Project 1012275

Formation and Reactivity of Biogenic Iron Microminerals

Beveridge, Terrance J.
University of Guelph

Ferris, F. Grant
Univeristy of Toronto

RESULTS TO DATE: Formation and Reactivity of Biogenic Iron Microminerals Interim Report

Terry Beveridge, Susan Glasauer, University of Guelph, Department of Microbiology

August 20, 1999

Objective

The overall purpose of the project is to explore and quantify the processes that control the formation and reactivity of biogenic iron microminerals, and the impact of these processes on the solubility of metal contaminants, e.g., uranium, chromium and nickel. The research addresses how surface components of bacterial cells, extracellular organic material, and the aqueous geochemistry of the DIRB microenvironment impacts the mineralogy, chemical state and micromorphology of reduced iron phases.

Towards achieving these goals, we have been investigating the formation of iron minerals, and their structure at the bacteria-solution interface, in the presence of Ni as an initial contaminant. The effects of Ni in the absence of iron were also studied in order to quantify the toxic effects of this metal. Towards understanding the role of functional groups on the bacterial surface, we have been working to isolate membrane vesicles (MVs) and lipopolysaccharides (LPS) for use in metal sorption studies. MVs are a natural product from these gram-negative cells which contain all of the normal surface components; they may also have reductase activity. LPS is the major surface constituent and must be chemically extracted from the bacteria.

Approach

Shewanella putrefaciens (strain CN32) was grown in tryptic soy broth (TSB) and then transferred to a mineral medium designed to maintain the bacteria without significant growth. The medium contained iron oxides as hydrous ferric oxide (HFO) and Ni2+. The cultures were grown in an anaerobic chamber under an Ar/H2 atmosphere. Bacteria and precipitates were observed by transmission electron microscopy (TEM), and chemical elements were identified by electron diffraction and microbeam x-ray detection techniques.

Shewanella was also grown under aerobic conditions without HFO, using the same mineral medium as above, with and without phosphate and Ni2+. The pH and light scattering (to monitor bacterial growth) were measured at intervals over 12 days, and viability of the cultures was established by plate count methods. Cells and precipitates were studied by TEM.

Because the project focuses on reactions at bacterial surfaces, using cell wall material rather than the entire bacterium will help in studying the identity of surface sites active in metal accumulation. Membrane vesicles (MVs) are segments of membrane that have budded off the cell and formed independent surface units. As a simplified analog of bacteria-associated membrane, they represent an ideal surface for studying metal reactions. MVs have been extensively studied for Pseudomonas aeruginosa; we have been refining methods used for isolating and concentrating these in order to obtain MVs from S.

putrefaciens for metal adsorption studies. These MVs must also be characterized to establish their chemical, structural and enzymatic properties.

Analogous to separating MVs, isolating LPS for use in sorption studies will help quantify their role in the immobilization of metals. LPS were isolated from two strains of S. putrefaciens (CN32 and MR-1), using an established protocol frequently used in our laboratory for whole-cell lysates and proteinase K digestion.

Progress

Electron microscopy on unstained cells treated with HFO and Ni2+ showed that precipitates containing Fe and Ni were concentrated in the periplasmic space of the cell wall. The membranes of the most heavily mineralized cells appeared to be discontinuous, yet the cells retained an intact shape. Electron diffraction on the precipitates gives evidence for green rust, a mixed Fe2+-Fe3+ mineral. The presence and location of this reduced Fe phase supports the view that HFO was reduced and associated with the bacteria. Reports of green rust in soils are rare, probably due to the rapid oxidation of Fe2+, which implies that the phase we observed has been stabilized. Together with the heavily mineralized cells, we observed cells with few precipitates that appeared intact; these may be cells in a resting state, or active cells that have shed the post-reduction metal products.

S. putrefaciens cells grown in the presence of Ni and P had higher survival rates than cells grown with Ni alone, shown by cell counts that were 2 orders of magnitude higher after 12 days. Numbers of cells grown with P only did not change. TEM on unstained cells grown with Ni + P appeared healthier and less electron dense than those grown with Ni only; no obvious mineral precipitates were observed in association with the cells from any treatment. Energy dispersive x-ray spectroscopy will be used to locate Ni and P in these samples. By the new year, we hope to have a new cryoTEM-ESI unit in place so that electron spectroscopic imaging of these elements can be done.

TEM analysis of Shewanella cells showed abundant MVs, which had a multilamellar structure. MVs isolated using traditional methods resulted in poor yield and several of the manipulations during isolation had to be revised, especially since there was flagella contamination in the initial preparations. A different technique, which used concentrated cell biomass, resulted in higher yields and these MVs were associated with abundant cytochromes. This may confirm previous reports of cytochrome-containing outer membrane in this species.

When LPS was extracted from both S. putrefaciens strains and run by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), only the so-called "rough" variety (no O-side-chains) was detected. No significant changes were found when the strains were grown using different carbon and energy sources, aerobically or anaerobically. The same result was obtained when cultures were grown in the presence of a mineral phase (iron minerals, glass beads, hydroxylapatite, etc.), although this may be due to incomplete extraction of LPS adsorbed to the solid phase. This work continues.

Future work

TEM observations and quantification of metals associated with the bacterial surface are continuing, using chromium and uranium in combination with HFO. To identify membrane sites reactive to HFO, we will study adsorption of HFO alone to cells of S. putrefaciens in different metabolic states, using freeze-fracture and freeze-substitution techniques to keep the relationship of cells to the iron hydroxide and any newly-formed minerals as intact as possible. We plan to use synchrotron microbeam methods to study the interaction of metals with reactive groups of the bacterial surface to determine nucleation sites which initiate mineral phases. At the same time we plan to establish the cellular microenvironments which help determine and drive these phases over time.

Work on isolating MVs and LPS and studying the composition of LPS from different iron-reducing species (i.e., Geobacter) will also continue. The cell wall components will be used for back titration and biomineralization experiments to determine charge and reactive sites on these membrane fractions.