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Formation and Reactivity of Biogenic Iron Microminerals

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RESULTS TO DATE: Formation and Reactivity of Biogenic Iron Microminerals

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The overall purpose of the project was to explore and quantify the processes that control the formation and reactivity of biogenic iron microminerals and their impact on the solubility of metal contaminants. The research addressed 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.

In the previous report (August 8, 2000), we established a protocol for growth of S. putrefaciens CN32 that more closely mimics natural conditions than do methods that have been previously used for culturing this bacterium. We then quantified the attachment of preformed amorphous and crystalline Fe(hydr)oxide minerals under conditions of growth in the defined medium (DM) under both aerobic and anaerobic conditions, and initiated studies on FeIII mineral reduction. In the present report, we describe investigations on the reduction of hydrous ferric oxide (HFO), goethite and hematite by CN32 during slow growth in DM (#1). Once the conditions for reduction were established, the biogenic minerals that formed subsequent to FeIII reduction were characterized and their spatial relationship to the cells was observed (#2). CN32 cells were also grown in a medium containing soluble FeIII to compare the influence of a fast Fe reduction rate on mineral precipitation (#3).

Approach for #1: Fe(hydr)oxide mineral reduction. Conditions for growth of the cells and methods for preparation of the hydrous ferric oxide (HFO), goethite and hematite used in the reduction studies were described in our previous report. Planktonic cells of CN32 were grown under anaerobic conditions in the presence of fumarate (an organic electron acceptor), HFO, nano-crystalline and micro-crystalline goethite, and nano-crystalline hematite, in DM. For the minerals, the order of increasing crystal order is: ferrihydrite < nano-goethite < micro-goethite @ hematite. The treatments were monitored for viable cell numbers by plate counts (CFUs) on trypticase soy agar (TSA), for Fe2+ by the ferrozine method using 0.5 M HCl extracts, and for lactate and acetate (an end-product of lactate catabolism) by HPLC measurements of filtered (0.2 mm) cell extracts. The appearance of the cells and their relationship to the FeIII minerals was followed by transmission electron microscopy (TEM) on cells prepared by whole mount and conventional embedding methods for thin sectioning.

Approach for #2: Biogenic minerals. After it was determined that CN32 would only reduce the FeIII contained in HFO, and not in the more crystalline minerals, a series of experiments was carried out under anaerobic conditions in which the bacteria were grown exclusively on HFO in DM. This was to determine which biogenic minerals were formed and whether their formation was reproducible. The treatments were monitored for cell numbers and Fe2+ continuously during growth, and samples were removed at regular intervals for examination by TEM. Once cell numbers had declined to < 10-3 CFU/ml (colony-forming units/ml) the precipitates were washed and dried in an anaerobic atmosphere (4% H2:96% Ar), and

powder X-ray diffraction (XRD) was carried out in an N2 atmosphere on samples lightly pressed into an aluminum topfill holder.

Approach for #3: Growth in FeIII-citrate. CN32 was grown under anaerobic conditions on a medium similar to the DM, with the addition of vitamins. FeIII-citrate (56 mM) served as the electron acceptor. Previous experiments have shown that CN32 grows rapidly on this medium with concomitant copious mineral precipitation. The aim of our experiments was to establish spatial relationships between the cell surface and the minerals formed during the rapid growth of the cells. The treatments were monitored for cell numbers (CFUs) and for Fe2+ (ferrozine method), and samples were removed at regular intervals for TEM examination.

Progress for #1. In contrast to what has been found in other investigations of FeIII mineral reduction by CN32, we determined that the bacteria did not reduce crystalline minerals; i.e., there was no significant Fe2+ detected with the goethite and hematite experiments. There was also no significant production of acetate, an indication that the cells were not metabolically active. Cell numbers declined over the 46-day length of the experiment from an initial inoculum of 109 CFU/ml. After 46 days, however, viable cells still existed in the treatments with the nano- and micro-crystalline goethite. No viable cells were found in the hematite treatment. Observations by TEM showed that the cells were initially closely associated with the minerals, similar to what was described in the previous report. Over time, however, some cells appeared to form dense clusters that were surrounded at their periphery by minerals. The formation of flocs is well known as a survival mechanism of bacteria, similar to the formation of biofilms, and may explain why the cells in some treatments remained viable under unfavorable conditions.

In contrast, approximately 50% of the HFO-Fe was reduced and cell numbers after 46 days were 2 orders of magnitude higher than in the treatments with goethite. The production of acetate indicated that the cells were active. Studies by TEM indicated that a variety of minerals formed during (or as a consequence of) reduction and this was further investigated as described in the next section. Survival and activity of the cells was highest overall in the treatment with fumarate.

The fact that CN32 did not reduce the crystalline FeIII minerals indicates that understanding the growth of the cells is crucial to the relevance of metal reduction studies. This bacterium has been implicated as being of great significance to the cycling of Fe in anaerobic sediments, via the weathering of formed minerals and the precipitation of biogenic minerals. It is known, however, that the formation of cell factors essential for reduction (e.g., membrane-bound cytochromes) is determined by the nutrients the cell receives. The influence that the nutritional status of cells has on Fe reduction has been given little attention and deserves further study.

Progress for #2. Over the course of 9 months, cells were repeatedly grown in anaerobic DM with HFO as the electron acceptor. We found that, even under identical conditions, the experiments were never perfectly reproducible; this lack of reproducibility has been observed by others working with the same bacterium and indicates the need for repeated studies within a given system. Clearly, some unknown factor(s) is at play which regulates Fe metabolism or the general growth of this bacterium.

The variability was demonstrated by the growth of the cells and by the minerals that formed during Fe reduction. The mineral products of the experiments fall into two rough groups: vivianite was formed when Fe reduction was relatively fast, and a variety of minerals formed when Fe reduction was slower. These minerals were magnetite, vivianite, green rust and goethite, as determined by XRD. In all cases where vivianite formed, the mineral crystals were large and extremely crystalline, as shown by the narrowness of the XRD peaks. The green rust was similarly crystalline; its formation was of particular interest because little is known about this mineral, which is metastable and highly sensitive to oxygen. The magnetite crystals were very small (< 10 nm), which correlates well with what has been previously found for biogenic magnetite. Goethite most likely formed by the abiotic transformation of the HFO. Such a variety of highly crystalline minerals was previously found (in a similar growth medium) only when the electron shuttle AQDS was included and was explained as occlusion of the surface by Fe2+ or steric effects that prevent the cells from accessing more than the periphery of the HFO aggregates. The fact that all or most

of the initial HFO was removed during the reduction proves that cells are able to access all of the Fe contained in the HFO and supports either an attachment mechanism or the existence of highly mobile cells that ?graze? the aggregate surface.

Observations by TEM support the attachment mechanism. Individual cells or cells in flocs were found tightly attached to the HFO, using both whole mount and conventional embedding techniques. Magnetite enclosed within the aggregates of HFO was detected by electron diffraction on whole mount samples. In contrast, vivianite and green rust were generally only observed by TEM at some distance from the cells. Vivianite appeared to form sphaerulitic crystals in the matrix of HFO; this habit results from fast crystal growth. In summary, the minerals appeared to form by diffusion of Fe2+ away from the cells and reflect the microheterogeneity of the environment created by cells actively respiring on HFO. The rates at which Fe is reduced by CN32 and Fe2+ is removed or diffuses from the cell surface are key to the mineral phases that result, and deserve further study.

At the same time that extracellular minerals were forming during FeIII reduction, we observed clusters of membrane-bound Fe-oxide granules of approximately 30-50 nm diameter in the bacterial cytoplasm near the cell poles. The granules appeared in the cells just after Fe reduction began and were apparent in > 90% of the cells we observed. We are currently conducting high energy electron diffraction studies to determine the mineralogy of these intracellular Fe granules. Because of the important role that iron metabolism plays in this bacterium, it is quite possible that these internal Fe-oxide granules are essential for or are by-products of growth. This is only the second report of the direct formation of an intracellular Fe mineral by a prokaryote. (Magnetite formed by magnetotactic bacteria was the first reported intracellular Fe mineral).

Progress for #3. The type of mineral product formed in CN32 culture in the FeIII-citrate medium depended on the growth phase of the bacteria and, hence, on the bacterium?s physiological status and the rate of FeIII reduction. For most of the cells, extensive mineralization was not observed during the active (exponential) growth phase. However, TEM observations on cells prepared as whole mounts indicated that approximately 10% of the cells were completely mineralized. Isolation of the mineral phase by differential centrifugation yielded a dense black precipitate which had a metallic luster. Examination of thin sections by TEM revealed that the cytoplasmic and periplasmic spaces of the mineralized bacteria were completely filled with an electron-dense material and no extracellular minerals were observed. The quantity of the mineral decreased when the culture reached the early stationary growth phase and disappeared at the late stationary phase. Formation of a light colored abundant mineral precipitate, which similar investigations have shown to be vivianite, started only when the culture reached stationary phase and more than 90% of the ferric iron was reduced. This coincided with a decrease in the number of viable cells. Observations on cells prepared as thin sections revealed a number of mineralization phenomena; i.e., clusters of feather-like minerals that were not associated with cells, completely mineralized cells which appeared to act as nucleation centers for crystallization, and needle-like minerals attached to the cell walls of lysed cells. No minerals were associated with viable cells which were determined to be those cells with an intact and relatively homogeneous cytoplasm. The appearance of apparently different kinds of minerals with different modes of precipitation, and their association with particular growth stages of the cells, has not been observed before. Of particular interest is the localization of the mineral precipitate during the active growth phase to only a small percentage of the cells. A similar phenomenon has been observed during our work on mineral sorption by cells and may indicate a ?suicide? mechanism by which some cells are sacrificed to maintain suitable growth conditions for the rest of the population.

Further work to characterize the minerals will require in situ methods with high spatial resolution. The mineral products will be identified and quantified by selected area electron diffraction (SAED), X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDS), and X-ray absorption fine structure spectroscopy (XAFS).

DELIVERABLES:

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