsenate reduction at the beginning of the year.

An estimate of the contribution made by microbial arsenate reduction to the mineralization of organic carbon formed annually by the photosynthetic activity of phytoplankton was made [57]. This was achieved by dividing annual primary productivity (as electron equivalents) by the extrapolated integrated rates for water column arsenate reduction as well as sulfate reduction (Table 1). The results indicated that 8–14% of carbon fixed by phytoplankton primary productivity would be oxidized back to CO_2 by the in situ activities of arsenate-respiring bacteria in the anoxic monimolimnion. Another 41% could be mineralized by sulfate reduction. Ignoring any aerobic degradation in the oxic mixolimnion, this means that about half of the sinking phytoplankton bloom would be oxidized to CO_2 in the anoxic monimolimnion before it settled onto the bottom sediments.

Table 1
Potential contribution of integrated monimolimnion arsenate reduction and sulfate reduction to the mineralization of phytoplankton primary productivity in Mono Lake[a]

Process	Mol/m^2	e$^-$ Mol equiv./m^2	% mineralized
Primary production	22.4[b]	89.6	–
Arsenate reduction	6.4	12.7	14.1
Sulfate reduction	4.6	36.8	41.0

[a] Based on the equation [57]: $CH_2O + 2H_2AsO_4^- \rightarrow HCO_3^- + 2H_2AsO_3^- + H^+$.
[b] Ref. [33].

5. Manipulation experiments with Mono Lake water

Lab-based time course incubation experiments with anoxic Mono Lake water were performed to gain greater insight into what types of electron donors fueled in situ arsenate reduction, and the anions that could inhibit this process [56,57,65]. This included attempts at using "specific" inhibitors of sulfate-reduction (e.g., molybdate, tungstate) to help reveal the physiological types of microbes involved in arsenate reduction [56,57]. Preliminary experiments [57] were conducted with bottom water (24 m) to which 0.25 mM arsenate was added, and arsenate reduction was measured with ^{73}As-arsenate. No obvious stimulation of either arsenate- or sulfate-reduction was achieved with addition of lactate or acetate as substrate amendments, implying that these processes were not electron donor limited. Arsenate itself caused a significant inhibition of sulfate reduction, which was only partially inhibited by tungstate. Curiously, the rates of arsenate reduction were five to sixfold higher than the in situ rates for this depth, but equaled the maximum displayed at 17 m (Fig. 4). It appears that dissimilatory arsenate reduction at 24 m was primarily limited by the availability of arsenate.

Another series of experiments were conducted without radiotracer in which the consumption of added 1.0–2.0 mM arsenate was followed [56]. The rates of arsenate reduction in these experiments were typically 30–50-fold faster (150–250 μmol l^{-1} d^{-1}) than those observed previously at ambient arsenate or with ~0.25 mM arsenate additions, reinforcing the idea of kinetic limitation by arsenate. Rates of arsenate reduction were slightly slower in samples amended with tungstate or molybdate, implying the involvement of sulfate-reducers in a small portion of the overall activity. Since some sulfate-reducers have been shown to respire arsenate [66,67], this was a reasonable interpretation, especially in light of

analyses of the ensuing populations of microbes which showed that sequences for sulfate-reducers appeared in some of the arsenate-amended lakewater samples (see Section 6). No strong stimulation of arsenate reduction was noted by amendment with acetate or lactate, or for that matter with glucose, succinate, or malate again reinforcing the concept that arsenate-reduction in the monimolimnion is not limited by the availability of organic substrates.

The waters of Mono Lake contain an unusually high concentration of dissolved organic carbon, ~6.7 mM. Although DOC is usually composed of refractory organics that are resistant to bacterial attack, it is possible that some portion of this DOC is "labile" in that it fuels arsenate reduction, especially when associated with the senescence of phytoplankton blooms. Alternatively, the monimolimnion also contains high levels of reduced inorganic compounds (sulfide, ammonia) as well as methane (Fig. 3) and it is possible that one or more of these substances serve as electron donors for dissimilatory arsenate reduction. Preliminary experiments have shown that this occurs with sulfide, and a pure culture of a bacterium (strain MLMS-1) was isolated by S.E. Hoeft that demonstrated growth via the sulfide-arsenate couple.

Arsenate reduction was strongly inhibited by the addition of nitrate ions to incubated bottom water [56]. However, rather than acting as a preferred electron acceptor to arsenate by virtue of its higher electrochemical potential (Table 2), nitrate was found to couple to the biological oxidation of arsenite back to arsenate. Hence, the rate of arsenite oxidation essentially equaled that of arsenate reduction, thereby giving the appearance of no loss of added arsenate. In addition to nitrate, other potential biological oxidants included nitrite and to a lesser extent, chelated Fe(III) [65]. Rapid oxidation of arsenite with MnO_2 addition was also observed, but this proved to be of a chemical rather than biological nature. Investigation of in situ arsenite oxidation in lakewater, either with oxygen or nitrate as the electron acceptor, remains a topic for future investigation.

Table 2
Electrochemical potential of some environmentally important redox pairs[a]

Chemical pair	E'_0 (mV)
O_2/H_2O	+818
SeO_4^{2-}/SeO_3^{2-}	+440
NO_3^-/NO_2^-	+430
Fe^{3+}/Fe^{2+}	+100
$HAsO_4^{2-}/H_3AsO_3$	+60
SO_4^{2-}/HS^-	−220
CO_2/CH_4	−244
S^0/HS^-	−270

[a] Compiled from Bard et al. [71], Thauer et al. [72] and Macy [73].

6. Arsenic-metabolizing bacteria in Mono Lake

Two As(V)-respiring anaerobic bacilli, *B. arsenicoselenatis* (strain E1H) and *B. selenitireducens* (strain MLS10), were isolated from Mono Lake sediments using lactate as the electron donor and either selenate (E1H) or selenite (MLS10) as the electron acceptor. Both strains were also capable of anaerobic growth using As(V) as the electron acceptor for the oxidation of lactate [68]

$$\mathrm{Lactate^-} + 2\mathrm{HAsO_4^{2-}} + \mathrm{H^+} \rightarrow \mathrm{Acetate^-} + \mathrm{HCO_3^-} + 2\mathrm{H_2AsO_3^-} \quad (\Delta G'_0 = -156.8\ \mathrm{kJ\,mol^{-1}}).$$

The bacilli proved to be haloalkaliphiles as they exhibited growth maxima at ~60 g l^{-1} salinity and pH at ~9.5, and were therefore well adapted to withstand the chemical conditions of the lake (Fig. 1). Other electron acceptors and donors used for growth are shown in Table 3.

Arsenate-respiring bacteria, as well as sulfate reducers, were also found in the water column of Mono Lake as determined by most probable number culturing methods (Table 4). Not surprisingly, arsenate respirers were most abundant in the anoxic bottom waters where they were about 20-fold more abundant than sulfate reducers. Both types of bacteria were present in the anoxic water column during meromictic as well as monomictic periods. The phylogenetic affiliation of the arsenate reducers cultivated by MPN methods was not pursued. To gain some insight into this question, we performed cold (6 °C) incubations of anoxic bottom water amended with 1 mM arsenate followed by denaturing gradient gel electrophorsis (DGGE) of the extracted DNA [56]. Unique bands that appeared in the arsenate-enriched water yielded sequence data that affiliated them with either ε- or δ-Proteobacteria in the *Thiomicrospira* and *Desulfovibrio* groups, respectively, clades in which arsenate respirers have not as yet been described. However, some 16 species of arsenate-respirers have been reported in the literature since 1994 [24], and the list is growing even as this manuscript was being written (e.g., [69]). Taxonomically, these organisms can be found within several diverse divisions of prokaryotes (e.g., β-, γ-, δ-, and ε-Proteobacteria, low G+C gram-positive bacteria, and Crenarchaea), and therefore it is not surprising that novel clades within these broad divisions responded to arsenate-enrichment, implying that there are new species within these divisions that are yet to be cultivated in pure culture.

These results, of course, did not prove that populations found in these enrichments were representative of the natural arsenate-respiring flora in Mono Lake water. Indeed, as reviewed in Section 3 above, a survey of microbial diversity made by extraction of ambient DNA in the lake water (i.e., no arsenate enrichment or

Table 3
Some general characteristics of three arsenic-metabolizing anaerobic bacteria isolated from Mono Lake[a]

Organism	Taxonomy	e^- donors	e^- acceptors	pH optimum	Salinity optimum
Bacillus selenitireducens strain MLS10	Gram+low G+C	Lactate, pryruvate, fructose, glucose starch	Se(IV), Se(0), S(0), As(V), fumarate, nitrate, nitrite, TMAO, low O_2	9.8 range: 7.3–11.2	60 range: 20–220
Bacillus arsenicoselenatis strain E1H	Gram+low G+C	Lactate, malate, citrate, starch	Se(VI), As(V), nitrate, Fe(III), fumarate	9.5 range: 7.3–10.1	60 range: 10–140
Strain MLHE-1	γ-Proteobacteria	As(III), H_2S^{2-}, acetate, H_2	Nitrate, air		

[a] Compiled from Switzer Blum et al. [68]; Oremland et al. [65], and Herbel et al. [74].

incubation) demonstrated that anoxic bottom waters were dominated by low G+C gram-positive bacteria of the *Bacillus*/*Clostridium* families [43]. This would suggest that organisms like *B. arsenicoselenatis* and *B. selenitireducens* are more typical of water column arsenate-reducers. Nonetheless, to prove this point, future studies will need to be performed using molecular techniques that are more specific than phylogenetic probes like fluorescence in situ hybridization (FISH). One possibility would be to target the RNA for expression of dissimilatory arsenate reductase. However, before this can be attempted it will first require the purification of their arsenate-reductase and identification of this enzyme's encoding genes (see Section 7).

An anaerobic arsenite-oxidizing bacterium, strain MLHE-1, capable of chemoautotrophic growth with arsenite, sulfide, or hydrogen as its electron donor and nitrate as its electron acceptor, was isolated from Mono Lake bottom water [65]. Growth in mineral salts medium on arsenite plus nitrate conforms to the following stoichiometry:

$$H_2AsO_3^- + NO_3^- \rightarrow H_2AsO_4^- + NO_2^-$$
$$(\Delta G_0' = -87.16 \text{ kJ mol}^{-1}).$$

Strain MLHE-1 was capable of dark $^{14}CO_2$ fixation with arsenite, and contained a Form 1 RuBisCo gene most similar to that of an unidentified *Thiobacillus* sp. (86% similarity). Strain MLHE-1 was also able to grow as a heterotroph with acetate as its electron donor either as an aerobe or with nitrate under anaerobic conditions. However, no growth on arsenite or its oxidation occurred under aerobic conditions. Strain MLHE-1 is a member of the *Ectothiorhodospira* clade of the γ-Proteobacteria, but is incapable of photosynthetic growth and lacks pigments. Previously, all other bacteria capable of autotrophic growth on arsenite were found in the α-Proteobacteria, and they are obligate aerobes [70].

Table 4
Cell densities of arsenate- and sulfate-reducing bacteria in the water column of Mono Lake as determined by MPN culture serial dilutions[a]

Cells ml^{-1}		
Depth (m)	SRBs	AsRBs
5	0.4 (0.05–1.23)	4.3 (1.03–13.8)
18	2.4 (0.48–9.65)	42.7 (10.3–139)
24	21.5 (3.45–89.8)	427.3 (103–1,385)

[a] Oremland et al. [57]; values in parentheses represent 95% confidence intervals.

7. Arsenic enzymes and genes

The isolation of the two Bacillus species from Mono Lake (*B. selenitireducens*, *B. arsenoselenatis*) provided the opportunity to investigate the pathway of arsenate reduction in these strains. Initial investigations indicated that they both possessed a membrane-associated enzyme that could couple the oxidation of methyl viologen to arsenate reduction. The respiratory arsenate reductase from *B. selenitireducens* was subsequently purified [75]. Its characterization has revealed some features in common with the enzyme that has been previously purified from the arsenate-respiring bacterium *Crysiogenes arsenatis* [76], but has some distinctions. It is tightly associated with the cytoplasmic membrane and requires solubilization with detergent (i.e., 2% Triton X-100). The active enzyme was purified in a heterodimeric state, with subunits of 110 and 34 kDa. This compares with the heterodimer of 87 and 29 kDa for *C. arsenatis*. The large subunit, ArrA, contains the catalytic site, while the smaller subunit, ArrB, is an iron-sulfur-cluster protein assumed to be involved in electron shuttling. The enzyme has a significantly higher affinity for arsenate (K_m of 34 μM as compared to 300 μM for *C. arsenatis*) and exhibits an optimal activity at pH 9.5 and salinity of 90 g l^{-1}. The enzyme is also able to oxidize methyl viologen with selenate, selenite, and arsenite but not nitrate, nitrite, or fumarate. Thus it shows broader substrate specificity than *C. arsenatis* which was reported to be arsenate specific [76]. Comparison of the N-terminal amino acid sequence of *B. selenitireducens* indicated a high degree of identity with the known amino acid sequence of *C. arsenatis*. Thus, the *B. selenitireducens* ArrA (20 amino acids) had a 50% identity and 85% similarity to that of *C. arsenatis*, while the ArrB (31 amino acids) had a 48% identity and 87% similarity.

Using the N-terminal sequence data, degenerate primers were designed to PCR amplify the gene encoding *arrA*. A 2.5 kb amplicon was obtained which con-

tained all but the 5′ end of *arrA*, an intergenic region of 29 nucleotides, and 30 nucleotides of the 5′ end of *arrB* [75]. The inferred protein had a molecular mass of 90,980 Da and a p*I* of 5.12. It shares a 56.7% identity with the *arrA* from *Shewanella* sp. strain ANA-3 [77]. The highest degree of identity was with a gene that encodes a hypothetical protein from the genome of *Desulfitobacterium hafniense*. *D. hafniense*, a low mole G+C gram-positive bacterium, was shown to be capable of using arsenate as a terminal electron acceptor, although its arsenate reductase was not identified [78]. Analysis of the contig revealed that the putative *arr* operon contains at least seven genes. The first three genes are putative regulatory elements and include a sensory kinase and a response regulator. These are followed by a gene that encodes a protein of unknown homology but is predicted to be membrane integral, then *arrA* and *arrB* [75]. The last gene before the contig ends encodes a chaperon-like protein similar to TorD and SerD. This operon is unlike the *arr* operon from *Shewanella* sp. strain ANA-3 that consists only of *arrA* and *arrB* [79]. Whether the *arr* operon from *B. selenitireducens* contains additional genes remains to be determined.

The phylogenetic diversity of prokaryotes capable of arsenate respiration minimizes the usefulness of molecular probes based on 16S rDNA sequence. However, the high degree of identity between the respiratory arsenate reductases from phylogenetically distant organisms suggests it will possible to develop biochemical (i.e. immunohistochemical) and molecular probes. Indeed, alignment of *arrA* from *B. selenitireducens*, *Shewanella* sp. strain ANA-3, and *D. hafniense* reveal several regions with high identity that differentiate this protein from other related members of the dimethyl sulfoxide (DMSO) reductase class of enzymes. We are currently investigating whether these regions will provide useful epitopes for both Western blot analysis and fluorescent in situ labeling of cells. The development of molecular probes may present a greater challenge as coding bias is evident in the few gene sequences currently available. While comparison of *arrA* from two species, such as *B. selenitireducens* and *D. hafniense* may show a high degree of sequence identity (e.g., 60%), this identity drops to 43% when the sequence from *Shewanella* sp. strain ANA-3 is added to the mix. Nevertheless, degenerate oligonucleotide primers have been used successfully by D.K. Newman to clone the putative *arrA* from a number of diverse species.

While there are more than 30 species of bacteria known to oxidize arsenate, only seven are chemolithoautotrophs that can grow by coupling the energy gained from this oxidation to the incorporation of CO_2 into cellular material [24]. The arsenite oxidizing bacterium strain MLHE-1, isolated from Mono Lake, was the first to couple the oxidation of arsenic to the reduction of nitrate [65]. To date only the arsenite oxidase from the heterotrophic bacterium *Alcaligenes faecalis* has been purified and fully characterized (including the crystal structure) [80]. A mononuclear molybdenum enzyme belonging to the DMSO class, it is comprised of two subunits. The catalytic subunit (~85 kDa) contains molybdenum bound to two pterin cofactors and a [3Fe-4S] cluster. The associated subunit (~14 kDa) presumably functions as an electron shuttle and has a Rieske-type [2Fe-2S] cluster. More recently, the genes encoding the catalytic and Rieske subunits have been cloned and sequenced from the beta Proteobacterium *Cenibacterium arsenoxidans* strain ULPAs1 [81] and identified in the genomes of several other organisms including *Chloroflexus auranticus*, *Sulfolobus tokodaii*, and *Aeropyrum pernix* [82]. A comparison of the amino acid sequence of the catalytic subunit (AoxA) from these five organisms shows they share only 15.7% identity and 42% similarity. While the Rieske subunit (AoxB) shows slightly better homology (23% identity and 48% similarity), this is due to the highly conserved nature of the iron sulfur cluster binding site. Thus, whether biochemical and molecular probes that specifically recognize arsenite oxidases can be successfully developed remains to be determined. An interesting finding was that the evolutionary lineage of the AoxA subunit seemed to occur prior to the taxonomic split between the Bacteria and Archaea [82]. This suggests that arsenite oxidation is an ancient trait that developed quickly in the Earth's early biosphere.

8. Organic arsenicals

In this review we have focused upon the energy-yielding transformations of inorganic arsenic in Mono Lake that are mediated by prokaryotes. This has constrained the discussion to the reduction of arsenate to arsenite and the corresponding reverse oxidation reaction. We were justified in this approach because Anderson and Bruland [17] have shown that organo-arsenicals are not abundant in the lake relative to its large inorganic pool. However, the analytical technique employed by these workers was essentially overwhelmed by the great size of the inorganic arsenic pool, thereby limiting its ability to directly detect any organo-arsenicals in Mono Lake surface water. Thus, their reported values were "less than 100 nM" for monomethylarsonic acid (MMAA) and dimethylarsenic acid (DMAA). For comparison, they also reported quite respectable concentrations of MMAA (8.6 nM) and DMAA (33 nM) in the saline Salton Sea, where the inorganic arsenic concentration is only 0.13 μM, or over three orders of magnitude less than that of Mono Lake. It seems possible that significant concentrations of organo-arsenicals occur in Mono Lake, and that a future lake-wide survey aimed at describing their molecular

identification, distribution, and seasonal variability would be justified.

Although little is known about organoarsenicals in alkaline soda lakes, the marine environment has been well-studied in this regard. Therefore, we can use what we know about marine systems as a departure point from which to formulate hypotheses concerning the biogeochemical cycling of organoarsenicals in Mono Lake. For example, it is well known that methylated arsenicals are synthesized by marine phytoplankton [83] and that these substances are present in ocean water [84] and anoxic marine sediments [84,85]. Of the various marine organoarsenicals, one of the most widespread forms is arsenobetaine. Arsenobetaine, an analog of the organic osmolyte glycine betaine, is commonly found in a diversity of marine animals including fish and invertebrates [86]. It would make considerable "sense" if the microalgae and zooplankton in Mono Lake employed arsenobetaine as an osmolyte, because its biosynthesis would additionally confer a protective mechanism to counter the toxicity of inorganic arsenic ions entering the cell. Preliminary measurements of samples of the brine shrimp *Artemia monica* taken from Mono Lake failed to detect much arsenobetaine; however, high levels of MMAA and DMAA were detected by W.C. Cullen in the extracted tissues. Glycine betaine is an important osmolyte in the eukaryotic picoplankter *Picocystis* strain ML from Mono Lake [59], but it is not known if cells contain arsenobetaine. Strain ML-D, an aerobic bacterium isolated from Mono Lake accumulated arsenobetaine when it was provided externally, accounting for as much as 28% of the internal osmolyte pool which also consisted of ectoine, DMSP (dimethylsulfoniopropionate), and glycine betaine [87]. It is not known as yet if the organisms living in Mono Lake actually synthesize and re-cycle arsenobetaine in situ.

The pelagic and littoral sediments of Mono Lake have active methanogenic activity [88–90]. The high concentration of sulfate in the lake competitively eliminates acetate and H_2 from methanogenic pathways, which shifts methane precursors to non-competitive methylotrophic substrates like trimethylamine or dimethylsulfide. Anaerobic degradation of arsenobetaine could follow pathways analogous to that for glycine betaine [91] resulting in the formation of trimethylarsine rather than trimethylamine. Methanogenic degradation of ^{14}C-trimethylamine was detected in Mono Lake pelagic sediments [90] but was inhibited when littoral sediments were incubated with 10 mM trimethylamine, probably due to the buildup of ammonia as an end product, which is toxic at this pH [91]. It is not known if methylotrophic methanogens can degrade trimethylarsine since the endproduct, arsine gas (H_3As), would be even more toxic than the corresponding concentration of ammonia.

The subject of microbial methylation of arsenic and related metalloids was recently reviewed by Bentley and Chasteen [92]. Methylotrophic methanogens can demethylate metalloids like dimethylselenide, which is an analog of dimethylsulfide, one of their recognized growth substrates [93]. However, in the case of arsenic, McBride and Wolfe [94] demonstrated the opposite, namely that whole cells and extracts of hydrogen-oxidizing *Methanobacterium bryantii* could form dimethylarsine from externally supplied arsenate ions. It appears that the ability of various species of methanogenic archaea to either methylate or demethylate toxic elements like arsenic is an area ripe for closer scrutiny. Certainly novel isolates from arsenic rich environments like Mono Lake would be worth testing in this capacity.

9. Future directions

Mono Lake has provided us with a wealth of new insights with regard to the diversity of microbes carrying out arsenic metabolism, their novel biochemical reactions, and the importance of these reactions to the biogeochemical cycles occurring in the lake, including the carbon cycle. The first two haloalkaliphiles isolated from the lake, *B. selenitireducens* and *B. arsenicoselenatis* grow as conventional heterotrophs while respiring arsenate. However, two novel strains, MLHE-1 and MLMS-1, provide an autotrophic-based linkage of arsenic to the nitrogen and sulfur cycles of the lake. In the case of MLHE-1, nitrate-linked oxidation of arsenite has already been demonstrated for more conventional freshwater environments, such as Upper Mystic Lake [62], and a subsurface aquifer in Bangladesh [95]. Clearly, work in such an extreme environment like Mono Lake has technical transferability to our understanding of hydrologic systems of more concern to human health.

Future research work in the lake should focus on using molecular tools, such as functional gene probes, for enumerating arsenate-respiring and arsenite-oxidizing populations. Such efforts will directly rely on progress made in the laboratory on the biochemistry and genetics of arsenic metabolism of Mono Lake arsenotrophs, as well as with isolates from freshwater systems. It also will be important to develop radiotracer assays to quantify the rates of arsenate reduction and arsenite oxidation occurring in the lake's shallow sediments. Although a potential impediment may be the adsorptive binding of the ^{73}As-arsenate radiotracer with any Fe(III) present in the sediments [96], for Mono Lake in particular this may be more than offset by the large amount of available phosphate that would compete with arsenate for binding sites. Such research may ultimately lead to practical techniques that can be adapted to freshwater systems and drinking water aquifers. Therefore, Mono Lake and similar soda lakes are a good departure points to initiate such an endeavor.

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References

[1] Nriagu, J.O. (2002) Arsenic poisoning through the ages. In: Environmental Chemistry of Arsenic (Frankenberger Jr., W.T. Ed.), pp. 1–26. Marcel Dekker, New York.
[2] Bagla, P. and Kaiser, J. (1996) India's spreading health crisis draws global arsenic experts. Science 274, 174–175.
[3] Nickson, R., McArthur, J., Burgess, W., Ahmed, K.M., Ravenscroft, P. and Rahman, M. (1998) Arsenic poisoning of Bangladesh groundwater. Nature 395, 338.
[4] McArthur, J., Ravenscroft, P., Safiulla, S. and Thirwall, M.F. (2002) Arsenic in groundwater: testing pollution mechanisms for sedimentary aquifers in Bangladesh. Water Resources Res. 37, 109–117.
[5] Cullen, W.R. and Reimer, K.J. (1989) Arsenic speciation in the environment. Chem. Rev. 89, 713–764.
[6] Zobrist, J., Dowdle, P.R., Davis, J.A. and Oremland, R.S. (2000) Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. Environ. Sci. Technol. 34, 4747–4753.
[7] Kneebone, P.E. and Hering, J. (2000) Behavior of arsenic and other redox-sensitive elements in Crowley Lake, CA: a reservoir in the Los Angeles aqueduct system. Environ. Sci. Technol. 34, 4307–4312.
[8] Wilkie, J.A. and Hering, J. (1998) Rapid oxidation of geothermal arsenic (III) in streamwaters of the eastern Sierra Nevada. Environ. Sci. Technol. 32, 657–662.
[9] National Research Council, 1999. Arsenic in Drinking Water. National Academy Press, Washington, DC, pp. 310.
[10] Seyler, P. and Martin, J.M. (1989) Biogeochemical processes affecting arsenic species distribution in a permanently stratified lake. Environ. Sci. Technol. 23, 1258–1263.
[11] Spliethoff, H.M., Mason, R.P. and Hemond, H.F. (1995) Interannual variability in the speciation and mobility of arsenic in a dimictic lake. Environ. Sci. Technol. 29, 2157–2161.
[12] Levy, D.B., Schramke, J.A., Esposito, K.J., Erickson, T.A. and Moore, J.C. (1999) The shallow ground water chemistry of arsenic, fluorine, and major elements: Eastern Owens Lake, California. Appl. Geochem. 14, 53–65.
[13] Ryu, J-H., Gao, S., Dahlgren, R.A. and Zierenberg, R.A. (2002) Arsenic distribution, speciation, and solubility in shallow groundwater of Owens Dry Lake, California. Geochim. Cosmochim. Acta 66, 2981–2994.
[14] Nordstrom, D.K. (2002) Worldwide occurrences of arsenic in ground water. Science 296, 2143–2144.
[15] Welch, A.H. and Lico, M.S. (1998) Factors controlling As and U in shallow ground water, southern Carson Desert, Nevada. Appl. Geochem. 13, 521–539.
[16] Smedley, P.L., Nicolli, H.B., Macdonald, D.M.J., Barros, A.J. and Tullio, J.O. (2002) Hydrochemistry of arsenic and other inorganic constituents in groundwaters from La Pampa, Argentina. Appl. Geochem. 17, 259–284.
[17] Anderson, L.C.D. and Bruland, K.W. (1991) Biogeochemistry of arsenic in natural waters: the importance of methylated species. Environ. Sci. Technol. 25, 420–427.
[18] Rosen, B.P. (2002) Biochemistry of arsenic detoxification. FEBS Lett. 529, 86–92.
[19] Mukhopadhyay, R., Rosen, B.P., Phung, L.T. and Silver, S. (2002) Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. 26, 311–325.
[20] Newman, D.K., Ahmann, D. and Morel, F.M.M. (1998) A brief review of arsenate respiration. Geomicrobiol. J. 15, 255–268.
[21] Stolz, J.F. and Oremland, R.S. (1999) Bacterial respiration of arsenic and selenium. FEMS Microbiol. Rev. 23, 615–627.
[22] Oremland, R.S., Newman, D.K., Kail, B.W. and Stolz, J.F. (2001) Bacterial respiration of arsenic and its significance in the environment. In: Environmental Chemistry of Arsenic (Frankenberger Jr., W.T. Ed.), pp. 273–296. Marcel Dekker, New York.
[23] Ehrlich, H.L. (2001) Bacterial oxidation of As(III) compounds. In: Environmental Chemistry of Arsenic (Frankenberger Jr., W.T. Ed.), pp. 313–327. Marcel Dekker, New York.
[24] Oremland, R.S. and Stolz, J.F. (2003) The ecology of arsenic. Science 300, 939–944.
[25] Christensen, M.N., Gilbert, C.M., Lajoie, K.R. and Al Rawi, Y. (1969) Geological-geophysical interpretation of Mono Basin, California – Nevada. J. Geophys. Res. 74, 5221–5239.
[26] Hill, D.P., Bailey, R.A. and Ryall, A.S. (1985) Active tectonic and magmatic processes beneath Long Valley caldera, eastern California: an overview. J. Geophys. Res. 90, 11,111–11,120.
[27] Sieh, K. and Bursik, M. (1986) Most recent eruption of the Mono Craters, eastern central California. J. Geophys. Res. 91, 12,539–12,571.
[28] Simpson, H.J., Trier, R.M., Olsen, C.R., Hammond, D.E., Ege, A., Miller, L. and Melack, J.M. (1980) Fallout plutonium in an alkaline, saline lake. Science 207, 1071–1073.
[29] Simpson, H.J., Trier, R.M., Toggweiler, J.R., Mathieu, G., Deck, B.L., Olsen, C.R. and Hammond, D.E. (1982) Radionuclides in Mono Lake, California. Science 216, 512–514.
[30] Anderson, R.F., Bacon, M.P. and Brewer, P.G. (1982) Elevated concentrations of actinides in Mono Lake. Science 216, 514–516.
[31] Oremland, R.S., Miller, L.G. and Whiticar, M.J. (1987) Sources and flux of natural gases from Mono Lake, California. Geochim. Cosmochim. Acta 51, 2915–2929.
[32] Joye, S.B., Connell, T.L., Miller, L.G., Jellison, R. and Oremland, R.S. (1999) Oxidation of ammonia and methane in an alkaline, saline lake. Limnol. Oceanogr. 44, 178–188.
[33] Jellison, R. and Melack, J.M. (1993) Meromixis in hypersaline Mono Lake, California: 1. Vertical mixing and density stratification during the onset, persistence, and breakdown of meromixis. Limnol. Oceanogr. 38, 1008–1019.
[34] Jellison, R., Romero, J. and Melack, J.M. (1998) The onset of meromixis during restoration of Mono Lake, California: unintended consequences of reducing water diversions. Limnol. Oceanogr. 43, 706–711.
[35] Jellison, R. and Melack, J.M. (1988) Photosynthetic activity of phytoplankton and its relation to environmental factors in hypersaline Mono Lake, California. Hydrobiologia 158, 69–88.
[36] Jellison, R. and Melack, J.M. (1993) Algal photosynthetic activity in response to meromixis in hypersaline Mono Lake, California: nitrogen fluxes. Limnol. Oceanogr. 38, 818–837.
[37] Jellison, R., Miller, L.G., Melack, J.M. and Dana, D.L. (1993) Meromixis in hypersaline Mono Lake, California. 2. Nitrogen fluxes. Limnol. Oceanogr. 38, 1020–1039.
[38] Miller, L.G., Jellison, R., Oremland, R.S. and Culbertson, C.W. (1993) Meromixis in hypersaline Mono Lake, California: 3. Biogeochemical response to stratification and overturn. Limnol. Oceanogr. 38, 1040–1051.
[39] Maest, A.M., Pasilis, S.P., Miller, L.G. and Nordstrom, D.K. (1992) Redox geochemistry of arsenic and iron in Mono Lake, California, USA. In: Water–Rock Interaction, WRI-7 (Kharaka, Y.K. and Maest, A.M., Eds.), pp. 507–511. Balkema, Netherlands.

[40] Horikoshi, K. and Akiba, T. (1982) Alkaliphilic Microorganisms. Springer, Berlin.
[41] Grant, W.D., Mwatha, W.E. and Jones, B.E. (1990) Alkaliphiles: ecology, diversity, and applications. FEMS Microbiol. Rev. 75, 255–270.
[42] Oren, A. (2002) Halophilic Microorganisms and their Environments. Kluwer Academic Publishers, Dordrecht, NL.
[43] Humayoun, S.B., Bano, N. and Hollibaugh, J.T. (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. Appl. Environ. Microbiol. 69, 1030–1042.
[44] von Wintzingerode, F., Göbel, U.B. and Stackebrandt, E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. 21, 213–329.
[45] Delong, E.F. (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA 89, 5685–5689.
[46] Raskin, L., Stromley, J.M., Rittmann, B.E. and Stahl, D.A. (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. Appl. Environ. Microbiol. 60, 1232–1240.
[47] Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W. and Saunders, J.R. (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. Appl. Environ. Microbiol. 62, 668–675.
[48] Good, I.J. (1953) The population frequencies of species and the estimation of the population parameters. Biometrika 40, 237–264.
[49] Singleton, D.R., Furlong, M.A., Rathbun, S.L. and Whitman, W.B. (2001) Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Appl. Environ. Microbiol. 67, 4374–4376.
[50] Curtis, T.P., Sloan, W.T. and Scannell, J.W. (2002) Estimating prokaryotic diversity and its limits. Proc. Natl. Acad. Sci. USA 99,, 10494–10499.
[51] Torsvik, V., Goksoyr, J. and Daae, F.L. (1990) High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56, 782–787.
[52] Torsvik, V., Sorheim, R. and Goksoyr, J. (1996) Total bacterial diversity in soil and sediment communities – a review. J. Ind. Microbiol. 17, 170–178.
[53] Torsvik, V., Ovreas, L. and Thingstad, T.F. (2002) Prokaryotic diversity – magnitude, dynamics, and controlling factors. Science 296, 1064–1066.
[54] Hollibaugh, J.T., Wong, P.S., Bano, N., Pak, S.K., Prager, E.M. and Orrego, C. (2001) Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event. Hydrobiologia 446, 45–60.
[55] MacIntyre, S. and Jellison, R. (2001) Nutrient fluxes from upwelling and enhanced turbulence at the top of the pycnocline in Mono Lake, California. Hydrobiologia 446, 13–29.
[56] Hoeft, S.E., Lucas, F., Hollibaugh, J.T. and Oremland, R.S. (2002) Characterization of bacterial arsenate reduction in the anoxic bottom waters of Mono Lake, California. Geomicrobiol. J. 19, 1–19.
[57] Oremland, R.S., Dowdle, P.R., Hoeft, S., Sharp, J.O., Schaefer, J.K., Miller, L.G., Switzer Blum, J., Smith, R.L., Bloom, N.S. and Wallschlaeger, D. (2000) Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. Geochim. Cosmochim. Acta 64, 3073–3084.
[58] Cloern, J.E., Cole, B.E. and Oremland, R.S. (1983) Seasonal changes in the chemical and biological nature of a meromictic lake (Big Soda Lake, Nevada, USA). Hydrobiologia 105, 195–206.
[59] Roesler, C.S., Culbertson, C.W., Etheridge, S.M., Goericke, R., Kiene, R.P., Miller, L.G. and Oremland, R.S. (2002) Distribution, production, and ecophysiology of *Picocystis* strain ML in Mono Lake, California. Limnol. Oceanogr. 47, 440–452.
[60] Cherry, J.A., Shaikh, A.U., Tallman, D.E. and Nicholson, R.V. (1979) Arsenic species as an indicator of redox conditions in groundwater. J. Hydrol. 43, 373–392.
[61] Newman, D.K., Beveridge, T.J. and Morel, F.M.M. (1997) Precipitation of As_2S_3 by *Desulfotomaculum auripigmentum*. Appl. Environ. Microbiol. 63, 2022–2028.
[62] Senn, D.B. and Hemond, H.F. (2002) Nitrate controls on iron and arsenic in an urban lake. Science 296, 2373–2376.
[63] Rochette, E.A., Bostick, B.C., Li, G. and Fendorf, S. (2000) Kinetics of arsenate reduction by dissolved sulfide. Environ. Sci. Technol. 34, 4714–4720.
[64] Wilkin, R.T., Wallschläger, D. and Ford, R.G. (2003) Speciation of arsenic in sulfidic waters. Geochem. Trans. 41, 1–7.
[65] Oremland, R.S., Hoeft, S.E., Santini, J.M., Bano, N., Hollibaugh, R.A. and Hollibaugh, J.T. (2002) Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. Appl. Environ. Microbiol. 68, 4795–4802.
[66] Newman, D.K., Kennedy, E.K., Coates, J.D., Ahmann, D., Ellis, D.J., Lovley, D.R. and Morel, F.M.M. (1997) Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. Arch. Microbiol. 168, 380–388.
[67] Macy, J.M., Santini, J.M., Pauling, B.V., O'Neill, A.H. and Sly, L.I. (2000) Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Arch. Microbiol. 173, 49–57.
[68] Switzer Blum, J., Burns Bindi, A., Buzzelli, J., Stolz, J.F. and Oremland, R.S. (1998) *Bacillus arsenicoselenatis* sp. nov., and *Bacillus selenitireducens* sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch. Microbiol. 171, 19–30.
[69] Takai, K., Kobayashi, H., Nealson, K.H. and Horikoshi, K. (2003) *Deferribacter desulfuricans* sp. nov., a novel sulfur-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent. Int. J. Syst. Evol. Microbiol. 53, 839–846.
[70] Santini, J.M., Sly, L.I., Wen, A., Comrie, D., De Wulf-Durand, P. and Macy, J.M. (2002) New arsenite-oxidizing bacteria isolated from Australian gold mining environments–phylogenetic relationships. Geomicrobiol. J. 19, 67–76.
[71] Bard, A.J., Parsons, R.S. and Jordon, J. (1985) Standard Potential in Aqueous Solutions. Marcel Dekker, New York.
[72] Thauer, R.K., Jungermann, K. and Decker, K. (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41, 100–180.
[73] Macy, J.M. (1994) Biochemistry of selenium metabolism by *Thauera selenatis* gen. nov., sp. nov., and use of the organism for bioremediation of selenium oxyanions in San Joaquin Valley drainage water. In: Selenium in the Environment (Frankenberger Jr., W.T. Ed.), pp. 421–444. Marcel Dekker, New York.
[74] Herbel, M.J., Switzer Blum, J., Borglin, S.E. and Oremland, R.S. (2003) Reduction of elemental selenium to selenide: experiments with anoxic sediments and bacteria that respire Se-oxyanions. Geomicrobiol. J. 20, 587–602.
[75] Afkar, E., Lisak, J., Saltikov, C., Basu, P., Oremland, R.S. and Stolz, J.F. (2003) The respiratory arsenate reductase from the haloalkaliphilic bacterium *Bacillus selenitireducens* strain MLS10. FEMS Lett. 226, 107–112.
[76] Krafft, T. and Macy, J.M. (1998) Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. Eur. J. Biochem. 255, 647–653.
[77] Saltikov, C.W. and Newman, D.K. (2003) Genetic identification of a respiratory arsenate reductase. Proc. Natl. Acad. Sci. USA 100, 10983–10988.
[78] Niggenmeyer, A., Spring, S., Stackenbrandt, E. and Rosenzweig, R.F. (2001) Isolation and characterization of a novel As(V)-reducing bacterium: Implications for arsenic mobilization and the genus *Desulfitobacterium*. Appl. Environ. Microbiol. 67, 5568–5580.
[79] Saltikov, C.W., Cifuentes, A., Venkateswaran, K. and Newman, D.K. (2003) 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. 69, 2800–2809.

[80] Anderson, G.L., Williams, J. and Hille, R. (1992) The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. J. Biol. Chem. 267, 23674–23682.
[81] Muller, D., Lievremont, D., Simeonova, D.D., Hubert, J.C. and Lett, M.C. (2003) Arsenite oxidase *aox* genes from a metal-resistant β-proteobacterium. J. Bacteriol. 185, 135–141.
[82] Lebrun, E., Brugna, M., Baymann, F., Muller, D., Lievremont, D., Lett, M.C. and Nitschke, W. (2003) Arsenite oxidase, an ancient bioenergetic enzyme. Mol. Biol. Evol. 20, 686–693.
[83] Andreae, M.O. and Klumpp, D. (1979) Biosynthesis and release of organoarsenic compounds by marine algae. Environ. Sci. Technol. 13, 738–741.
[84] Andreae, M.O. (1979) Arsenic speciation in seawater and interstitial waters: the influence of biological-chemical interactions on the chemistry of a trace element. Limnol. Oceanogr. 24, 440–452.
[85] Reimer, K.J. and Thompson, J.A.J. (1988) Arsenic speciation in marine interstitial water. The occurrence of organoarsenicals. Biogeochemistry 6, 211–237.
[86] Hanaoka, K., Yamamoto, H., Kawashima, K., Tagawa, S. and Kaise, T. (1988) Ubiquity of arsenobetaine in marine animals and degradation of arsenobetaine by sedimentary micro-organisms. Appl. Organomet. Chem. 2, 371–376.
[87] Ciulla, R.A., Diaz, M.R., Taylor, B.F. and Roberts, M.F. (1997) Organic osmolytes in aerobic bacteria from Mono Lake, an alkaline, moderately hypersaline environment. Appl. Environ. Microbiol. 63, 220–226.
[88] Kiene, R.P., Oremland, R.S., Catena, A., Miller, L.G. and Capone, D.G. (1986) Metabolism of reduced methylated sulfur compounds by anaerobic sediments and a pure culture of an estuarine methanogen. Appl. Environ. Microbiol. 52, 1037–1045.
[89] Oremland, R.S. and King, G.M. (1989) Methanogenesis in hypersaline environments. In: Microbial Mats. Physiological Ecology of Benthic Microbial Communities (Cohen, Y. and Rosenberg, E., Eds.), pp. 180–189. ASM Press, Washington, DC.
[90] Oremland, R.S., Miller, L.G., Culbertson, C.W., Robinson, S.W., Smith, R.L., Lovley, D., Whiticar, M.J., King, G.M., Kiene, R.P., Iversen, N. and Sargent, M. (1993) Aspects of the biogeochemistry of methane in Mono Lake and the Mono Basin of California. In: Biogeochemistry of Global Change. Radiatively Active Trace Gases (Oremland, R.S., Ed.), pp. 704–741. Chapman & Hall, New York.
[91] King, G.M. (1984) On the metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. Appl. Environ. Microbiol. 53, 2426–2434.
[92] Bentley, R. and Chasteen, T.G. (2002) Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol. Mol. Biol. Revs. 66, 250–271.
[93] Oremland, R.S. and Zehr, J.P. (1986) Formation of methane and carbon dioxide from dimethylselenide in anoxic sediments and by a methanogenic bacterium. Appl. Environ. Microbiol. 52, 1031–1036.
[94] McBride, B.C. and Wolfe, R.S. (1971) Biosynthesis of dimethylarsine by *Methanobacterium*. Biochemistry 10, 4312–4317.
[95] Harvey, C.F., Swartz, C.H., Badruzzaman, A.B.M., Keon-Blute, N., Yu, W., Ali, M.A., Jay, J., Beckie, R., Niedan, V., Brabander, D., Oates, P.M., Ashfaque, K.N., Islam, S., Hemond, H.F. and Ahmed, M.F. (2002) Arsenic mobility and groundwater extraction in Bangladesh. Science 298, 1602–1606.
[96] Domagalski, J.L., Orem, W.H. and Eugster, H.P. (1989) Organic geochemistry and brine composition in Great Salt, Mono, and Walker Lakes. Geochim. Cosmochim. Acta 53, 2857–2872.