

Report as of FY2007 for 2005AZ114G: "Chemolithotrophic denitrification: The missing link in the biogeochemical cycle of arsenic"

Publications

- Conference Proceedings:
 - ◆ Sierra-Alvarez, R., W. Sun, P. Rowlette, I. Cortinas and JA Field. 2005. Anoxic Oxidation of Arsenite Linked to Denitrification. Eighth International In Situ and On-Site Bioremediation Symposium. June 6-9, 2005. Baltimore, MD. (Conference proceedings).

Report Follows

Progress Report: USGS-NIWR 104G Project # 2005AZ114G

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**Chemolithotrophic Denitrification:
The Missing Link in the Biogeochemical Arsenic Cycle?**

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Microorganisms are important in catalyzing conversions of arsenic between its two common oxidation states, arsenate (As(V)) and arsenite (As(III)). Recent evidence indicates that nitrate-reducing bacteria can oxidize As^{III} in anoxic environments. The objective of this study is to evaluate the importance of chemolithotrophic denitrifying bacteria in the biogeochemical cycle of arsenic. The proposed research will examine the direct microbial oxidation of As(III) with nitrate as electron acceptor, as well as the microbial oxidation of Fe(II) with nitrate and subsequent adsorption of As(V) by the iron oxides formed. The central question addressed in this proposal is whether anoxic oxidations of As(III) and Fe(II) are ubiquitous process in groundwater and surface waters controlling the mobility of arsenic.

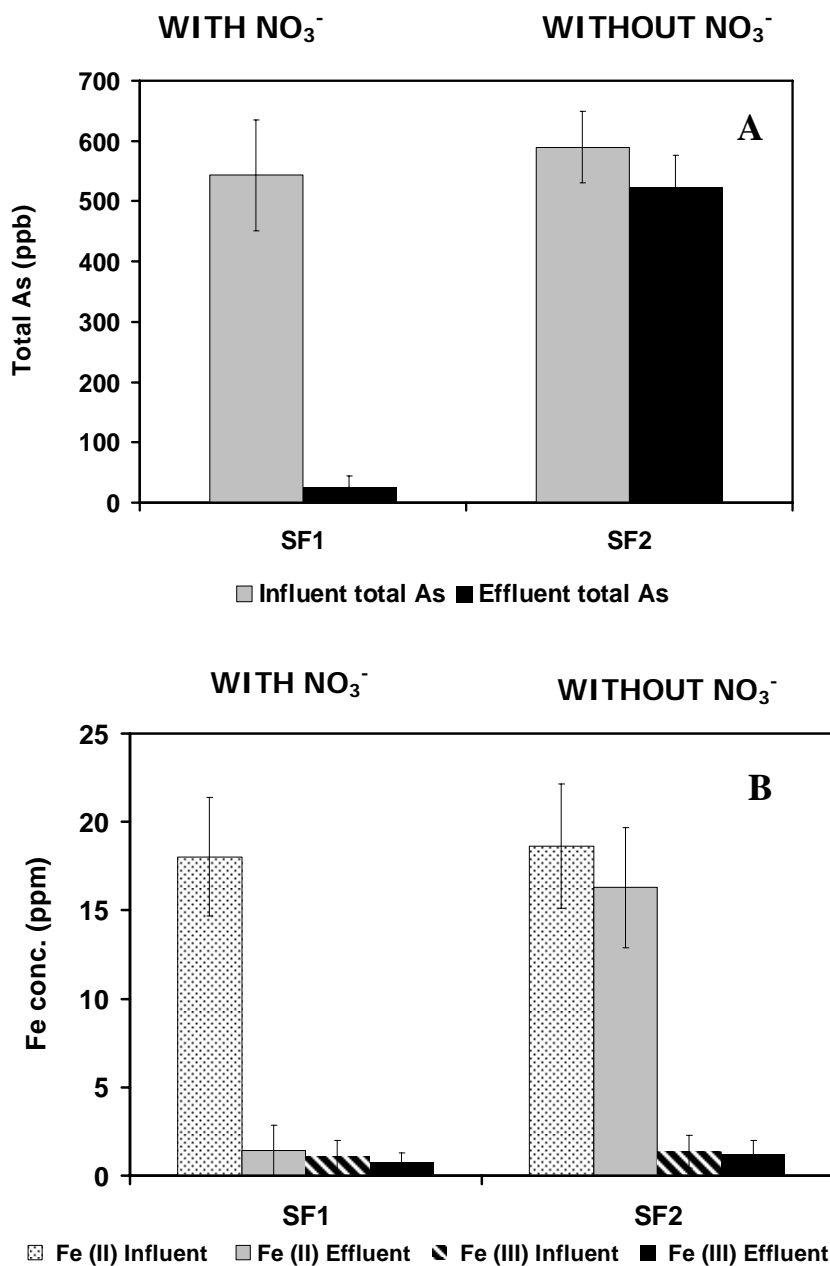
Research conducted during the first two year of this project has demonstrated that the anoxic oxidation of As(III) by chemolithotrophic denitrification is a relatively ubiquitous process that can be enriched from inocula in anaerobic sludges and sediments. The evidence indicates that As(III) oxidation is linked to the complete denitrification of NO₃⁻ to N₂ gas. The change in speciation from As(III) to As(V) by this anoxic process was shown to lower the mobility of arsenic in a model sediment column packed with aluminum oxides.

Work during the last year of the project tested the impact of nitrate in reducing the mobility of arsenic in a sand sediment column (SF1) fed with a mixture of Fe(II) and As(III), which represent the species of iron and arsenic in anoxic groundwater. A control column (SF2) fed with only nitrate and As(III) was run in parallel. The results of this continuous flow experiment confirmed that the oxidation of iron by chemolithotrophic denitrifiers leads to the formation of iron oxides that serve as sorbents of both As(III) and As(V) (Figures 1A and 1B).

The graph demonstrates that the iron in the effluent of SF2 was predominately composed of Fe(II); whereas the effluent of SF1 contained only small amount of Fe(III). The total iron concentration in the SF1 effluent was considerably lower than in the corresponding influent due to its continued adsorption/precipitation on the surface of sand. These findings indicate that in nature nitrate should be expected to decrease the mobility of arsenic by two mechanisms: 1) microbially catalyzed oxidation of arsenite to arsenate, 2) microbial oxidation of ferrous iron to ferric iron oxides which are effective arsenic sorbents. Furthermore, our results suggest that nitrate supplementation to groundwater could be used as an effective approach for the bioremediation of arsenic. Considering the levels of arsenic found in contaminated groundwater, the concentrations of nitrate are well below the federal drinking water standards for this nitrogen compound.

Significant effort was also dedicated to identify the microorganisms responsible for the anoxic oxidation linked to denitrification in four enrichment cultures developed in our research using inocula from different sources (anaerobic pond sediments, anaerobic reactor sludge; sludge from a continuous As-oxidizing denitrifying bioreactor). The different cultures were maintained in a mineral medium supplemented with As(III) and nitrate under a CO₂/N₂ atmosphere. No yeast or other organic amendments were added to the medium. Microbial cultures were characterized utilizing different microbial molecular ecology techniques, including denaturing gradient gel electrophoresis (DGGE), fluorescence in situ hybridization (FISH), sequencing of 16S DNA clone libraries. DGGE fingerprints revealed that in spite of the fact that the medium only contained nitrate and arsenite, the enrichments cultures consisted of various microorganisms. Sequencing of the 16S DNA clone libraries constructed for the different enrichment cultures revealed that sequences closely related to those of bacteria in two different bacterial genera were dominant in the cultures. The relative abundance of microorganisms in those two groups in the various cultures was determined by FISH utilizing specific probes designed in our study.

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Figures 1A and 1B. Average concentration of arsenic in the influent (grey block) and effluent (solid block) of reactors SF1 and SF2 (**Panel A**). Average concentration of ferrous iron (dotted block) and ferric iron (stripped block) in the influent, and average concentration of ferrous iron (grey block) and ferric iron (solid block) in the effluent of continuous bioreactors SF1 and SF2 (**Panel B**). SF1 and the control bioreactor SF2 were fed with As(III) (6.7 μ M) and Fe(360 μ M), in addition the feed of reactor SF2 was supplemented with nitrate (2.0 mM). Both reactors were packed with sand as 95% of the empty bed volume and inoculated with 2.94 g VSS/l anaerobic sludge obtained from a As(III)-oxidizing chemolithoautotrophic denitrifying reactor.