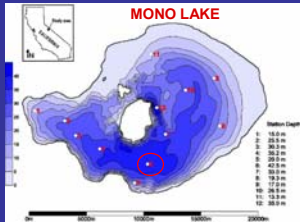


Role of sulfide, selenate and nitrate in arsenite oxidation in Mono Lake, CA

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AEROBIC ARSENITE OXIDATION

Arsenic is an important chemical constituent in Mono Lake where it occurs naturally at sufficient concentrations (~200 μM) to provide a source of energy for microbial metabolism (through oxidation or dissimilatory reduction). Much is known about the reductive portion of the microbial arsenic cycle in Mono Lake; however, less is known about the oxidation of As(III) to As(V). Experiments were conducted with surface water samples in April and August of 2004 to measure potential rates of aerobic As(III) oxidation to As(V) and to study the effect of co-oxidation of reduced S compounds. Oxidation was quantified by measurement of arsenate using the molybdate blue spectrophotometric method. Minimal production of arsenate in killed controls indicates that abiotic arsenite oxidation is slow. If similar rates occur *in situ*, all As(III) present in anoxic waters could easily be oxidized within a few days during lake turnover. Thus biological As(III) oxidation may be an important process over short time scales in the arsenic biogeochemistry of Mono Lake.

EXPERIMENT 1 : EFFECT OF SULFIDE

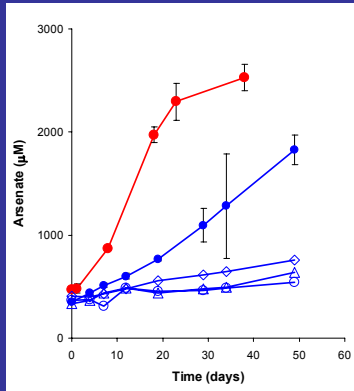
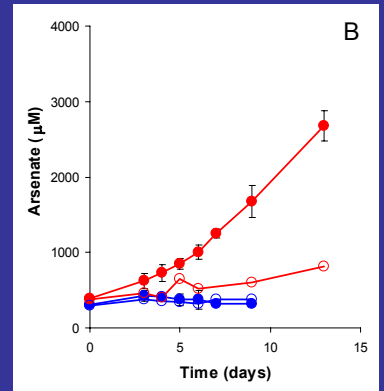
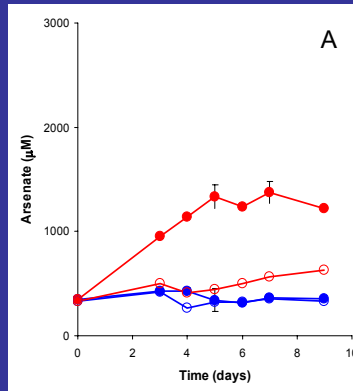


Figure 1. 2 mM arsenite (as NaAsO₂) was added to Mono Lake water. 2 mM sulfide (as Na₂S) was added to one set of samples. Oxidation was significantly faster in the arsenite + sulfide treatments. Filtered (0.22 μm), formalin-killed, and no arsenite controls did not exhibit significant oxidation of arsenite.

EXPERIMENT 2 EFFECTS OF SUBSTRATE CONCENTRATION



Figures 2a and 2b. Experiment 2 was conducted a) to confirm the results of Experiment 1 and b) to examine the effects of substrate concentration on arsenite oxidation rates. (A) 1 mM arsenite or 1 mM arsenite + sulfide or (B) 5mM arsenite or arsenite + sulfide were added to Mono Lake surface water samples. Abiotic arsenite oxidation was measured in formalin-killed controls. In both the 1 mM and 5mM treatments, arsenite was oxidized more rapidly than in treatments with no sulfide. Initial rates of arsenite oxidation were slower in the 5 mM treatment than in the 1 mM treatment.

HOW DOES SULFIDE STIMULATE As(III) OXIDATION?

Hypotheses:

- 1) Arsenite is oxidized by sulfide oxidizing bacteria, whose growth and metabolism are enhanced by the addition of sulfide
- 2) The addition of sulfide leads to the formation of thioarsenic compounds which are oxidized more rapidly than arsenite
- 3) Partial oxidation products of sulfide (e.g. thiosulfate, etc.) react with and oxidize As(III) abiotically

Experiment 3 Formation of arsenic-thiol compounds

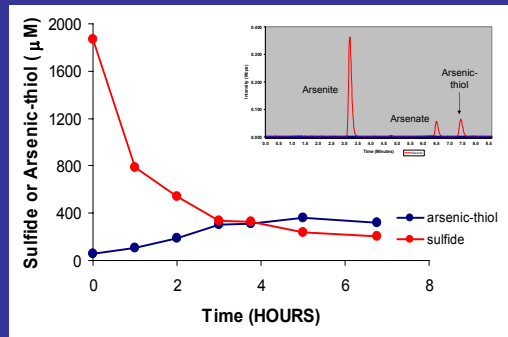
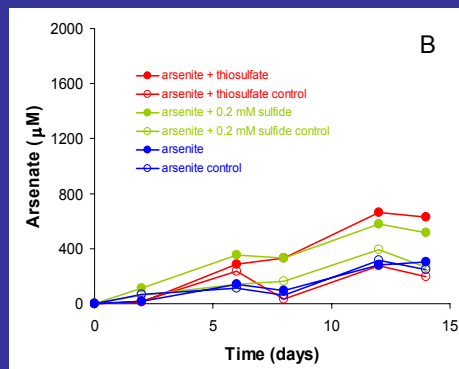
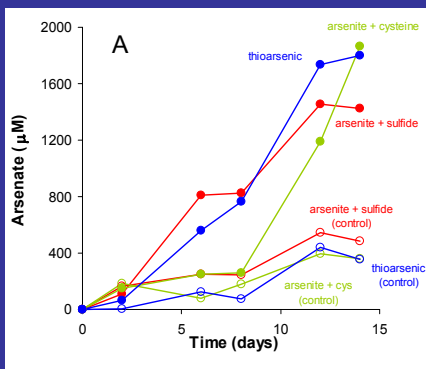


Figure 3. Consumption of sulfide via abiotic oxidation, biotic oxidation, and formation of arsenic-thiol compounds measured in an aerobic Mono Lake water enrichment culture. The culture was enriched with 1mM arsenite and 2 mM sulfide. Sulfide was measured using a sulfide electrode. The unknown arsenic-thiol compound was quantified using IC-ICP-MS. The inset panel shows a typical ICP-MS chromatogram.

Arsenic-thiol compounds may comprise >50% of ΣAs(III) in sulfidic Mono Lake waters (see J.T. Hollibaugh presentation for further details).

Effects of sulfur compounds on arsenite oxidation



Figures 4a and 4b. Various sulfur compounds were added to (2mM) arsenite oxidation experiments to determine the effects of reduced S compounds and sulfide oxidation products on arsenite oxidation rates. The greatest stimulation of As(III) occurred in treatments with 2 mM sulfide, 1 mM cysteine or 2 mM arsenic-thiol compound (Figure 4a). Thiosulfate (1 mM) and 0.2 mM sulfide increased As(III) oxidation rates over those of arsenite alone to a lesser degree (Figure 4b).

CONCLUSIONS

- Arsenite oxidation in Mono Lake is biologically controlled
- Presence of sulfide (or reduced sulfur compounds) significantly increases rates of biological arsenite oxidation
- Sulfide oxidation products enhance abiotic oxidation to a lesser degree
- Arsenic-thiol compounds can form spontaneously in Mono Lake water (even under aerobic conditions) and rapidly oxidize or decompose in the presence of bacteria
- Sulfide oxidation products enhance abiotic oxidation as well
- Rates of arsenite oxidation during lake turnover may depend on sulfide concentrations in mixing waters

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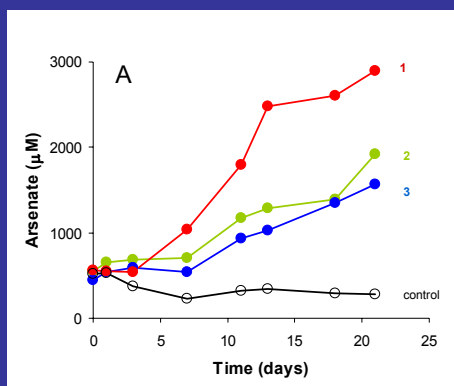
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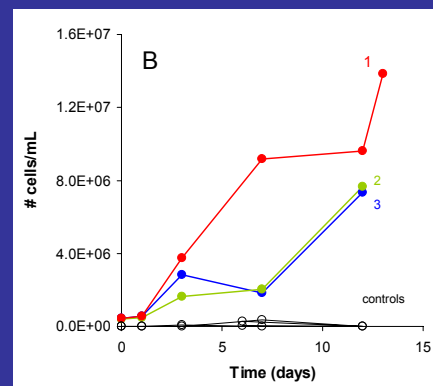
ANAEROBIC ARSENITE OXIDATION

Significant arsenate concentrations (5-20 μM) have been measured in the sulfidic bottom waters of Mono Lake on several occasions. This redox disequilibrium suggests that anaerobic oxidation of arsenite may also occur. Selenate, which is present at concentrations of $\sim 10 \mu\text{M}$ in the lake, was tested as an electron acceptor for arsenite oxidation. Anaerobic oxidation of arsenite (1 mM) using selenate (1 mM) as an electron acceptor occurred at a rate of $\sim 10 \mu\text{M}/\text{day}$ in April experiments. The oxidation of arsenite using selenate as the terminal electron acceptor represents a novel pathway for arsenite oxidation (and selenate reduction). Separate experiments with nitrate and selenate were conducted to compare the efficacy of the two electron acceptors. Rates were significantly faster in experiments conducted in August ($\sim 200 \mu\text{M}/\text{day}$). Potential rates of anaerobic arsenite oxidation using nitrate (5 mM) as an electron acceptor were nearly 1 mM/day after a 5 day lag period.

TESTING SELENATE AS T.E.A.

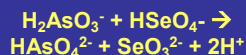


CELL GROWTH ON SELENATE AND ARSENITE



Figures 5a and 5b. 3 mM arsenite (as NaAsO_2) and 3 mM selenate (as Na_2SeO_4) were added to anaerobic Mono Lake water samples (1, 2, 3) and a 0.22- μm filtered control. Oxidation of arsenite was measured as production of arsenate. Rates of arsenite oxidation in the three samples were correlated to cell growth. (Cells were stained with DAPI and counted under an epifluorescence microscope.)

Arsenite oxidation with selenate:



$$(\Delta G^\circ = -94.5 \text{ kJ/mol})$$

AUTOTROPHIC ENRICHMENT

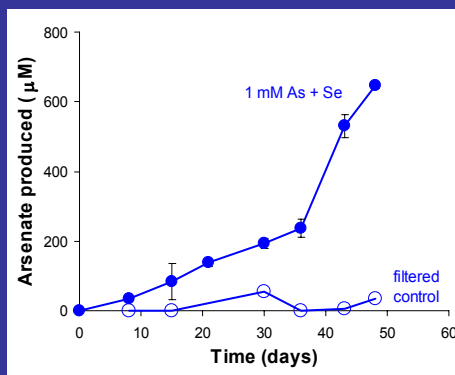


Figure 6. Autotrophic artificial Mono Lake water medium was inoculated with freshly collected lake water and enriched with 1 mM arsenite and selenate. Arsenite was oxidized at a rate of $\sim 10 \mu\text{M}/\text{day}$.

ARSENIC AND SELENIUM SPECIATION

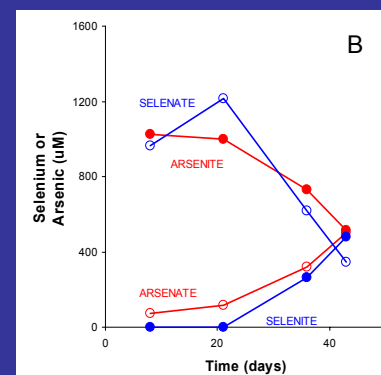
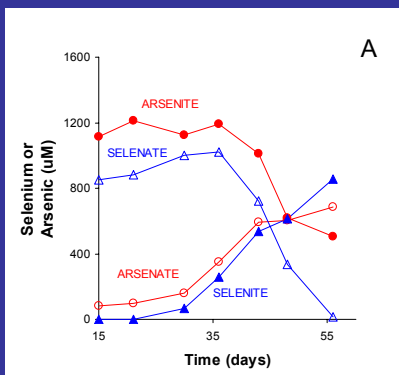


Figure 7a and 7b. Selected samples from the autotrophic enrichment were analyzed for arsenic and selenium speciation by IC-ICP-MS. Production of arsenate and selenite matched the disappearance of arsenite and selenate, indicating that the proposed reaction occurs as written.

EFFECTS OF SUBSTRATE CONCENTRATION

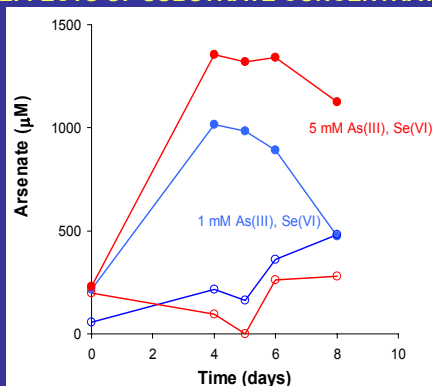


Figure 8. Effects of substrate concentration on anaerobic arsenite oxidation rates were tested with 1 mM arsenite + selenate and 5 mM arsenite + selenate. Increased substrate concentration increased the initial rate of arsenite oxidation. Some oxidation also occurred in filtered controls.

COMPARING NITRATE AS A T.E.A.

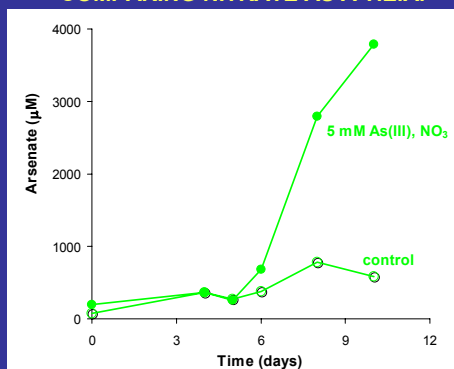


Figure 9. Nitrate (5 mM) and arsenite (5 mM) were added to Mono Lake water to compare rates of oxidation to those using selenate. After an initial lag time, arsenite was oxidized more rapidly than in the selenate experiment. Some oxidation also occurred in filtered controls.

CONCLUSIONS

- Oxidation of arsenite using selenate is a novel microbial metabolism
- Anaerobic arsenite oxidation using selenate can support cell growth
- Metabolism may be present in both autotrophic and heterotrophic bacteria
- Nitrate yields more rapid oxidation rates than selenate
- Anaerobic arsenite oxidation is less important than aerobic oxidation in the Mono Lake As cycle due to low in situ TEA concentrations

ACKNOWLEDGEMENTS

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