

Report as of FY2006 for 2005AZ114G: "Chemolithotrophic denitrification: The missing link in the biogeochemical cycle of arsenic"

Publications

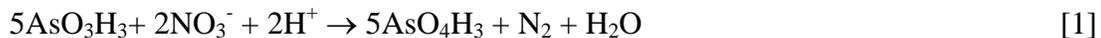
- Conference Proceedings:
 - Sierra-Alvarez, R., W. Sun, P. Rowlette, I. Cortinas and JA Field. 2005. Anoxic Oxidation of Arsenite Linked to Denitrification. Eighth International In Situ and On-Site Bioremediation Symposium. June 6-9, 2005. Baltimore, MD. (Conference proceedings).

Report Follows

Progress Report

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.

Inocula from different sources were tested for their ability to support the anoxic oxidation of As(III) with nitrate as the electron acceptor. Pond sediments and wastewater sludges were found to be capable of this reaction. The results indicate that chemolithotrophic denitrification utilizing As(III) as an energy source is a ubiquitous process in anaerobic environments. The microbial reactions could be sustained for long periods of time in upward flow anaerobic sludge bed reactors (in which biomass is immobilized as granular biofilms). An example of continuous reactor operation is shown in Figure 1 by the formation of As(V) from As(III). The reactor was able to tolerate up to 5 mM As(III); however when 7.5 mM As(III) was applied, there was a steady decline in activity that was corrected by lowering the influent As(III) concentration back to 3.75 mM. The molar ratio of As(V) formed compared to nitrate consumed (corrected for background removal by the endogenous substrate) was found to be 2.45. This is close the theoretical stoichiometry of 2.5 for complete denitrification as shown in equation 1 below:



To confirm that N₂ was indeed the end product of the reaction, a batch experiment was conducted with acclimatized continuous reactor sludge to test for N₂ production from NO₃⁻ with and without As(III) addition as electron donor. The results shown in Figure 2 indicate that N₂ gas was formed in response to As(III) addition. The ratio of As(V) formed to net NO₃⁻ consumption (corrected for endogenous nitrate consumption) was found to be 2.65, which was close to the theoretical value for complete denitrification (eq. 1). The molar yield of N₂-N compared to NO₃⁻-N consumed was 94.3%. This constitutes the first direct evidence that As(III) oxidation is linked to complete denitrification.

Several enrichment cultures have been developed from the original inocula or from biomass from the continuous reactors. Enrichments were made by repeated transfers (once every 3 weeks) into basal mineral medium with 0.5 mM As(III) and excess nitrate. Presently, three enrichment cultures are maintained as follows: 1) originating from an anaerobic biofilm from a reactor treating distillery wastewater; 2) originating from pond sediments; and 3) originating from biofilm from one of our continuous reactor converting As(III) to As(V) linked to denitrification. Although As(III) is readily used as an electron donor by the enrichment cultures, it is nonetheless an inhibitory compound. In batch experiments, rates of As(III)-linked denitrification are observed to increase with decreasing As(III) concentrations due to the lower inhibitory effect at lower concentrations.

As(III) is generally considered to be more mobile in the environment compared to As(V). The basis for this claim is that As(III) is less strongly adsorbed by certain common metal oxides

such as aluminum oxides. Adsorption isotherms of both As(III) and As(V) shown in Figure 2, illustrate that As(III) is less strongly adsorbed by activated alumina (AA) compared to As(V). This information taken in combination with the fact that NO_3^- is used by microorganisms to oxidize As(III) led us to the following hypothesis. Nitrate can potentially be used to oxidize As(III) in groundwater to decrease the mobility of arsenic. The As(V) formed would be more strongly retained by metal oxides in soil. We set out to test the hypothesis using columns packed with activated alumina. The columns (0.42 L) were continuously fed with approximately 500 $\mu\text{g/L}$ of As(III), column either in the presence of nitrate (column S1) or absence of nitrate (column S2). The columns were inoculated with an As(III) oxidizing-denitrifying enrichment culture. Figure 4 shows the release of arsenic (measured as total arsenic) was greater in S2 compared to S1 in accordance with the expectation. Column S2 broke-through on day 200; whereas in the same time period, only half the arsenic entering the column S1 was released. The differences in the release of arsenic from the two activated alumina columns could be attributed to the difference in speciation of arsenic resulting from the microbial oxidation of As(III) to As(V) by chemolithotrophic denitrifiers in column S1. Figure 5 illustrates the average speciation of arsenic in the influent and effluent of reactors S1 and S2 during days 202 to 215. The graph demonstrates that the arsenic in the influent and the effluent of S2 was predominately composed of As(III); whereas the effluent of S1 contained only As(V). There was incomplete recovery of As(V) in the S1 effluent due to its continued adsorption by activated alumina. The results taken as a whole suggest that microbial oxidation of As(III) linked to denitrification decreases the environmental mobility of arsenic.

In the coming period, we will test the impact of nitrate in reducing the mobility of arsenic in sand sediment columns fed with a mixture of Fe(II) and As(III), which would represent the species of iron and arsenic in anoxic groundwater. The expectation is that the oxidation of iron by chemolithotrophic denitrifiers will form iron oxides that can adsorb both As(III) and As(V).

In conclusion, the research so far 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 NO_3^- to N_2 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.

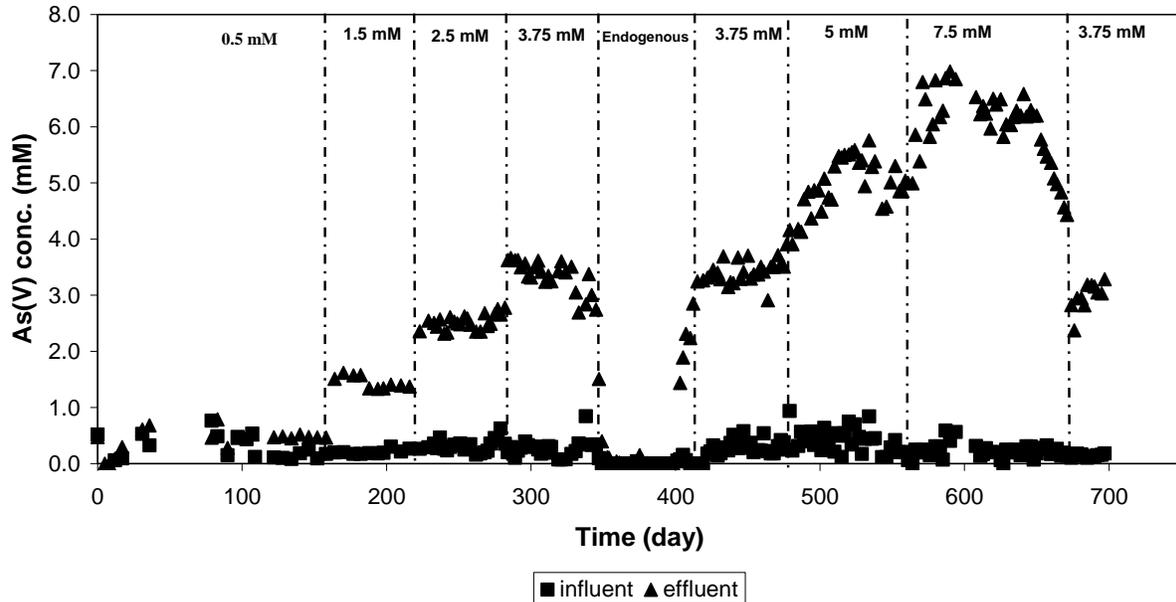


Figure 1. Influent and effluent concentrations of As(V) during the operation of a 2-L laboratory-scale upflow anaerobic sludge blanket reactor inoculated with 27 g volatile suspended solids/L of granular anaerobic biofilm from a full scale anaerobic reactor treating distillery wastewater (Nedalco). The reactor was fed with basal mineral medium and As(III) as the sole energy source and nitrate as the sole electron acceptor, the As(III) concentrations added in influent are indicated in the graph (endogenous means no addition of any electron donor). The hydraulic retention time was maintained at 1 day.

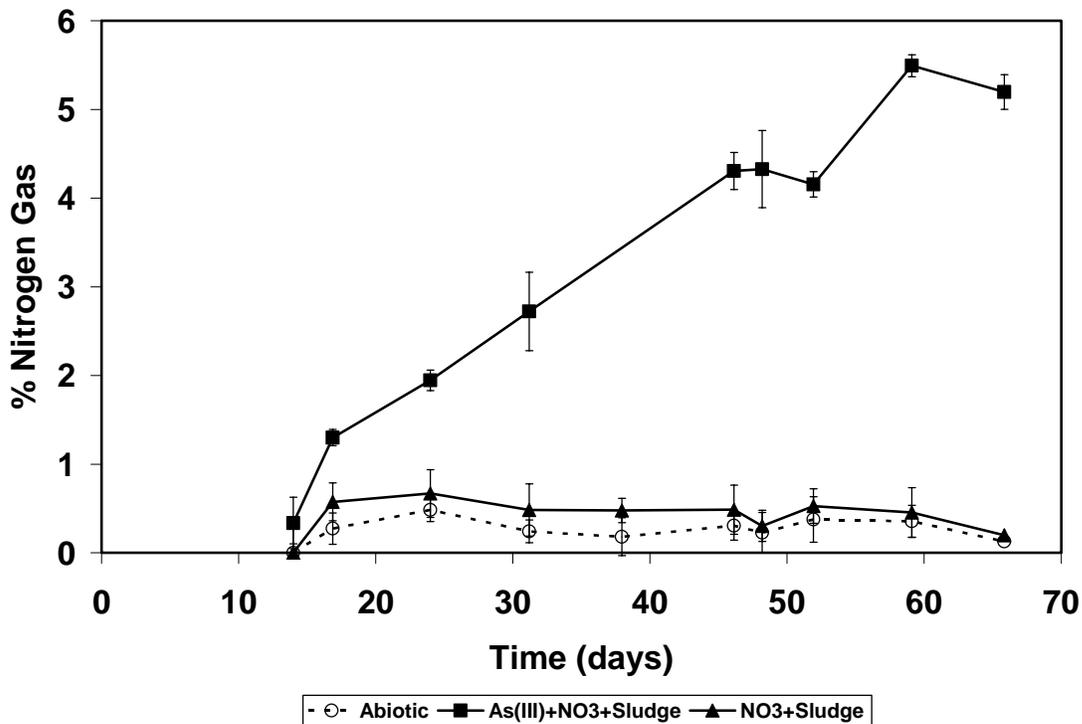


Figure 2. Formation of N_2 gas from the oxidation of As(III) linked to denitrification. Bottles were incubated with 3.5 mM As(III) with 0.6 g volatile suspended solids/L

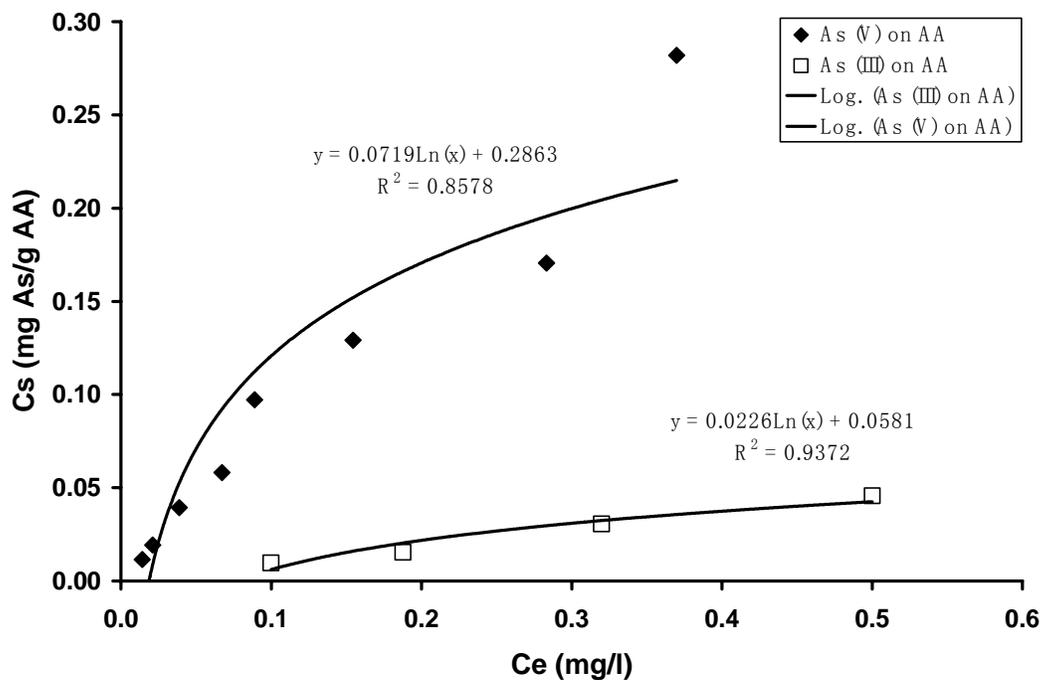


Figure 3. Adsorption isotherms of As(V) and As(III) on activated aluminum.

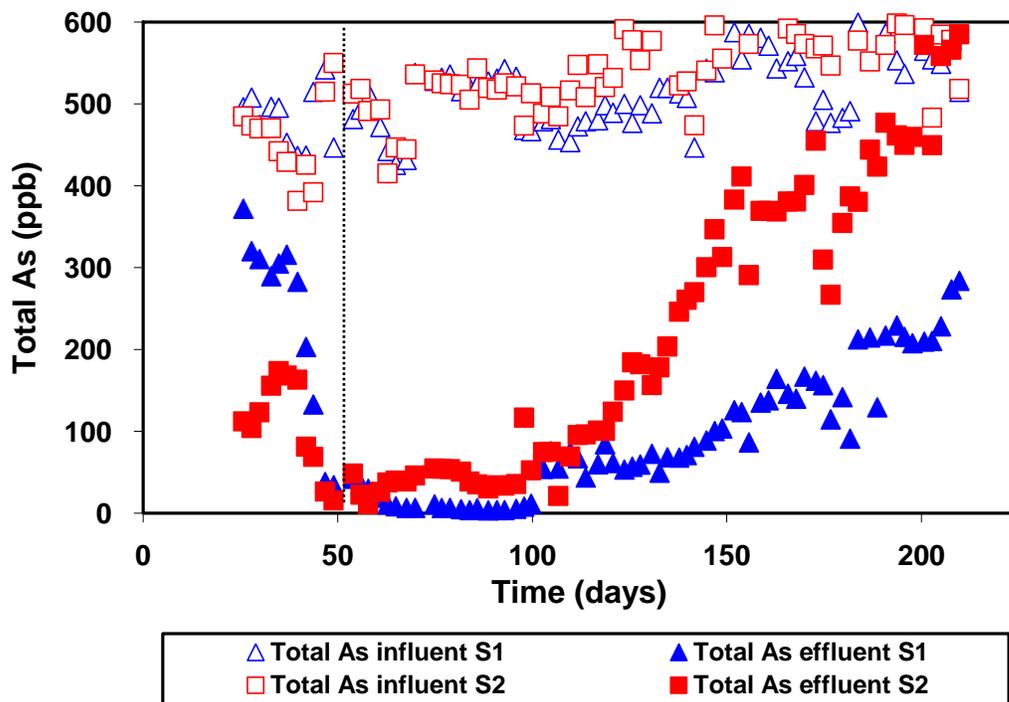


Figure 4. Influent and Effluent concentrations of total arsenic entering activated alumina packed bed columns S1 (with NO_3^-) and S2 (without NO_3^-). The vertical line on day 50 indicates the time point, after which the pH was controlled around 7.5, prior to day 50 the pH was alkaline (8 to 10).

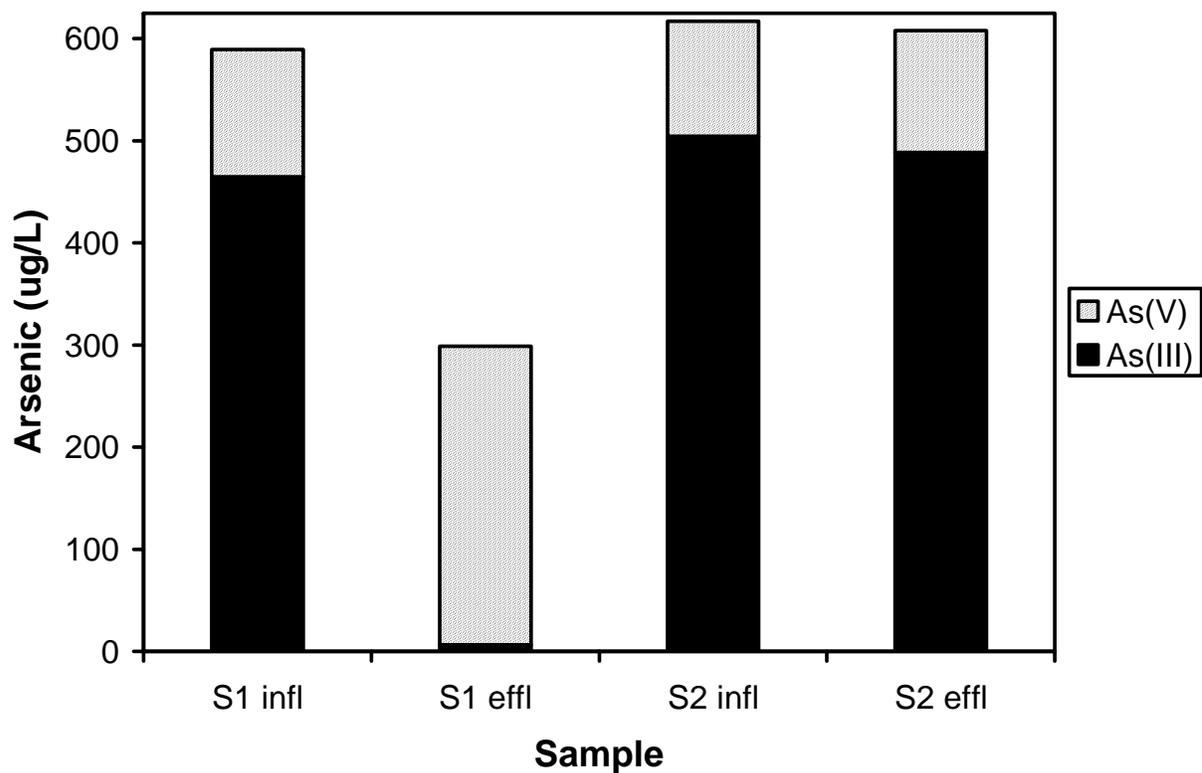


Figure 5. Speciation of arsenic in influent and effluent samples of activated alumina packed columns containing NO_3^- (S1) or lacking NO_3^- (S2).

Student Support

PhD Student:

Wenjie Sun

Undergraduate Assistants:

Pieter Rowlette
 Ivann Hsu
 Analucia Canizales
 Lily Milner