Project 1012275

Formation and Reactivity of Biogenic Iron Minerals

Ferris, F. Grant
University of Toronto

RESULTS TO DATE: Formation and Reactivity of Biogenic Iron Microminerals

Objective

This objective of this research is to evaluate the formation and reactivity of biogenic iron minerals produced by dissimilatory iron-reducing bacteria, particularly with respect to the solid phase incorporation of metal contaminants (e.g., uranium, chromium, and nickel).

Methods

· Dissimilatory Iron Reducing Bacteria

Shewanella putrefaciens strain CN-32 is a facultative iron reducing bacteria, and the primary test organism being used in this investigation. This organism was isolated from an anaerobic aquifer in northwestern New Mexico and has been deposited in the Subsurface Microbial Culture Collection located at the Oregon Graduate Institute (D. Boone, SMCC Curator). This organism is ideal for examining the impact of changes in metabolic activity on the formation of microminerals because it can grow heterotrophically (with lactate as electron donor and carbon source) or autotrophically with H2 and CO2. This organism can also use a variety of electron acceptors for growth, including Fe(III), Mn(IV), NO3-, fumarate, sulfur, and O2.

For experimental work, strain CN-32 has been grown routinely in aerobic shake cultures using Tryptic Soy Broth (TSB) as a complete growth medium. Mid-exponential phase cells were harvested by centrifugation and washed 3 times in 30 mM 1,4-piperazinediethanesulfonic acid (PIPES) buffer, pH 7. Cells prepared in this manner express iron reductase activity. Batch experiments were conducted using a chemically defined medium (sodium lactate 1.8mM, NH4Cl2 2.2mM, KCl 1.2mM, CaCl2 0.61mM, mineral salts solution, NaH2PO4 3.9mM, 4.5 mM PIPES buffer at pH 6.8, and hydrous ferric oxide - see below) in sealed culture tubes under an inert nitrogen atmosphere in an anaerobic chamber. Tubes were inoculated with washed cells to obtain a final cell concentration of 0.4 optical density at 600 nm (~ 10^8 cells per ml). To assess the solid phase partitioning of Ni2+, the metal was added to prior to inoculation of the culture tubes at concentrations of 10^-3 M, 10^-4 M, and 10^-5 M. The cultures were incubated without shaking to avoid disturbing the association of bacteria with minerals.

· Hydrous Ferric Oxide

The research has thus far focused on mineralogical transformations of poorly crystalline hydrous ferric oxide (HFO). HFO was prepared by neutralization of a FeCl3.6H2O solution with 1.0 N NaOH followed by repeated washing with ultra pure water (UPW) to remove residual chloride and sodium. HFO prepared in this manner was maintained as an aqueous suspension, and was purged with O2-free N2 before dilution to a final concentration of 45mM Fe (as HFO) in the culture medium.

· Analytical Methods

Reduction of the Fe (III) oxides were monitored over time by following the pH and formation of Fe (II) in both the solution and associated with the solid using Ferrozine as the colorimetric reagent. Samples were removed through the stoppers of sealed culture tubes using a needle and syringe that had been flushed

with O2-free N2. One mL of the was added to one ml of 1.0 N HCl and allowed to sit overnight before analyzing for Fe(II). The 1.0 N HCl extraction dissolves most ferrous iron forms expected in the samples. A second mL of the original sample was passed through a 0.22 um filter; this fraction comprised the soluble Fe (II) and dissolved Ni2+ fraction. The concentration of Ni2+ in solution was determined atomic absorption spectrometry. The viability of the cells were evaluated by epifluorescence microscopy using the Live Dead Baclight Bacteria Viability Kit (Molecular Probes, Inc. L-7007).

For scanning electron microscopy (SEM), cells were collected by centrifugation, washed with UPW, and vacuum filtered onto nucleopore 0.22 um polycarbonate membrane filters. The filters were then placed in vials containing 2% glutaraldehyde for one hour, rinsed with UPW, and then dehydrated through a graded series of ethanol (25%, 50%, 80%, 95%, 100%) before critical point drying using Smidri Critical Point Dryer. They were subsequently mounted on aluminum stubs and sputter coated with gold and examined using a JOEL scanning electron microscope equipped with an energy dispersive X-Ray spectrometer (EDX) at a probe current of 1 x 10-9 A and an accelerating voltage of 15KV. Major and minor mineral phases present in the samples were identified by X-ray diffraction (XRD) using a Philips PW1050 goniometer and PW1830/40 automated diffractometer operating with a high power Cu tube source (40 mA - 40 kV) and sealed Xe PW3011 detector with pulse height analysis levels at 32 to 80% to reduce the effects of Fe fluorescence.

Results

The reductive dissolution of HFO by S. putrefaciens CN-32 was revealed by a progressive increase in dissolved Fe (II) concentrations over time. At 15 days, the dissolved Fe (II) concentrations peaked near 3.5 mM, corresponding to 7.7 % of the total Fe in the culture tubes. Thereafter, dissolved Fe (II) concentrations decreased to less than 1.0 mM at 30 days. On the other hand, 1.0 N HCL extractable Fe (II) concentrations at 15 days were nearly 30 mM, corresponding to ca. 68 % of the total Fe, and remained at this level to the end of the experiments at 30 days. During the experiments, the pH of the cultures increased asymptotically to 8.3.

The introduction of Ni2+ into the cultures had a clear negative impact on HFO reduction. At 15 days with 10-3 M Ni2+, dissolved Fe (II) concentrations peaked at only 2.8 mM, compared with 3.0 mM for 10-4 and 10-5 M Ni2+. Concentrations of 1.0 N HCL extractable Fe (II) leveled off at day 5 near 20 mM with each of the three concentrations of Ni2+. Thus, only 51 % of the total Fe was reduced by CN-32 in the presence of Ni2+ compared with nearly 75 % in the absence of Ni2+. Similarly, culture pH only increased to 8.0, compared to 8.3 in the absence of Ni2+. The pronounced inhibitory affect of Ni2+ on HFO reduction was traced using epifluorescence microscopy to a 20 % decrease of viable cells in the cultures, suggesting that Ni2+ is directly toxic to the bacteria.

Although Ni2+ had an adverse impact on the progress of HFO reduction, dissolved Ni2+ concentrations decreased rapidly in an exponential fashion during the experiments. Assuming first order kinetics, rate constants for the decrease in Ni2+ concentrations were 0.12 day-1, 0.08 day-1, and 0.10 day-1 for 10^-3, 10^-4, and 10^-5 M Ni2+, respectively. Scanning electron microscopic and energy dispersive X-ray spectroscopic examination of the residual solids remaining in the cultures confirmed that Ni2+ had partitioned into a neogenic mineral phase arising from the reductive dissolution and transformation of HFO.

Identification of the neogenic mineral phase produced in response to HFO reduction was accomplished by X-ray diffraction. Early in the experiments, there were no peaks observed in the diffraction patterns, as expected for freshly precipitated HFO. However, by 5 days in the absence of Ni2+ a distinct peak (d-spacing of 1.1 nm) for the mixed Fe (II)/Fe (III) mineral known as green rust (GR) had evolved. Also, minor peaks for metavivianite (a hydrous ferrous phosphate mineral) were observed. The signal to noise ratio of the GR peaks increased progressively over the later stages of the experiment.

In samples with Ni 2+, the peak for GR was clearly visisble in X-ray diffraction patterns after only 5 days. In comparison the the control experiments, the signal to noise ratio of the GR peaks in the presence of Ni2+ were improved. This is consistent with other studies which suggest that divalent metals like Ni2+ can substitute into the crystal structure of GR. Thus, results obtained to date indicate that GR is not only a major product arising from bacterial reduction of HFO, but serves additionally as very efficient sink for the solid phase capture and immobilization of dissolved Ni2+.

Preliminary experiments with Cr3+ and UO2+2 indicate that these metals are also removed from solution in response to GR formation during the bacterial reduction of HFO.