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Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California

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Abstract—The stratified (meromictic) water column of alkaline and hypersaline Mono Lake, California, contains high concentrations of dissolved inorganic arsenic (~200 μmol/L). Arsenic speciation changes from arsenate [As (V)] to arsenite [As (III)] with the transition from oxic surface waters (mixolimnion) to anoxic bottom waters (monimolimnion). A radioassay was devised to measure the reduction of ^{73}As (V) to ^{73}As (III) and tested using cell suspensions of the As (V)-respiring *Bacillus selenitireducens*, which completely reduced the ^{73}As (V). In field experiments, no significant activity was noted in the aerobic mixolimnion waters, but reduction of ^{73}As (V) to ^{73}As (III) was observed in all the monimolimnion samples. Rate constants ranged from 0.02 to 0.3/day, with the highest values in the samples from the deepest depths (24 and 28 m). The highest activities occurred between 18 and 21 m, where As (V) was abundant (rate, ~5.9 μmol/L per day). In contrast, sulfate reduction occurred at depths below 21 m, with the highest rates attained at 28 m (rate, ~2.3 μmol/L per day). These results indicate that As (V) ranks second in importance, after sulfate, as an electron acceptor for anaerobic bacterial respiration in the water column. Annual arsenate respiration may mineralize as much as 14.2% of the pelagic photosynthetic carbon fixed during meromixis. When combined with sulfate-reduction data, anaerobic respiration in the water column can mineralize 32–55% of this primary production. As lakes of this type approach salt saturation, As (V) can become the most important electron acceptor for the biogeochemical cycling of carbon. *Copyright © 2000 Elsevier Science Ltd*

1. INTRODUCTION

Arsenic has trace abundance in the Earth's crust, yet it occurs widely in the environment, with localized high concentrations found in certain rocks, soils, and waters (Azcue and Nriagu, 1994). In nature it exists in four oxidation states: arsenate [As (V)], arsenite [As (III)], elemental arsenic, and arsine [As (-III)]. The later two occur only rarely, whereas As (V) and As (III) comprise the bulk of the inorganic speciation encountered in natural waters and sediments (Cullen and Reimer, 1989). Arsenate adsorbs strongly to mineral surfaces like ferrihydrite, whereas arsenite is much more mobile and toxic. Although the acute toxicity of arsenic to humans has been known since ancient times, both lethal and sublethal effects ("arsenicosis") associated with chronic ingestion of arsenic-contaminated well water currently occur over large regions, such as in the Ganges Delta (Nickson et al., 1998). This has focused concerns on water quality standards. Sources of arsenic in the environment include anthropogenic ones such as drainage from abandoned mines and mine tailings, usage as pesticides and biocides, as well as natural ones derived from hydrothermal leaching or weathering of arsenic minerals in rocks.

In stratified water bodies, arsenic typically exhibits a transition from As (V) to As (III) in depth profiles transiting from oxic to anoxic waters (Peterson and Carpenter, 1983; Aggett and Kriegman, 1988; Seyler and Martin, 1989; Maest et al., 1992; Kuhn and Sigg, 1993). Similar trends with depth are also observed in sediment cores taken from arsenic-contaminated reservoirs (Ficklin, 1990). Although As (V) can be reduced to As (III) by the presence of strong chemical reductants like sulfides (Kuhn and Sigg, 1993; Newman et al., 1997a,b), direct biochemical reduction of As (V) is also possible. Two mechanisms of biochemical reduction have been described: (1) a plasmid-encoded, detoxifying reductase (*arsC* enzyme) present in the cytoplasm of certain bacteria (e.g., *Escherichia coli* and *Staphylococcus aureus*), which reduces As (V) to As (III) for its rapid extrusion from the cell (Chen et al., 1986; Gladysheva et al., 1994; Ji et al., 1994), and (2) a respiratory ("dissimilatory") As (V) reductase present in the cell envelope of certain anaerobes that enables them to conserve the energy derived from the oxidation of organic substrates or H_2 (Krafft and Macy, 1998; Newman, D. K., Oremland, R. S., Dowdle, P. R., Morel, F. M. M., and Stolz, J. R., in prep.). Dissimilatory As (V) reduction is a newly discovered means of bacterial respiration, and several novel species of Gram-positive and Gram-negative Eubacteria have been isolated, which can achieve growth using As (V) as an electron acceptor (Ahmann et al., 1994; Laverman et al., 1995; Macy et al., 1996; Newman et al., 1997a,b; Switzer Blum et al., 1998; Stolz et al., 1999).

Dissimilatory As (V) reduction ("DAsR") to As (III) occurs in anoxic sediments that have been supplemented with millimolar As (V) (Rittle et al., 1995; Dowdle et al., 1996; Ahmann et al., 1997; Harringon et al., 1998). However, no studies have been done that examine the capacity of microbes to carry out DAsR at ambient concentrations of As (V) (e.g., nanomolar to micromolar), which would require the use of a radioisotope that does not significantly alter these ambient concentrations. Al-

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though ^{73}As is a gamma-emitting radioisotope, it has not as yet been used to study reduction of in situ concentrations of As (V) as has previously been done with sediments for selenium using ^{75}Se-selenate (Oremland et al., 1990). In addition, little is known about the mechanisms or rates of As (V) reduction in anoxic lake water. A radiotracer study of DAsR in anoxic waters circumvents technical problems associated with the binding of ^{73}As to solid mineral phases (e.g., ferrihydrite) that can occur in sediment assays. Furthermore, conducting such assays in a soda lake that has alkaline and carbonate-rich brinewater assures that any radiolabeled product, such as arsenic trisulfide ($^{73}As_2S_3$), formed by bacterial activity in the sulfide-rich bottom waters will remain in solution rather than precipitating as a solid (Newman et al., 1997a).

The anoxic waters of Mono Lake afford an idealized testing environment in which to devise a radioassay with ^{73}As. Because these waters have high sulfide concentration, the relative contributions of chemical and biological processes toward As (V) reduction can also be assessed. We undertook this investigation to devise such an assay and to determine whether it can be successfully applied to the anoxic waters of Mono Lake, an environment with an unusually high content of dissolved arsenic.

Mono Lake is an alkaline (pH 9.8; salinity 75–90 g/L) soda lake (dissolved carbonates 0.4 mol/L) located in central California on the eastern slope of the Sierra Nevada. The lake is usually monomictic, and undergoes one complete winter mixing event induced by the sinking of cold surface waters. However, inputs of large amounts of freshwater into the lake in the early 1980s and again in the late 1990s resulted in episodes of meromixis (persistent stratification) (Jellison and Melack, 1993a). Currently, Mono Lake is in a state of meromixis, which has been predicted to last for several decades (Jellison et al., 1998).

Meromixis results in the buildup of ammonia, sulfide, and methane in the anoxic monimolimnion (Miller et al., 1993). Annual primary productivity in the mixolimnion is diminished because of nitrogen limitation (Jellison and Melack, 1993b). Mono Lake contains exceptionally high concentrations of dissolved inorganic arsenic (~200 μmol/L), which is almost entirely in the form of As (V) and As (III) (Anderson and Bruland, 1991; Maest et al., 1992). The arsenic is derived from hydrothermal sources that enter the lake as hot springs and seeps. During the meromixis of the 1980s, a stoichiometric reduction of As (V) to As (III) was noted with transition from the oxic mixolimnion to the anoxic monimolimnion (Maest et al., 1992). Arsenate is potentially an important electron acceptor for bacterial respiration in the lake, having abundance (~200 μmol/L) comparable to that of dissolved oxygen (DO = ~150 μmol/L), but well below sulfate (~130 mmol/L). With the exception of DO and sulfate, these levels of As (V) greatly exceed those of any other potential electron acceptors, including nitrate ($\leq$1 μmol/L) and Fe (III) ($\leq$10 μmol/L) (Maest et al., 1992). In this paper we report on the successful development of a method to measure DAsR in Mono Lake, and its application to quantify the rates of As (V) reduction in the water column. The experimental data show that bacterial respiration of this toxic trace element holds the potential to account for a significant amount of carbon cycling in this ecosystem.

2. MATERIALS AND METHODS

2.1. Water Column Sampling

Water column samples were collected with a Van Dorn sampler on July 20, 1999, and on October 8, 1999, from the western basin of the lake (Oremland et al., 1987). An earlier sampling of the lake for the purpose of measuring water column sulfate-reduction was conducted in August 1986, during the previous meromictic event. Samples from discrete depths were stored in dark nalgene bottles (1 L) that were filled to overflowing with sample to minimize their exposure to oxygen. Samples were kept in a cooler until they were returned to shore (within ~3 h). Samples for arsenic speciation were collected in glass biological oxygen demand (BOD) bottles and stored at 4°C for overnight shipment to Frontier Geosciences. Upon arrival they were flash frozen (−60°C) and stored for 3 weeks before analysis.

2.2. Analysis of Lake Water Samples

Dissolved oxygen, conductivity, temperature, and turbidity measurements were made with in situ probes (YSI 158 model 5739 probe; Seabird conductivity probe, Martek temperature/turbidity probe). Dissolved methane and sulfide were collected and analyzed as given elsewhere (Miller et al., 1993). Sulfate was determined by ion chromatography (Switzer Blum et al., 1998). Particulate chlorophyll *a* was determined by solvent extraction of filtered volumes of lake water (Lorenzen, 1967). Bacterial population sizes were measured by acridine orange direct counts (Hobbie et al., 1977) as modified by Harvey et al. (1987).

Dissolved arsenic speciation in the samples collected in July 1999 was determined as follows. As (III) was measured directly upon receipt of the samples by hydride cryotrapping gas chromatography atomic absorption spectrometry (HG-CT-GC-AAS) at pH 6 (Andreae, 1977; Crecelius, 1978). However, because of some unknown interference occurring when the pH of Mono Lake samples was adjusted to 2, total As (ΣAs) could not be determined by this method. Therefore, ΣAs was determined by inductively coupled plasma emission spectroscopy mass spectrometry (ICP-MS), and As (V) was calculated as the difference between ΣAs minus As (III). The problem with this operationally defined speciation approach is that As (V) is not directly measured. In the anoxic waters where most of the ΣAs is in the form of As (III), the analytical uncertainty for the ΣAs and As (III) add up to increase the uncertainty of the calculated As (V) concentrations. In addition, a small systematic bias between the two techniques may distort the As (V) results because in the worst case, negative As (V) numbers may result if the ICP-MS measurement is low due to a bias relative to the HG-CT-GC-AAS determinations. To allow for the determination of small amounts of As (V) in the presence of large amounts of As (III), we switched to a direct As speciation technique for the samples collected in October 1999. We used ion chromatography to separate the As (III) from the As (V) species, and detected As (V) on-line by hydride generation atomic fluoresence spectrometry (IC-HG-AFS), with a method modified after Gomez-Ariza et al. (1998). Modifications included in-line helium flow to prevent oxidation of As (III) (D. Wallschlaeger and N. Bloom, in prep.).

2.3. Bacterial Enrichment Cultures

Estimates of bacterial population densities of sulfate-respiring bacteria (SRB) and arsenate-respiring bacteria (AsRB) were performed using most probable number (MPN) culturing techniques. The lactate-based medium for culturing Mono Lake haloalkaliphiles (Switzer Blum et al., 1998) was amended with yeast extract (0.5 g/L) and either 10 mmol/L Na_2SO_4 or 5 mmol/L NaH_2AsO_4 as electron acceptors. Anaerobic culture tubes containing 9 mL of medium were crimp-sealed under N_2 with butyl rubber stoppers. One-milliliter water samples from three depths (5, 18, and 24 m) were used to inoculate a decimal dilution series (highest dilution, 10^{-7}) by syringe. Three tube MPNs were analyzed for each depth. Inoculated tubes were incubated at room temperature (~20°C) for 3 weeks and then scored. For scoring, the pH of individual tubes was lowered to 6.5–7.5 by injection of 1 mL 1 N HCl, and Fe $(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (0.1 g/L) was injected into the tubes for the sulfate reducers. Tubes scoring positive for sulfate reduction

turned black, whereas those scoring positive for arsenate reduction turned yellow, due to the formation of iron sulfide and arsenic trisulfide, respectively.

2.4. Arsenate Radioassay With Cell Suspensions of *Bacillus selenitireducens*

The haloalkaliphilic, As (V)-respiring *B. selenitireducens* was used to develop an assay for reduction of ^{73}As (V) to ^{73}As (III). This organism as well as *B. arsenicoselenatis* were originally isolated from Mono Lake sediments (Switzer Blum et al., 1998). Cells were grown with 20 mmol/L lactate as electron donor and 5 mmol/L arsenate as electron acceptor, harvested, washed, and resuspended in mineral salts medium as described previously (Switzer Blum et al., 1998). Bacterial cell counts were made with acridine orange (Hobbie et al., 1977). Cells suspensions (25 mL in 50 mL serum bottles sealed under N_2; cell density, 10^8 cells/mL) were amended with 20 mmol/L lactate, 0.5 mmol/L arsenate, and 0.8 μCi ^{73}As (V) (specific activity, 158 Ci/mole). Controls consisted of sterile media. Samples were withdrawn by syringe during the incubation period and processed as described below. In a second experiment, washed cells were resuspended in surface water recovered from Mono Lake in 1996 (depth, 5 m; salinity, 75 g/L; stored for 3 yr at 5°C). Cells were added at three different population densities as determined by direct counts: $\sim 10^8$, 10^7, and 10^6 cells/mL. These cell suspensions were amended with 5 mmol/L lactate and 0.8 μCi ^{73}As (V) and incubated at ambient As (V) concentrations ($\sim$200 μmol/L). Cells were incubated in serum bottles (see above) at 20°C with constant rotary shaking (200 rpm). Suspensions were sampled by syringe (0.5 mL) during the incubation. Controls consisted of autoclaved and formalin (4% vol/vol final) killed cultures. Subsamples were centrifuge-filtered and then placed on ion-exchange resin columns for chromatographic separation of the radioactive arsenic species, followed by quantification by gamma spectrometry (see below).

2.5. Radioassays for Arsenate Reduction and Sulfate Reduction

Lake water samples from several depths of a vertical profile were returned to shore and subsampled within 4 h of collection. Water from discrete depths was temporaily drawn into 60 mL plastic syringes and 8 mL subsamples were injected into N_2-flushed Glaspak syringes (10 mL). The syringes containing the subsamples were capped with Teflon Mininert valves or with cutoff hubs filled with rubber (Smith and Oremland, 1987) and injected with 6.1 μCi ^{73}As (V) (specific activity, 158 Ci/mole; purity, >99%; Los Alamos National Laboratory, Los Alamos, NM). A filter-sterilized (0.2 μm; July samples) or formalin-killed (October samples) control was established for each depth in addition to triplicate sets of the experimental samples. At two depths (18 and 24 m), 60 h time course incubations were run during which subsamples (0.5 mL) were extruded from the syringes for filtration and chromatographic separation (see below).

Amendment experiments were performed with samples from these depths by adding 100 μL of the stock solutions given below (all in deionized water) to the 10 mL Glaspak syringes separately, before the injection of 5 mL of lake water (final concentrations): Na_2HAsO_4 (5 mmol/L), $NaNO_3$ (5 mmol/L), Na_2WO_4 (5 mmol/L), Na_2MoO_4 (5 mmol/L), Na lactate (1 mmol/L), and Na acetate (1 mmol/L). Samples were incubated in the dark in a refrigerator with an average temperature of 11°C (range, 7–15°C), which approximated the temperatures found below 15 m. Upon completion of the incubation, subsamples (0.5 mL) were filter-centrifuged (Dowdle and Oremland, 1998) and 200 μL of the filtrate was added to 4 mL of deionized water and adjusted to pH 3. These 4.2 mL volumes were placed on ion-exchange (resin used: AGI-X8, 50–100 mesh, Bio-Rad Labs, Hercules, CA) columns that separated ^{73}As (V) from ^{73}As (III) by elution with 30 mL of 0.12 N HCl (Ficklin 1983, 1990). Arsenite eluted in the first 6 mL, whereas arsenate eluted in the subsequent 24 mL. For the July incubation, we noted that a significant amount of the radioactivity ($\sim$50% of added) was retained on the columns in the case of samples taken from the sulfide-rich monimolimnion (24 and 28 m). This was found to be due to the formation of a solid (probably $^{73}As_2S_3$) at the low pH required for elution of ^{73}As (III) and ^{73}As (V) (Newman et al., 1997a). Because arsenic trisulfide is soluble in high pH carbonate solutions, we modified the protocol for the October sampling by adding 20 mL of a solution of 1 mol/L Na_2CO_3 (pH 11) to successfully elute these retained counts from the columns. These solubilized counts of $^{73}As_2S_3$ were added to those for the previously eluted ^{73}As (III) fraction to give the total amount of ^{73}As (III) produced from reduction of ^{73}As (V). All the eluted volumes were collected in scintillation vials and transported back to the laboratory for quantification by gamma spectrometry. ^{73}As has a half-life of 80.5 days, with 10.5% of its radioactive decay emitted as gamma rays (10.5% of activity) and the remainder (89.5%) associated with undetectable electron capture decay.

Sulfate reduction assays were conducted in concert with the arsenate reduction assays and in a similar fashion as given above with only minor differences: 5 mL volumes of lake water samples were dispensed into Glaspak syringes, and the syringe tips were sealed with rubber-filled cutoff needle hubs through which injections of amendments and isotope could be performed. Each sample was injected with of 100 μCi of $Na_2{}^{35}SO_4$ (Amersham; specific activity, 1.25 Ci/mol) were made to each sample. Incubations were terminated by injection of Zn acetate followed by freezing at −60°C. The digestion, liberation, and trapping of $H^{35}S^-$ was conducted in sealed serum bottles placed in a stripping-trapping train (Smith and Oremland, 1987). A water column sulfate-reduction profile was also obtained in August 1986 during Mono Lake's last episode of meromixis. The methods used were essentially the same as outlined above, except that the syringes were incubated in situ by returning them to their depths of collection. Rate constants for sulfate and arsenate reduction were calculated as the fraction of added label reacted per day. Rates were calculated by multiplying the rate constants by the ambient concentrations of sulfate or arsenate.

3. RESULTS

3.1. Development of the ^{73}As (V) Reduction Assay

3.1.1. Experiments with B. selenitireducens

Cell suspensions quantitatively reduced ^{73}As (V) to ^{73}As (III); the reduction of 5 mmol/L As (V) was nearly complete by 21 h (Fig. 1). No reduction occurred in the sterile controls. The initial reduction rate for As (V) removal (640 μmol/L per hour) was comparable to that of As (III) accumulation (504 μmol/L per hour) indicating the efficacy of the assay under these conditions. Addition of cells to aged Mono Lake surface water (5 m) also resulted in the reduction of ^{73}As (V) to ^{73}As (III) (Fig. 2). With an As (V) concentration of 200 μmol/L (Maest et al., 1992), initial arsenate reduction rates in dense cell suspensions (10^8 cells/mL) were equivalent to 50 μmol/L per hour for production of As (III) and 44 μmol/L per hour as measured by removal of As (V). Initial reduction rates at 10-fold lower cell densities (10^7 cells/mL) were lower by a factor of 10 ($\sim$4.12 μmol/L per hour); however, arsenate reduction rates in these samples declined after about 7 h incubation. No arsenate reduction was detected in lake water inoculated to a density of 10^6 cells/mL of *B. selenitireducens*, or in heat-killed controls of *B. selenitireducens* (initial population, 10^8 cells/mL), or in lake water incubated without *B. selenitireducens*. In both of the above experiments, loss of As (V) was in reasonable balance with production of As (III) (Figs. 1 and 2).

3.1.2. Laboratory experiments with freshly recovered lake water

Lake water that was taken from depths of 19 and 28 m in June and September 1999, shipped to Menlo Park and incubated at 20°C demonstrated active microbial As (V) reduction at both depths. After 67 h of incubation, activity was $\sim$10-fold higher at 28 m (6.4% reduction) than at 19 m (0.68% reduc-

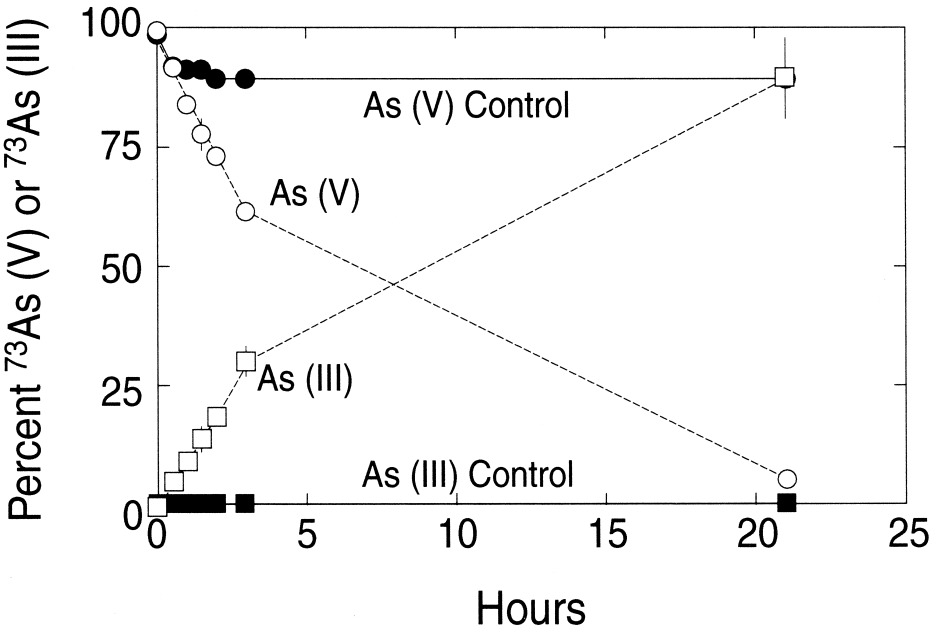

Fig. 1. Reduction of ^{73}As (V) to ^{73}As (III) by cell suspensions of *B. selenitireducens* (cell density, 10^8 mL^{-1}; medium contained 5 mmol/L arsenate). Open symbols: live cells; closed symbols: sterile medium. Symbols represent the mean of three cell suspensions and bars indicate ±1 standard deviation. Absence of bars indicates error was smaller than the symbols.

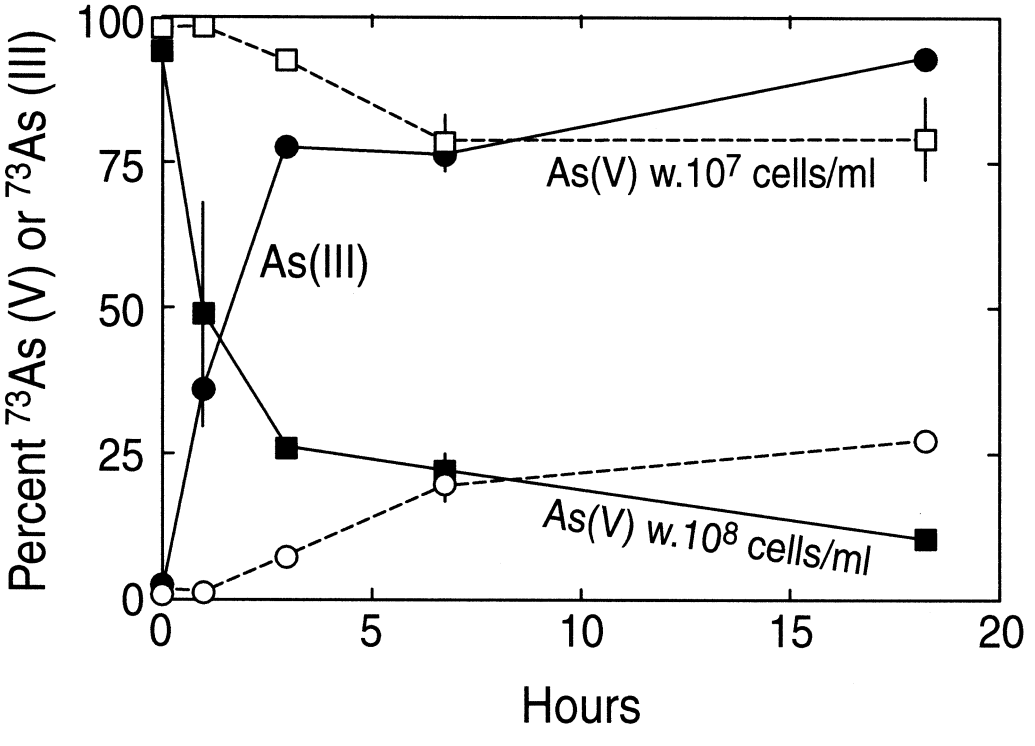

Fig. 2. Reduction of ^{73}As (V) to ^{73}As (III) in aged Mono Lake surface water (dissolved arsenate, ~200 μmol/L) by suspensions of *B. selenitireducens* at two different cell densities (incubation temperature, 20°C). Symbols: ■, As (V) with 10^8 cells mL^{-1}; ●, As (III) with 10^8 cells mL^{-1}; □, As (V) with 10^7 cells mL^{-1}; ○, As (III) with 10^7 cells mL^{-1}. Symbols represent the mean of three cell suspensions and bars indicate ±1 standard deviation Absence of bars indicates error was smaller than symbols.

Table 1. ^{73}As–arsenate reduction in lakewater samples taken from 19 m depth.[a]

Additions	% reduction[c]	μmoles As (V) reduced[c]
None	2.9 ± 2.1	5.8 ± 4.2
Lactate[b]	20.2 ± 3.7	40.4 ± 7.4
Lactate + As (V)[b]	25.3 ± 3.5	119.0 ± 16.0
Filter sterilized	0.23	0.50
Formalin killed	0.09	0.20

[a] Incubated for 139 h at 20°C; ambient As (V) = ~0.2 mmol/L.
[b] Final concentration: lactate = 1.0 mmol/L; arsenate = 0.25 mmol/L added + 0.2 mmol/L ambient = 0.45 mmol/L.
[c] Average of two samples ± range in values.

tion), whereas neglible amounts of chemical reduction occurred in the formalin-killed controls at 19 m (0.17%) and at 28 m (0.31%) (data not shown). The rate of As (V) reduction remained roughly linear over the 139 h incubation period, but there was a marked increase with time in reduction rates in samples amended with lactate and lactate plus As (V) from 19 m, presumably due to growth of As (V)-respiring bacteria (Table 1). In the above experiments, samples from 28 m did not include the $^{73}As_2S_3$ fraction, but the extraction step was included in all subsequent work given below.

In another experiment designed to approximate in situ conditions, we conducted time-course incubations of water from 24 m held at 10°C (Fig. 3). As (V) reduction assays were amended with 250 μmol/L As (V) (Fig. 3a), whereas no As (V) was added to sulfate reduction assays (Fig. 3b). The rates of As (V) removal (Fig. 3a) and As (III) production (data not shown) were linear over 70 h, although loss of As (V) exceeded the formation of As (III) by about 30%. The reason for this disparity is unclear, but we also noted this in our fieldwork (see below). It is likely that some other types of arsenite–sulfur compounds are formed in addition to $^{73}As_2S_3$ (e.g., $H^{73}AsS_3^{=}$) that are resistant to alkaline elution from the ion exchange columns. Killed controls produced only minor quantities of As (III) (1.3–1.5%) or removed only minor amounts of As (V) (0.45–7.5%) relative to the label initially added (not shown). For the above reasons we determined rate constants for DAsR by measuring the loss of ^{73}As (V) relative to abiotic controls in lieu of production of ^{73}As (III). A parallel incubation experi-

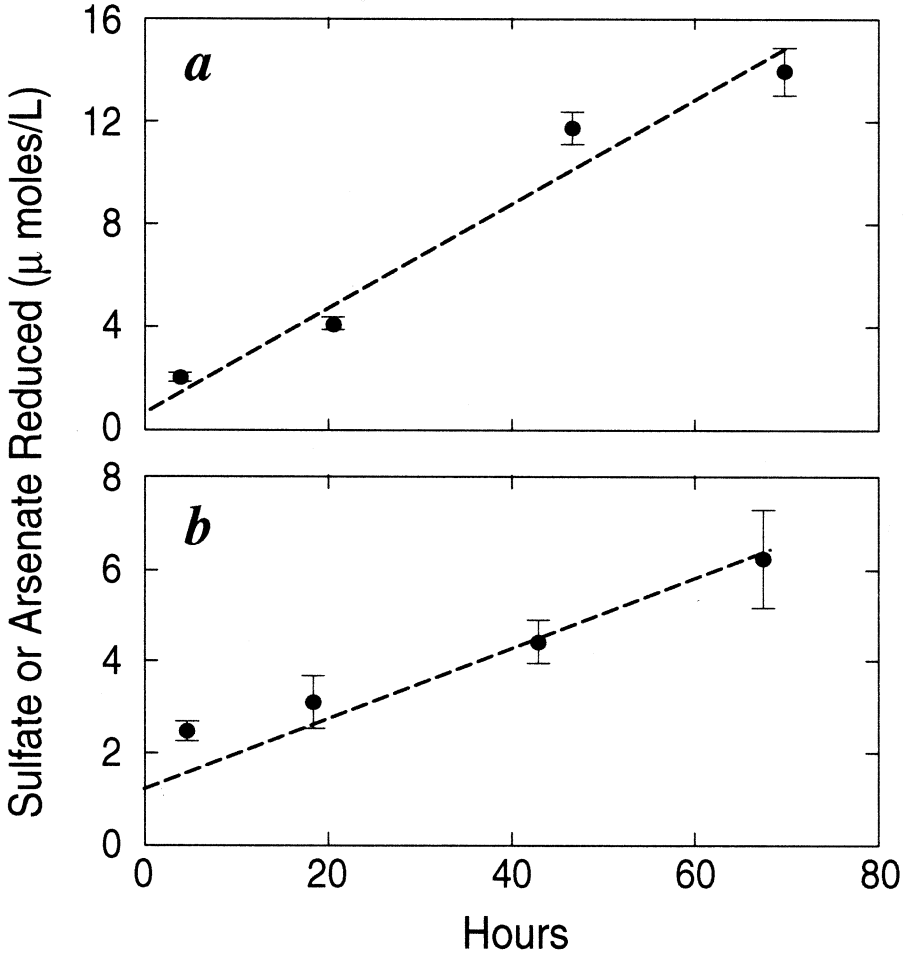

Fig. 3. Time courses of (a) ^{73}As (V) reduction (r^2 = 0.968) and (b) $^{35}SO_4^{=}$ reduction (r^2 = 0.866) with 24 m of water incubated at 10°C. Samples in the As (V)-reduction experiment were supplemented with 250 μmol/L As (V). Symbols represent the mean of three samples and bars indicate ±1 standard deviation.

Table 2. Effect of oxyanion inhibitors and organic acid substrates on arsenate reduction and sulfate reduction in anoxic monimolimnion water.[a]

Addition[b]	As (V) reduced (μmol/L)[c]	$SO_4^=$ reduced (μmol/L)[d]
None	10.9 ± 2.4	2.6 ± 0.3
Lactate	10.6 ± 1.1	2.7 ± 0.2
Acetate	13.9 ± 2.1	3.0 ± 0.2
Molybdate	71.9 ± 15.8	ND[e]
Tungstate	46.2 ± 5.8[f]	2.0 ± 0.1
Arsenate	ND	0.8 ± 0.1

[a] Water from 24 m depth incubated at 10°C; samples collected 9/1999.
[b] Final concentration of all amendments = 5 mmol/L.
[c] Production of ^{73}As(III) after 50 h; samples contained 250 μmol/L added As (V).
[d] Incubation time, 41 h.
[e] ND = not determined.
[f] Recovery of added ^{73}As label = 74% for tungstate; for all other conditions ≥93%.

ment of 24 m of water was analyzed for sulfate reduction, and the rate was linear over the 70 h time course (Fig. 3b).

3.1.3. Experiments with inhibitors and substrates

Table 2 presents results of amendment experiments with water samples from 24 m. In these experiments radioisotope recoveries at the end of the incubation were high, with ^{73}As (V) + ^{73}As (III) + $^{73}As_2S_3$ in live samples accounting for 90.7–95.8% of the initial amount of ^{73}As (V) added (not shown). A significant quantity (43.6–65.3%) of the As (III) recovered over the incubation was extracted in the high carbonate/high pH final elution, indicating that it was in the form of arsenic trisulfide. Arsenate reduction exceeded sulfate reduction by about fourfold in live samples. Neither lactate nor acetate stimulated either process. Tungstate enhanced As (V) reduction by about fourfold. This value for tungstate is probably an underestimate because we were unable to account for ~26% of the added counts, which likely were retained on the column as an arsenite–tungstate complex. In contrast, tungstate caused a 23% inhibition of $SO_4^=$ reduction. Earlier work with Mono Lake sediments had shown that 50 mmol/L tungstate inhibited ^{35}S-sulfate reduction by >80% (R. L. Smith, unpublished data). Molybdate markedly enhanced As (V) reduction (6.5-fold), whereas As (V) inhibited sulfate reduction by 65%. Use of formalin as a poison again proved effective as a control by eliminating 94–98% of As (V) and $SO_4^=$ reduction in the October 1999 profile (data not shown).

3.2. Field Results

3.2.1. Vertical profiles of chemical and biological gradients

Figure 4 shows the vertical profiles in Mono Lake obtained in July 1999 for various physical, chemical, and biological properties. A stepped thermocline was evident between 9 and 22 m of depth as were increases in density (Fig. 4d), and a subsurface oxygen maximum (~150 μmol/L) occurred at the top of the upper thermocline, below which oxygen steeply declined. The water column became anoxic at 18 m (Fig. 4a). Transparency decreased rapidly between 16 and 21 m (Fig. 4a), and a strong pycnocline was present at 20 m (Fig. 4d). Both methane and sulfide began to increase at 20 m, attaining their highest values at 28 m (Fig. 4c). Dissolved methane concentrations in the bottom waters have increased nearly eightfold since July 1995, when the lake was monomictic (Joye et al., 1999). Current bottom water sulfide and methane concentrations are comparable to the maximum levels attained during the last episode of meromixis in January 1987 (Miller et al., 1993). A peak of chlorophyll *a* at 24 m (Fig. 4a) is associated with a dense layer (~10^5 cells/mL) of *Picocystis* sp., an unusual green algal picoplanter (Roesler et al., 1999). Bacterial abundance was on the order of 10^7 cells/mL throughout the water column (Fig. 4e), with abundances in the monimolimnion from two- to sixfold higher than in the mixolimnion.

Total inorganic arsenic concentrations were ~200 μmol/L throughout the water column, with As (V) and As (III) as the dominant species present in the mixolimnion and the monimolimnion, respectively (Fig. 4b). Arsenite concentrations above the pycnocline were significant, ranging between 0.43 and 0.60 μmol/L (not discernable from the scale in Fig. 4b). However, no trend was obvious in the depth profile (not shown), and these levels of As (III) could reflect detoxifying *arsC* activity of the microbial flora (Chen et al., 1986). A transition from As (V) to As (III) occurred between 20 and 22 m of depth, and these speciation values are in agreement with an earlier investigation (Maest et al., 1992). Methylated forms of arsenic were below detection limits (~0.5 μmol/L; N. Bloom, unpublished data) throughout the water column, which also agrees with a previous analysis of surface water (Anderson and Bruland, 1991). The high levels of dissolved As (V) detected at 24 and 28 m during July are an artifact of the analytical technique that substracts one large number [As (III)] from another (ΣAs). To gain accurate values of As (V) at depths below 20 m, we used the ICP-HG-AFS method for the samples collected in October. These revealed the presence of low (2.05–5.28 μmol/L) but detectable levels of As (V) in the monimolimnion (Fig. 5). The full suite of physical/chemical/biological properties were not measured during October, but the vertical position of the pycnocline was the same as in July, although the thermocline had broken down to the extent that dissolved oxygen had penetrated to 19 m of depth as opposed to 18 m in July (data not shown). The depths below 15 m, however, had extremely low dissolved oxygen: 59, 32, 29, 27, and 0 μmol/L at 16, 17, 18, 19, and 20 m, respectively compared with 156 μmol/L at 15 m.

Low, but significant numbers of sulfate-respiring and arsenate-respiring bacteria were present throughout the water column (Table 3). The abundance of each group increased ~10-fold with the transition from surface water (5 m) to the chemocline (18 m), and from the chemocline to the monimolimnion (24 m). Arsenic respirers were consistently >10-fold more abundant than sulfate respirers throughout the water column.

3.2.2. Arsenate and sulfate reduction rates

No reduction of ^{73}As (V) was detected in the water samples taken in the oxygenated mixolimnion (5–17 m) during July, but activity was present in the samples taken from 18 m and below (Table 4). Only small quantities of radioisotope were recovered

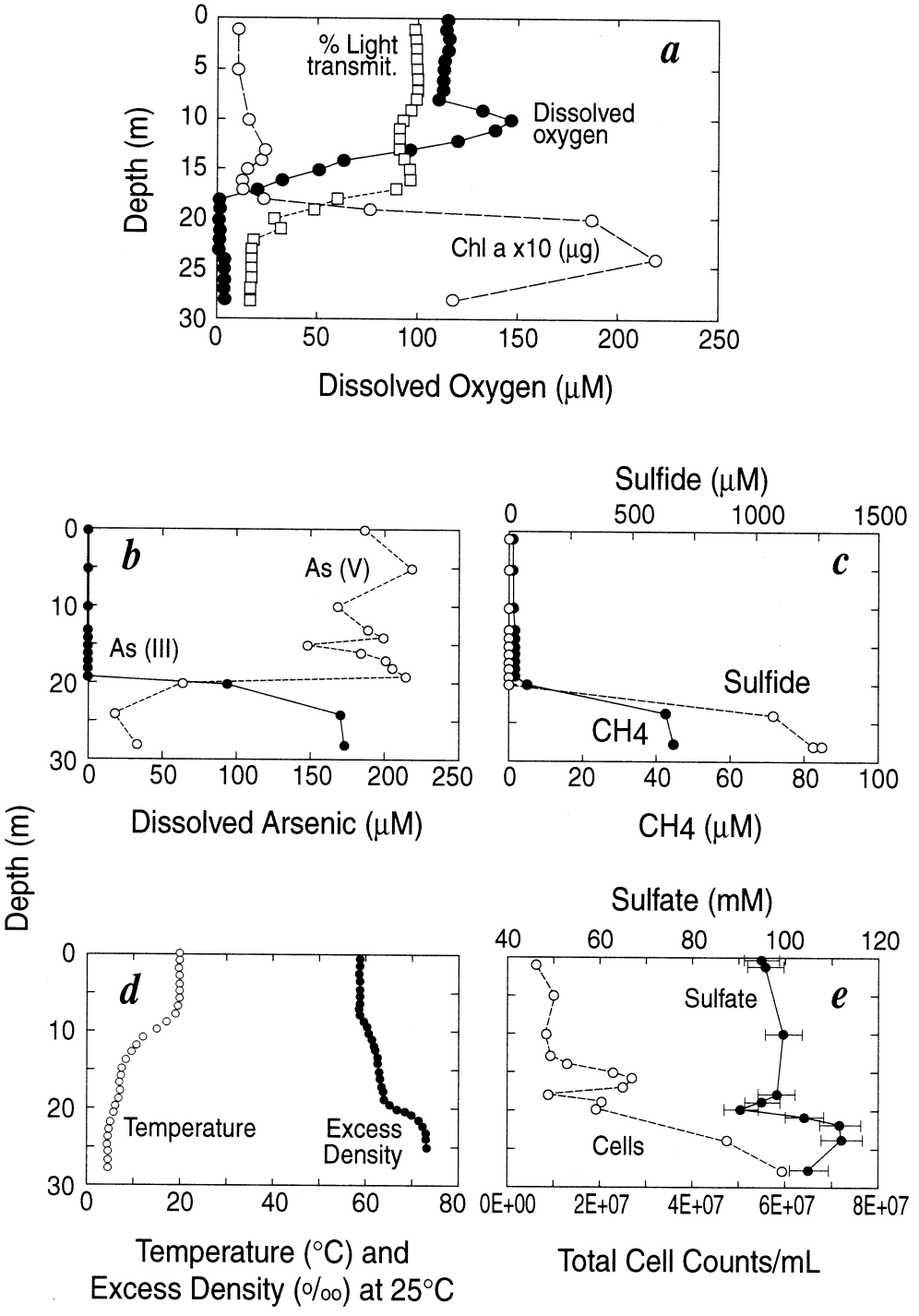

Fig. 4. Vertical depth profiles of water column physical, chemical, and biological parameters made in Mono Lake during July, 1999.

on filters (1.3–2.4% of the total counts added) and there were no differences between live and sterilized samples (not shown). Therefore, we attribute this to a small amount of adsorptive binding of ^{73}As (V) to the filters rather than any biogenic formation of labeled particulates. The bulk of the arsenic recovered was either in the form of dissolved As (III) or as unreacted As (V). Recovery of counts as column-eluted ^{73}As (V) + ^{73}As (III) + ^{73}As filters averaged 94.7 ± 1.2% (n = 29) for the samples taken between 5 and 20 m of depth. No significant differences between live and filter-sterilized controls could be detected as loss of ^{73}As (V) in the samples from 18, 19, and 20 m, but a small (<0.2% of counts added) and significant amount of ^{73}As (III) production was noted relative to the sterile controls for samples from these depths. Therefore,

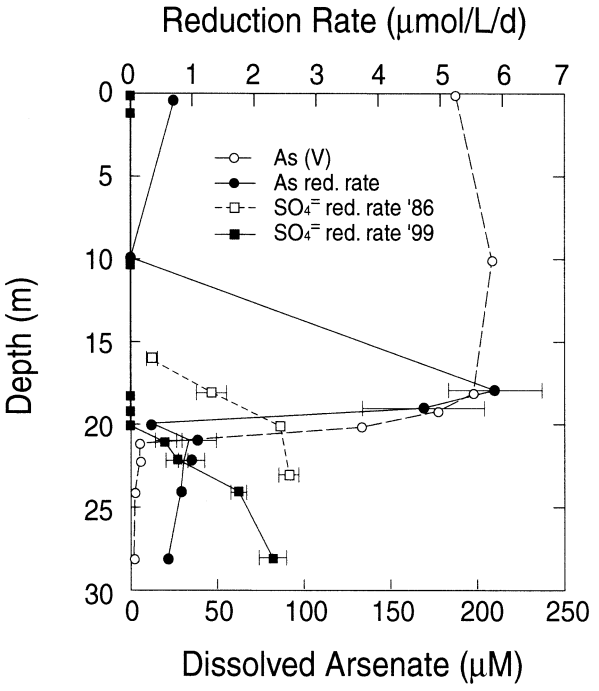

Fig. 5. Vertical depth profiles of arsenate reduction rates, sulfate reduction rates, and dissolved arsenate obtained in October 1999. A comparison is made of the sulfate reduction rates obtained in August 1986 when the lake was last meromictic, but 3 m shallower. Symbols represent the mean of three samples and bars indicate ±1 standard deviation.

rate constants for As (V) reduction from these depths were based on the production of As (III), and they were all low. Correspondingly, calculated turnover times for As (V) pools were long: 20, 2.7, and 3.8 yr for the samples from 18, 19, and 20 m, respectively.

The October sampling yielded somewhat different results for the upper water column (Table 4). First, a small amount of As (V) reductase activity was detected in the sample taken from the surface (0 m). Second, although the rate constants determined by the production of ^{73}As (III) generally agreed with those found for July, with values ranging from 0.00016 to 0.00070 per day (turnover time, 3.8–17 yr) for the depths above 20 m (data not shown), we did observe significant loss of ^{73}As (V) relative to the formalin-killed controls. For example, after 48 h of incubation of the 18 and 19 m samples, we recovered 0.10% and 0.15% of the added label as ^{73}As (III), but loss of ^{73}As (V) relative to killed controls yielded 6.2% and 5.6%. This

Table 3. Cell densities of sulfate-respiring bacteria (SRBs) and arsenate-respiring bacteria (AsRBs) in the Mono Lake water column as determined from the MPN cultures.

Depth (m)	SRBs[a]	AsRBs[a]
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] Values in parentheses indicate 95% confidence level interval.

Table 4. Rate constants for reduction of ^{73}As (V) in water column samples taken during July 1999 and October 1999.[a]

Depth (m)	k (d^{-1}): July 1999	k (d^{-1}): Oct. 1999
0	ND[b]	0.0038
5	0	ND
10	ND	0
15	0	ND
16	0	ND
17	0	ND
18	0.00014	0.0299
19	0.001	0.0267
20	0.0073	0.0024
21	ND	0.207
22	ND	0.173
24	0.290	0.277
28	0.280	0.290

[a] Data represent the net loss of ^{73}As (V) relative killed controls. Mean of three samples; incubation time, 42 h at 10°C.
[b] ND = not determined.

As (V) loss resulted in calculation of higher rate constants (Table 4), which translate into much shorter As (V) turnover times (0.1–1.1 yr).

For the anoxic monimolimnion, there was significant production of ^{73}As (III) in the samples taken from 24 and 28 m in the July sampling (~10.4% of label added by 42 h). However, much higher levels of reduction were observed as loss of ^{73}As (V) relative to the filtered controls (48.8–50.7% by 42 h). We attribute this disparity to the formation of $^{73}As_2S_3$ that was not eluted from the ion exchange columns in the July experiments. When we sampled the anoxic monimolimnion again in October, $^{73}As_2S_3$ was eluted from the columns and added to the values for ^{73}As (III). There was close agreement between the amount of arsenic recovered as ^{73}As (III) + $^{73}As_2S_3$ when compared to the amount of ^{73}As (V) consumed: 93, 98, 90, and 89% for samples from 21, 22, 24, and 28 m, respectively. The rate constants for both sampling dates, as determined by loss of ^{73}As (V), were in excellent agreement. Calculated turnover times agreed closely, and ranged between 3.4 and 5.8 days. No sulfate reduction was detected at 20 m of depth, but sulfate reduction rate constants ranged between 0.000005 and 0.00002 per day and corresponding to turnover times of 137–538 yr for sulfate were measured at depths of 21 m and below.

The vertical profiles of As (V) reduction and $SO_4^=$ reduction obtained in October 1999, as well as the $SO_4^=$ reduction profile for August 1986 are shown in Figure 5. Highest rates of As (V) reduction occurred at 18 and 19 m, and significant but decreasing levels of activity also occurred at greater depths. Sulfate reduction rates increased with depth, with highest rates found at 28 m for the 1999 data set. There was excellent agreement between the 1986 and 1999 data sets, the only difference being the vertical deepening of the profile owing to the ~3 m lake level rise by 1999. In August 1986, the water column was suboxic (DO, <5 µmol/L) at 15–16 m and anoxic at 17 m. The integrated rate of $SO_4^=$ reduction in 1999 was 12.6 mmoles m^{-2} day^{-1} for the 21 to 28 m depth interval, and the integrated rate of As (V) reduction was 17.3 mmoles m^{-2} day^{-1} for the 18 to 28 m depth interval.

4. DISCUSSION

Dissimilatory reduction of metals and metalloids has become recognized as an important process in their biogeochemical cycling (Lovley, 1995). For metals with high crustal abundances like iron and manganese, bacterial reduction of the most oxidized forms also represents an important mechanism for cycling carbon in anoxic sediments. Thus, Fe (III) reduction in selected sediments can prove more important in this regard than methanogenesis and sulfate reduction combined (Roden and Wetzel, 1996). However, because of the limited solubilities of Fe (III) and Mn (IV), their significance as electron acceptors in anoxic water columns has not been assessed, although they are clearly reduced in the suboxic depths of stratified water bodies such as the Black Sea (Nealson and Myers, 1992). Because questions of toxicity are often associated with many trace metals, attention on these substances has focused on their bacterial reduction in sediments as a means by which they are either solubilized (e.g., arsenic), or immobilized by precipitation (e.g., selenium). For example, selenate is rapidly immobilized as elemental selenium in agricultural evaporation ponds, but it is far less important as an oxidant of organic matter than are the concurrent dissimilatory processes of sulfate reduction and denitrification (Oremland et al., 1990). However, Mono Lake has unusually high concentrations of dissolved inorganic arsenic compared to other water bodies (Anderson and Bruland, 1991). We developed the radioassay in this investigation to address three questions concerning arsenic in Mono Lake: (1) Is the reduction of As (V) to As (III) a biological or a chemical phenomenon? (2) If the reduction is biological, then what is its quantitative significance to the mineralization of primary productivity? (3) Which bacterial processes are responsible for the observed As (V) reduction?

The evidence that most of the As (V) reduction is biological and not chemical rests on the finding of a lack of reduction with formalin-killed cells or in filter-sterilized reaction mixtures in the laboratory (Tables 1 and 2), and in the field (Table 4). It also rests on the observation that pronounced stimulation of As (V) reduction occurred in the presence of the electron donor lactate during prolonged incubation of 19 m water (Table 1). Lactate is a favored substrate for two species of As (V)-respiring bacteria from Mono Lake (Switzer Blum et al., 1998) and growth of these types of microbes probably occurred during the 139 h incubation at 20°C. Additional evidence consists of the presence of significant populations of culturable As (V)-respiring bacteria in the anoxic portions of the water column (Table 3). Although As (V) can be reduced to As (III) by the millimolar levels of sulfide in Mono Lake bottom waters, rapid chemical reduction is favored under strongly acidic rather than the prevalent highly alkaline conditions (Cherry et al., 1979).

The fact that we measured such high rate constants (~0.2/day) for DAsR in the anoxic waters at 21 m and below (Table 4) suggests that some physical, chemical, or biological mechanisms must also exist to resupply the As (V). Integrated rates of water column As (V) reduction at 18 m and below during October 1999 (Fig 5) were 17.3 mmoles m^{-2} day^{-1}. The contribution of As (V) to the monimolimnion by vertical mixing can be determined using eddy flux calculations. Values of eddy diffusivity (K_z) attained across the chemocline during summers in the last episode of meromixis ranged broadly between a low of 0.01×10^{-7} m^2 s^{-1} to a high of 8.39×10^{-7} m^2 s^{-1} in 1986 and 1984, respectively (Jellison et al., 1993). Downward flux (J) of As (V) from 18 to 21 m can be calculated from the equation: $J = K_z [\delta As/\delta z]$. Using the range of values for K_z given above, we calculate downward As (V) fluxes of between 0.06 and 48.3 mmoles As (V) m^{-2} day^{-1}. This broad range indicates that mixing has the potential to transport sufficient As (V) into the bottom waters to satisfy consumptive demand. However, this downward transport appears to be highly episodic, with large annual or seasonal inputs countered with prolonged periods of neglible flux. Aside from physical mixing of the water column, regeneration of As (V) from biological or chemical oxidation of As (III) may also occur. Bacterial oxidation of As (III) with oxygen as the electron acceptor could conceivably occur in the suboxic waters between 16 and 19 m (Wilkie and Herring, 1998; Santini et al., 2000). Thus, the concentration of As (V) at those depths would reflect the net balance between vertical mixing, DAsR, and bacterial oxidation occurring at very low DO, but some other oxidant would be required at greater depths. One possibility is Mn (IV), which can efficiently oxidize As (III) in aquatic environments (Oscarson et al., 1981). In addition to oxidative recycling of As (III), a likely source of As (V) is from transport into the monimolimnion by the sinking of As (V)-saturated ferrihydrite particles or arsenate-containing minerals like scorodite. The dissolution of these particles by dissimilatory Fe (III)-reducing bacteria would release the associated As (V) into the bottom waters (Cummings et al., 1999).

The significance of As (V) respiration to the mineralization of carbon in this system can be calculated by balancing the four electrons derived from the oxidation of carbohydrate ("CH_2O") to CO_2 with the two-electron reduction of As (V) to As (III) by the equation:

$$CH_2O + 2\ H_2ASO_4^- \longrightarrow HCO_3^- + 2\ H_2AsO_3^- + H^+$$

Annual primary productivity of Mono Lake during the 1984–1988 meromixis was 22.4–38.5 mol C m^{-2}, which represents a decline of ~36% from normal monomictic conditions (Jellison and Melack, 1993b). If we assume that current conditions in Mono Lake reflect the 1984–1988 carbon fixation estimates, then oxidation of carbohydrate derived from primary productivity would generate 89.6–154 mole equivalent of electrons per year.

Extrapolating the integrated October 1999 As (V) reduction profile (Fig. 5) of 17.3 mmoles m^{-2} day^{-1} gives 6.3 moles As (V) reduced m^{-2} y^{-1}, which would act as a sink for 12.6 mole equivalent electrons. Thus, respiration of As (V) in the anoxic water column can potentially mineralize 8.2–14.1% of annual pelagic primary productivity during meromixis, clearly a significant quantity for a "trace" element. A similar calculation for sulfate reduction is made using the equation:

$$2\ CH_2O + SO_4^= \longrightarrow 2\ HCO_3^- + HS^- + H^+$$

The integrated daily sulfate reduction value for the anoxic waters (12.6 mmol m^{-2} day^{-1}) extrapolates to 4.6 moles $SO_4^=$ reduced m^{-2} yr^{-1}, which would act as a sink for 36.8 mole equivalent of electrons. Thus, water column sulfate reduction can mineralize 23.8–41.2% of annual pelagic primary produc-

tivity, which when combined with As (V) respiration indicates that water column dissimilatory reduction can mineralize 32.1–55.3% of annual primary productivity. We can factor in an estimate of the sediment contribution to carbon mineralization by using a data set obtained in August 1986 (R. L. Smith, unpublished). Vertical profiles of ^{35}S-sulfate reduction show that activity was highest (220 μmol L^{-1} day^{-1}) in the 0 to 5 cm subsurface interval. Integrated sediment sulfate reduction is 13.4 mmol m^{-2} day^{-1}, which translates to an annual electron sink of 39.1 mole equivalents. Combining this sediment data with those from the water column illustrates that these anaerobic respiratory processes (not incuding methanogenesis) can mineralize 57.5–98.9% of pelagic primary productivity occurring during meromixis. Our use of the sediment $SO_4^=$ reduction data from a 1986 fieldtrip to make these calculations appears justified because the water column profiles were quite similar for 1986 and 1999 (Fig. 5) in all aspects except their vertical displacement owing to the recent lake level rise of 3 m. We stress that these calculations refer only to the stratified portion of the pelagic water column, and do not account for lake-wide primary productivity that would encompass the oxic, shallow regions as well as the pelagic ones. In addition, our estimates need to be verified by conducting these assays seasonally over an interval of a few years duration.

Attributing As (V) reduction to a particular group of microorganisms in Mono Lake raises the question of whether it was sulfate reducers like *Desulfotomaculum auripigmentum* (Newman et al., 1997a,b) or nonsulfate reducers like *B. selenitireducens* and *B. arsenicoselenatis*, the latter two species being originally isolated from Mono Lake (Switzer Blum et al., 1998). The higher abundance of As (V) respirers throughout the water column argues for *B. selenitreducens*-type organisms, which achieved maximal abundance in the bottom waters (Table 3). The population density of both sulfate-reducing bacteria (SRB) and arsenate-respiring bacteria (AsRB) were much lower than total live bacterial counts obtained with acridine orange (Fig. 4), accounting for only ~0.001% of the total population in the bottom water for AsRB. However, MPN data reflect the ability of organisms to actually grow in the medium devised and therefore, can greatly underestimate population sizes. Indeed, a comparison of the cell-normalized rates of As (V) reduction obtained in the laboratory with *B. selenitireducens* (50 nmol 10^8 $cells^{-1}$ h^{-1}) are much lower than the highest extrapolated rates in the water column (58 μmol 10^8 $cells^{-1}$ h^{-1}). This suggests that the actual population size of AsRB may be underestimated by as much as two to three orders of magnitude. AsRB in contaminated lake sediments as determined by an MPN technique ranged between 10^3 and 10^4 mL^{-1}, and were roughly two orders of magnitude lower than SRB (Harrington et al., 1998). Our values of ~4×10^2 cells/mL for the bottom waters are therefore not unreasonable for MPN methodology, but the fact that AsRB outnumbered SRB may be a unique facet of the microbial ecology of Mono Lake.

The results obtained with the group VIA oxyanion inhibitors (Table 2) are more difficult to interpret (Oremland and Capone, 1988). The results suggest a competition between SRB and AsRB in that tungstate inhibited sulfate reduction, whereas molybdate and tungstate each enhanced As (V) reduction. This appears to illustrate a classic competitive shift away from a thermodynamically less favored, but abundant electron acceptor (sulfate) to another that generates more energy (arsenate) (Newman et al., 1998), but is present at only limited concentrations in the bottom waters. This is also supported by the strong inhibition of sulfate reduction by arsenate. However, other interpretations are also possible. For example, the results could reflect an internal biochemical shift within the SRB themselves (rather than the overall microbial flora) by which they channel electrons to As (V) when confronted with a toxic sulfate analog like tungstate (or arsenate) to conserve ATP. Internal ATP pools used for sulfate activation are severely depleted when an analog like molybdate (or tungstate) is used to inhibit SRB (Taylor and Oremland, 1979). A second explanation is that molybdenum and tungsten are both limiting cofactors in the As (V) reductases of the population in question, and their addition may promote As (V) reduction. Both molybdenum and tungstate are relatively abundant in Mono Lake's waters (Mo = 0.71 μmol/L; W = 8.6 μmo/L; L. G. Miller, unpublished data), which makes the concentration limitation unlikely. Tungstate should act as an antagonist of molybdenum-containing enzymes, and effect inhibition rather than stimulation of As (V) reduction, whereas molybdate should have no effect on AsRBs. This was the pattern achieved in salt marsh sediments (Dowdle et al., 1996), and molybdate had little effect on As (V) reduction in contaminated lake sediments (Harrington et al., 1998). Hence, the stimulatory effects of tungstate and molybdate on DAsR may be only an experimental artifact caused by displacement of ^{73}As (V) retained on the ion exchange columns by these group VIA divalent oxyanions, making it appear in the eluted fractions assigned to ^{73}As (III). Overall, however, the results suggest that both SRB and AsRB carry out As (V) reduction in the bottom waters of Mono Lake, but more work is needed with other methods, along with a more refined use of inhibitors to better quantify their relative contributions. Future use of molecular techniques to probe the genomes of the resident bacterial flora might help to clarify this question. Clearly, neither sulfate reduction nor arsenate reduction was stimulated by acetate or lactate, indicating that neither process was electron donor limited in short-term incubations. Mono Lake contains rather high levels of DOC (~90 mg/L; Oremland et al., 1987) that would suggest that appreciable levels of fatty acid anions might comprise a part of this pool.

It is interesting to speculate about the potential significance of As (V) respiration in water bodies that are at or near salt saturation. At these very high salinities, SRB are constrained by the high energy requirement for maintenance of their internal salt balance exceeding those gleaned from metabolism of electron donors with sulfate as the oxidant (Oren, 1999). Hence, sulfate reduction is not detected in the Dead Sea or the Orca Brine. Oxidation of electron donors like H_2 with arsenate is thermodynamically far more favorable than that with sulfate, as was illustrated by Newman et al (1998) who used ion concentrations of 1 μmol/L and $10^{-6.6}$ atmospheres for H_2 (pH 7):

$$SO_4^= + H^+ + 4\,H_2 \longrightarrow HS^- + 4\,H_2O$$

$$\Delta G' = -0.42 \text{ kjoule/mole electrons}$$

$$H_2AsO_4^- + H^+ + H_2 \longrightarrow H_3AsO_3 + H_2O$$

$$\Delta G' = -23.0 \text{ kjoule/mole electrons}$$

Primary production occurs in salt-saturated brines because certain microalgae adapt by synthesizing internal osmolytes (Oren, 1999). It is therefore possible that As (V) may assume an even greater importance as an electron acceptor for the mineralization of photosynthetic carbon in As-rich soda lakes that have salinities considerably higher than that of Mono Lake. Indeed, because soda oceans may have typified the Archean (Kempe and Degens, 1985), an argument can be made for very saline soda lakes being the last biome present on an increasingly arid Martian surface after its early, wet "Noachian" period (Cattermole, 1992; Carr, 1996). The heat flow from Martian shield volcanoes could have introduced hydrothermal arsenic into these lakes (assuming the regolith-contained arsenic), and if some oxygen was also present, As (III) would be oxidized to As (V) (Wilkie and Herring, 1998) by chemoautotrophs (Santini et al., 2000). Theoretically, a biogeochemical cycling of arsenic between its +3 and +5 oxidation states could sustain the anaerobic decomposition processes of such a residual, extremely hypersaline ecosystem. The microorganisms in that milieu would not only need to be adapted to high pH and salinity, but would also have to evolve mechanisms for enduring high concentrations of arsenic. Microbes that can diminish arsenic toxicity by forming As-containing osmolytes (e.g., arsenobetaine) or that can exploit the energy of arsenic redox chemistry would have survival advantages.

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