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Advanced Experimental Analysis of Controls on Microbial Fe(III) Oxide Reduction

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Research Objective

Understanding factors which control the long-term survival and activity of Fe(III)-reducing bacteria (FeRB) in subsurface sedimentary environments is important for predicting their ability to serve as agents for bioremediation of organic and inorganic contaminants. This project seeks to refine our quantitative understanding of microbiological and geochemical controls on bacterial Fe(III) oxide reduction and growth of FeRB, using laboratory reactor systems which mimic to varying degrees the physical and chemical conditions of subsurface sedimentary environments. Methods for studying microbial Fe(III) oxide reduction and FeRB growth in experimental systems which incorporate advective aqueous phase flux are being developed for this purpose. These methodologies, together with an accumulating database on the kinetics of Fe(III) reduction and bacterial growth with various synthetic and natural Fe(III) oxide minerals, will be applicable to experimental and modeling studies of subsurface contaminant transformations directly coupled to or influenced by bacterial Fe(III) oxide reduction and FeRB activity.

Research Progress and Implications

This report summarizes research accomplished after approximately 1.5 yr of a 3-yr project. A central hypothesis of the research is that advective elimination of the primary end-product of Fe(III) oxide reduction, Fe(II), will enhance the rate and extent of microbial Fe(III) oxide reduction in open experimental systems. This hypothesis is based on previous studies in our laboratory which demonstrated that association of evolved Fe(II) with oxide and FeRB cell surfaces (via adsorption or surface precipitation) is a primary cause for cessation of Fe(III) oxide reduction activity in batch culture experiments. Semicontinuous culturing was adopted as a first approach to test this basic hypothesis. Synthetic goethite or natural Fe(III) oxide-rich subsoils were used as Fe(III) sources, with the Fe(III)-reducing bacterium Shewanella alga as the test organism.

Average residence times of 90, 18, 9, or 4.5 d were established by replacing 1, 5, 10 or 20 mL of the aqueous phase of the cultures (30 mL total volume) with fresh sterile, anaerobic culture medium every 3 d. Consistent with our basic hypothesis, replacement of 5-20 mL of medium (4.5-18 d residence time) resulted in a 2-4 fold increase in the cumulative amount of Fe(II) produced in the cultures over a 2 month incubation period, relative to that occurring in parallel batch cultures. Fe(III) reduction in the semicontinuous cultures showed little or no sign of slow-down at the end of the experiment, whereas reduction had virtually ceased in the batch cultures. A two-fold increase in the extent of synthetic goethite reduction was also observed in an experiment conducted with a continuous-flow stirred reactor (CFSTR) with a residence time of 2-3 d.

In all cases the enhanced reduction was accounted for by generation of dissolved Fe(II) which was periodically removed from the cultures during aqueous phase replacement. The Fe(II) content of the solid-phase (sorbed or precipitated) was lower in the semicontinuous and CFSTR systems, which suggested that aqueous phase flux led to significant Fe(II) desorption or reduced solid-phase association. Together these results indicate that the primary basis for the stimulation of Fe(III) oxide reduction was removal of the major end-product of oxide reduction, i.e. Fe(II). Our findings demonstrate that aqueous phase transport and attendant elimination of reaction end-products could play an important role in governing the in-situ rate and persistence of microbial Fe(III) oxide reduction activity in anaerobic subsurface sediments. This in turn may have an important impact on the survival

and activity of FeRB to be exploited for bioremediation purposes (e.g. the reductive immobilization of uranium, chromium, cobalt, and other metal/radionuclide contaminants; oxidation of hydrocarbon contaminants; reductive dechlorination of chlorinated solvents). An important implication of these findings is that models describing bacterial Fe(III) oxide reduction in subsurface environments will have to account for the fate of evolved Fe(II), and relate this in some manner to the abundance of available Fe(III) reduction sites and the viability of Fe(III)-reducing bacteria.

Other significant research accomplishments generated by this project include: (1) a detailed analysis of the growth rate and biomass yield (derived from measurements of cell protein production) of two representative FeRB (S. alga and Geobacter metallireducens) growing with different forms of synthetic Fe(III) (ferric citrate, amorphous Fe(OH)3, a-FeOOH) as an electron acceptor. Results indicate that despite considerable differences in the rate and pattern of Fe(III) reduction and FeRB growth, an approximately consistent long-term biomass yield of 5 to 15 mg protein per mmol Fe(III) reduced was observed with the different forms of Fe(III). These results provide a useful point of departure for quantitative modeling of FeRB growth and metabolism in various types of experimental and in-situ anaerobic sedimentary systems; (2) a preliminary examination of the use of ^{3}H-leucine incorporation for measuring short-term (essentially instantaneous) FeRB (G. metallireducens and S. alga) growth rates during reduction of soluble and solid-phase Fe(III). Results suggest that G. metallireducens, unlike S. alga, lacks the ability to express a high rate of exogenous leucine incorporation (probably due to the lack of an "active" cell membrane leucine uptake system). An important implication of this finding is that ^{3}H-leucine incorporation may not be an appropriate means of estimating FeRB growth rates in natural sediments in which Geobacter dominates Fe(III) reduction activity.

Another important implication of this study is that rates of S. alga biomass production estimated from 3H-leucine incorporation with 10-1000 nM total leucine concentration were much lower than those inferred from independent measurements of biomass yield during Fe(III) reduction, probably because of isotope dilution by de novo leucine biosynthesis. A subsequent experiment indicated that additions of at least 1 mM total leucine are required to repress de novo biosynthesis during short-term (0.5-2 hr) ^{3}H-leucine incorporation experiments in culture medium. Rates of protein production during growth of S. alga on ferric citrate estimated from ^{3}H-leucine incorporation with 1 mM total leucine agreed within a factor of 2 with independent estimates of bacterial protein production. This result indicates that ^{3}H-leucine incorporation can provide a quantitatively valid method for tracking instantaneous bacterial growth rates in experimental Fe(III) reduction systems.

Planned Activities

Manuscripts on the initial semicontinuous culture work and the FeRB growth/biomass production study are in preparation and will be submitted for publication by September 1998. A detailed series of experiments using the semicontinuous culture systems is in progress, examining the influence of various aqueous phase components (chelators, dissolved inorganic carbon, humic substances) on the rate and extent of natural and synthetic Fe(III) oxide reduction; this work will form the basis of a manuscript to be submitted for publication early next year. Completion of two papers dealing with ^{3}H-leucine incorporation by FeRB and the use of ^{3}H-leucine incorporation to measure FeRB growth rates is anticipated by the end of the year. A broad-ranging study of the rate/extent of natural and synthetic Fe(III) oxide reduction and associated FeRB growth will be initiated this summer to examine how the mineralogy and Fe(III) oxide abundance of natural soils and sediments may quantitatively control the growth and metabolism of these organisms; this study will combine measurements of gross biomass production with ^{3}H-leucine based estimates of instantaneous FeRB growth rates during the active growth phase of batch culture systems. Initiation of experiments on Fe(III) oxide reduction and FeRB growth in CFSTR and column reactor systems will occur early this Summer; the main thrust of research activity on this project will become focused on these experimental systems starting in the Fall of 1998.

Other Access To Information

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