Biological Controls on the Precipitation of Chromium in Harbor Sediments

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LONG-TERM GOALS

My long term goal is to understand the mechanisms controlling the cycling of metals in the marine environment, more specifically the role of microbial activities in the precipitation of metals and mineral formation. It is important to be able to distinguish biotic from abiotic processes and to determine the relative contribution of the different mechanisms to metal cycling and the microbial populations involved.

OBJECTIVES

The overall goal of this project is to evaluate the processes involved in the attenuation of chromium (Cr) contamination in harbor sites. Specifically our objectives are to 1) evaluate the mechanisms of Cr(VI) reduction in harbor sediments; 2) evaluate whether bacteria can couple their growth on organic matter to Cr(VI) reduction and if so, whether there is a hierarchy in the use of electron acceptors by these bacteria; and 3) to determine the effects of Cr(VI) on metal- and sulfate-reducing activities and consequential precipitation of Cr.

APPROACH

1) We hypothesize that Cr(VI) can be reduced by sulfate-reducing and metal-reducing bacteria by two pathways, 1) indirectly through chemical precipitation via the metabolic products HS^- and Fe^{2+}, and 2) direct use of Cr(VI) as an electron acceptor. We also hypothesize that the relative importance of sulfate-reducing and metal-reducing bacteria for Cr precipitation will be different depending on the levels of Cr(VI). To demonstrate that sulfate-reducing and metal-reducing bacteria reduce Cr(VI) directly or indirectly we are using consortia and pure cultures obtained from polluted sediments and measuring the disappearance of Cr(VI) or precipitation of solid phase Cr (Cr(III)) with time in defined media in the presence and absence of SO_4^{2-} and Fe(III). Bacterial growth and the metabolic products, HS^- and Fe(II), are measured. The effect of different concentrations of Cr(VI) on the relative rates (i.e., kinetics) of Cr(VI) reduction by sulfate-reducing and metal-reducing bacterial cultures will be measured. An analogous set of experiments will be performed with natural and mesocosm sediment slurries. This work is primarily being carried out by the postgraduate researcher, Dr. Anna Obraztsova

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and graduate student Ms. Meriah Arias, a graduate student working on this project but supported by a NIH fellowship.

2) We hypothesize that the potential energy that can be obtained from the utilization of Cr(VI) as an electron acceptor can partially outweigh the toxic effects of the metal on these bacteria and that the selection among potentially competing electron acceptors is concentration dependent. Since recent evidence suggests that at least some bacteria appear to grow at the expense of Cr(VI) reduction, we plan to elucidate the hierarchy among Cr(VI), Fe(III), and/or SO_4^{2-} with pure cultures by comparing the rates of metal reduction and bacterial growth in experiments with various ratios of Cr(VI) and Fe(III) or SO_4^{2-} in a defined medium. Dr. Obraztsova is working on this aspect of the project.

3) The activities of metal- and sulfate-reducing microorganisms are involved in metal reduction/detoxification processes and are important factors controlling the fate of metals in polluted areas. We hypothesize that metals may affect the diversity and therefore the relative activity of metal- and sulfate-reducing microbial populations. Field and laboratory (pure culture, consortia, and mesocosm) experiments will be conducted to measure ferric iron and sulfate reduction rates. Experiments with natural sediments, both from polluted and non-polluted control sites and with laboratory mesocosms set up with a range of added amounts of Cr will be conducted and compared to evaluate whether the activities of SRB or MRB dominate under different conditions. The effect of Cr(VI) in the overlying water column on bacterial diversity and profiles of redox sensitive species (O_2, H_2S, and Fe(II)) will be evaluated. Bacterial diversity will be examined by denaturing gradient gel electrophoresis (DGGE) and the chemistry will be measured using microelectrodes. The microbiology work is primarily being carried out by graduate student Ms. Meriah Arias, a graduate student working on this project but supported by a NIH fellowship. The microelectrode work will be carried out by a new graduate student in the lab, Ms. Karen Murray, who is not directly supported by this project.

WORK COMPLETED

We have isolated several strains of bacteria that appear to use Cr(VI) as an electron acceptor for growth.

Two stable anaerobic consortia from Mare Island Naval Shipyard and one from Point Magu have been tested for their ability to reduce Cr(VI) at different concentration and DGGE experiments are in progress to assess the populations that are resistant to and/or reduce Cr(VI).

Laboratory mesocosms to evaluate the effect of Cr(VI) on microbial processes and populations in sediments have been designed and their assembly is almost complete.

We have purchased the electrochemical analyzer and are learning how to make measurements of O_2 and H_2S.

RESULTS

We examined Cr(VI) reduction by marine bacteria to evaluate which groups of organisms are likely important players in Cr(VI) reduction in marine environments. We identified several bacteria that appear to couple growth to the reduction of Cr(VI) under anaerobic conditions. We have obtained one isolate from Salt Pond (a coastal pond near Woods Hole, MA) that is closely related to *Pantoea agglomerans* (strain SP1) and another isolate from Marine Island Naval Shipyard (San Francisco Bay) that is a strain of *Shewanella alga* (strain FMI). Both of these strains can grow either aerobically or anaerobically with nitrate, various metals including Cr(VI), and sulfur compounds as electron acceptors. Strain FMI was able to reduce Cr(VI) up to concentrations of ~1 mM and was able to reduce Cr(VI) even when other electron acceptors (O_2, NO_3^-, and S^0) were present, although the rates were different.

To further investigate the role of bacteria in Cr(VI) reduction, two stable anaerobic consortia from metal-contaminated sediments at Mare Island Naval Shipyard, one of which produced hydrogen sulfide, were obtained and grown with varying concentrations of Cr(VI). Both consortia reduced ~1 mM Cr(VI) within 96 hours. The microbial compositions of the consortia grown in the presence and absence of Cr(VI) were investigated by denaturing gradient gel electrophoresis of ribosomal small subunit (16S) PCR products. The dominant organisms in the presence of Cr(VI) were most similar to *Pantoea, Enterococcus, Pseudomonas* and *Shewanella* species. A third stable anaerobic consortium from Cr-contaminated soils at Point Mugu capable of reducing ~2 mM Cr(VI) was also obtained. This consortium, when grown in the presence of Cr(VI) was also composed of organisms similar to *Pseudomonas* and *Shewanella* species. This suggests that Cr(VI) reduction in these consortia was not due to sulfate-reducing organisms.

IMPACT/APPLICATIONS

The overall goal of this project is to address the problem of heavy metal contamination in Navy facilities, specifically chromium (Cr) in harbor sites. Traditional methods for treating contaminated waters, soils, and sediments such as dredging are often expensive, laborious, and may have adverse environmental impacts. The proposed research will help elucidate the complex interplay between direct and indirect effects of microbial processes on Cr precipitation in marine sediments and improve our understanding of some key factors that affect Cr mobility. Ultimately, this information may lead to new strategies for controlling or remediating metal pollution in marine sediments. Specifically, we expect that our data will provide the scientific basis for predicting the extent and pathways for the natural attenuation of Cr in harbor sediments.

TRANSITIONS

None at present.

RELATED PROJECTS

We have performed some preliminary experiments to examine the mechanisms of Cr(VI) reduction by *Shewanella* strain FMI and *Pantoea* strain SP1 using protein polyacrylamide gel electrophoresis. It is important to elucidate the biochemistry of Cr(VI) reduction in bacteria that grow with Cr(VI) in order to fully understand the mechanism of Cr(VI) reduction. A proposal to fund this work has been submitted.

PUBLICATIONS

Francis, C. A., A. Y. Obraztsova and B.M. Tebo. Dissimilatory metal reduction by the facultative anaerobe *Pantoea agglomerans* SP1. *Applied and Environmental Microbiology*. Submitted.