FACTORS LIMITING MICROBIAL ACTIVITY IN VOLCANIC TUFF AT YUCCA MOUNTAIN

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ABSTRACT

Samples of tuff aseptically collected from 10 locations in the Exploratory Shaft Facility at the site of the proposed high-level nuclear waste repository at Yucca Mountain, Nevada Test Site were analyzed for microbiological populations, activities, and factors limiting microbial activity. Radiotracer assays (^{14}C-labeled organic substrate mineralization), direct microscopic counts, and plate counts were used. Radiolabeled substrates were glucose, acetate, and glutamate. Radiotracer experiments were carried out with and without moisture and inorganic nutrient amendments to determine factors limiting to microbial activities. Nearly all samples showed the presence of microorganisms with the potential to mineralize organic substrates. Addition of inorganic nutrients stimulated activities in a small number of samples. The presence of viable microbial communities within the tuff has implications for transport of contaminants.

I. INTRODUCTION

The proposed Yucca Mountain high-level nuclear waste repository site has been undergoing intense site characterization, including detailed microbiological study of the volcanic tuff. Use of Yucca Mountain at the Nevada Test Site for storage of spent nuclear fuel rods and other high-level nuclear waste will require assurance that any spill that might occur in the unsaturated zone of the site will have a very low probability of threatening underlying groundwater resources. Previous studies of the microbiology of other volcanic tuff environments (Rainier Mesa, Nevada Test Site, and Pajarito Plateau, Los Alamos, New Mexico) have shown the presence of significant populations of microorganisms.[1-4] The presence of microorganisms within geological material in the vicinity of a waste repository has potential implications for the transport of contaminants, including transuranic radionuclides. Microbes could accelerate transport by solubilizing or chelating metal contaminants; alternatively contaminants could become sorbed to microbial biomass, thereby retarding transport. The purpose of this study was to quantify indigenous microorganisms and their activities in Yucca Mountain volcanic tuff and to determine factors (nutrients and water availability) that may be limiting to microbial activities.

II. WORK DESCRIPTION

Samples were collected from February to November 1995 during the drilling of the Exploratory Shaft Facility (ESF). Samples were collected from ten sites within the ESF where small alcoves had been recently excavated perpendicular to the ESF tunnel. Samples were collected from freshly exposed rock faces using a sterilized hammer and chisel or a sterilized pneumatic chisel. Four samples were collected from each alcove. Fluorescent microbead tracers were used to determine whether excavation was sufficiently deep to avoid any surface contaminants. One of each of the four samples was used as a negative control (autoclaved) or as a positive control (spiked with a live culture). Portions of each sample were shipped to New Mexico Institute of Mining and Technology for microbiological analyses.

Volcanic tuff samples were crushed aseptically to a uniform powder using a combination of a sterilized hydraulic rock press and a sterilized hammer and stainless steel pan. All sample handling was carried out in a sterile transfer hood under HEPA-filtered air.

Direct microscopic counts of microorganisms were performed using the acridine orange method, as described by Kieft et al.[2, 5] Culturable aerobic heterotrophs were quantified by a plate count procedure on R2A agar (Difco, Detroit). Potential microbial activities were quantified by a ^{14}C-organic substrate mineralization procedure, as previously described[2, 5] The ^{14}C-labeled substrates were glucose, acetate, and glutamate, in separate treatments. 10-g samples of crushed rock were placed into 60-ml serum vials. Approximately 3.7 kBq (0.1 μCi) of labeled substrate (1.25 mM) were added to each vial. To test the effects of moisture limitation, some vials were wetted to approximately 50% water holding capacity with sterile distilled water. Nutrient limitation was tested by moistening the samples with

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an N and P amendment (0.1 mM NH_4Cl, 0.05 mM K_2HPO_4). To test whether the concentration of substrate added might be too low to stimulate activity, separate treatments were spiked with 100x the concentration of unlabeled glucose as labeled glucose). Substrate mineralization to $^{14}CO_2$ was captured in a 0.3 N NaOH trap and quantified by liquid scintillation counting. Mineralization was quantified weekly for three weeks. All treatments with all samples were performed in triplicate. Poisoned controls (3.7% formaldehyde) were used to quantify abiotic substrate mineralization.

III. RESULTS

Direct counts of microorganisms were below detection ($<3 \times 10^4$ cells g^{-1} wet weight of sediment) in nearly all samples. Sterilized control samples were all below detection. The highest number of microbes (6.1×10^5 cells g^{-1} dry weight) were counted in a non-control samples was found in a sample from the Pah Canyon ash flow.

Standard plate counts of non-control samples ranged from below detection ($<10^1$ CFU g^{-1} wet weight of sediment) to 8.5×10^4 CFU g^{-1} dry weight in a sample from the contact between the Tiva Canyon and Pah Canyon tuffs. Autoclaved control samples generally showed few, if any colonies on plate counts. Positive controls showed prolific growth.

Mineralization of radiolabeled organic substrates showed a wide range of activities among samples, among treatments, and among substrates. Percent mineralizations ranged from below detection (after subtraction of sterile control values) to over 70% mineralization during three weeks incubation. Moisture was limiting in most samples. Highest values were found for acetate, followed by glucose and glutamate. Addition of inorganic nutrients (N and P) resulted in stimulation of microbial activity in some, but not all samples. Additional unlabeled substrate did not increase mineralization of labeled substrate in most samples.

IV. CONCLUSIONS AND DISCUSSION

The general conclusions that can be drawn from these data are that microbes are present in the volcanic tuff and that the abundance of these microorganisms varies among geologic formations within Yucca Mountain. Moisture can be considered to be limiting in most of the samples. However, the moisture limitation is probably due to low nutrient diffusion in the unsaturated environment and not due to severe desiccation stress.[2] The inorganic nutrient limitation is not surprising, given the generally low nutrient availability in the tuff. The negligible potential for transport of either nutrients or microbes within the unsaturated rock matrix suggests that the inhabitant microbes have been surviving in situ by endogenous metabolism for extremely long time periods.

The finding of significant populations of viable microorganisms in the vicinity of the proposed Yucca Mountain repository has important implications for transport modeling and risk assessment. While it's nearly impossible to predict the influence of microorganisms on transport of radioactive waste, one can be nearly certain that there will be an effect. The nature of a contaminant mixture will determine whether existing populations will grow, thereby increasing their impact. If the spill contains readily degradable organic C along with moisture and other nutrients, the microbial impact could be profound. These preliminary findings demonstrate a critical need for further microbial characterization and experimentation, as well as for inclusion of microbial influences in geochemical and geohydrological modeling.

ACKNOWLEDGEMENT

The research reported in this paper was supported by the Yucca Mountain Site Characterization Office as Part of the Civilian Radioactive Waste Management Program, managed by the U. S. Department of Energy, Yucca Mountain Site Characterization Project.

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