N-026 Potential for Microbial Stimulation in Deep Vadose Zone Sediments by Gas-Phase Nutrients

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Abstract

Viable microbial populations are low, typically 10^4 cells per gram, in deep vadose zones in arid climates. There is evidence that microbial distribution in these environments is patchy. In addition, infiltration or injection of nutrient-laden water has the potential to spread and drive contaminants downward to the saturated zone. For these reasons, there are uncertainties regarding the feasibility of bioremediation of recalcitrant contaminants in deep vadose zones. The objectives of this study were to investigate the occurrence of denitrifying activity and gaseous carbon-utilizing activity in arid-climate deep vadose zone sediments contaminated with, and/or affected by past exposure to, carbon tetrachloride (CT). These metabolisms are known to degrade CT and/or its breakdown product chloroform under anoxic conditions. A second objective was to determine if CT would be degraded in these sediments under unsaturated, bulk-phase aerobic incubation conditions. Both denitrifier population (determined by MPN) and microbial heterotrophic activity (measured by mineralization of 14-C labeled glucose and acetate) were relatively low and the sediments with greater in situ moisture (10-21% versus 2-7%) tended to have higher activities. When sediments were amended with gaseous nutrients (nitrous oxide and triethyl/tributyl phosphate) and gaseous C sources (a mixture of methane, ethane, propylene, propane, and butane) and incubated for 6 months, approximately 50% of the samples showed removal of one or more gaseous C sources, with butane most commonly used (44% of samples), followed by propylene (42%), propane (31%), ethane (22%), and methane (4%). Gaseous N and gaseous P did not stimulate removal of gaseous C substrates compared to no addition of N and P. CT and gaseous C sources were spiked into the sediments that removed gaseous C sources to determine if hydrocarbon-degraders have the potential to degrade CT under unsaturated conditions. In summary, gaseous C sources – particularly butane and propylene – have promise for increasing the numbers and activity of indigenous microbial populations in arid-climate deep vadose zone sediments.

Introduction

There are large inventories of contaminants in deep vadose zones in the semiarid western U.S. Ongoing contamination of the saturated zone by contaminants migrating through these deep vadose zones further compounds the problem. In situ bioremediation of contaminants can offer advantages in cost, speed, public acceptance, and final cleanup levels achieved relative to physical removal methods. However, there are uncertainties regarding the feasibility of bioremediation of recalcitrant contaminants in deep vadose zones where microbial populations are low and discontinuous, and how hydrologic features of the vadose zone control microbiological processes.

Subsurface sediments were sampled from the Z9 trench area of the Department of Energy Hanford Site in Washington state. The Z-9 trench was used to dispose of liquid wastes to the soil column from 1955 to 1962. Wastes included large quantities of plutonium-contaminated wastewater and up to 79,000 gallons of carbon tetrachloride (CT), 7,000 gallons of tributyl phosphate, and 12,000 gallons of dibutyl phosphate. CT has migrated approximately 220 ft to the groundwater and produced a plume of approximately one square mile. A soil vacuum extraction system removed an estimated 53,000 kg of CT from the vadose zone between 1993 and 1999. However, residual CT exists in and adjacent to the fine-grained sediments and is a continuing source of groundwater contamination.

Boreholes were drilled in 2001 to more fully characterize the distribution of vadose zone CT and the associated microbiology. Because injection of water containing nutrients would tend to push vadose zone contaminants to the aquifer, gaseous nutrients (carbon/energy sources, nitrous oxide, and triethyl/tributyl phosphate) were considered as a potential means to stimulate microbial growth and activity. The ability of vadose zone microbial communities to utilize gaseous carbon sources and degrade CT was tested, and is reported in this poster.

Materials and Methods

• **Sediments:** twelve sediments varying in depth were collected from each of two holes adjacent to trench Z-9 (Table 1 and Figure 1).

• **Denitrifying activities:** denitrifiers were enumerated by the MPN method using 3 tubes/3 dilutions (10-fold) in R2A medium plus 0.05% KNO_3.

• **Microbial heterotrophic activities**: measured by mineralization of ^{14}C-labeled glucose and sodium acetate (0.25 μCi each) in reaction bottle with 10 g sediment.

• **Microbial stimulation by gas-phase nutrients**: serum bottles (160 ml size) crimp-sealed with aluminum caps and thick rubber stoppers were used as reaction vessels. Sediments (50 g) were amended with N_2O (41 μmoles) and triethyl/tributyl phosphate (7 μmoles each). Gaseous C sources (mixture of methane, ethane, propylene, propane, and butane, each at 82 μmoles) were also injected into the bottles. A set of positive controls received traditional nutrients (40 μmoles NH_4NO_3 and 7 μmoles K_2HPO_4) plus the gaseous C mixture. A set of negative controls received the gaseous C mixture but no N or P sources. Experiments were set up in duplicate for each of the 3 treatments from each of 12 sediment depths from both holes, for a total of 144 bottles. Representative sediments were autoclaved and served as sterile controls. Bottles were incubated in the dark at ambient temperature for 6 months and analyzed for loss of gaseous C sources by gas chromatography (GC).

• **Carbon tetrachloride (CT) addition:** After initial microbial stimulation by gaseous C, bottles that showed removal of at least one carbon source (37 from Hole 1 and 35 from Hole 2) (Table 2A) were selected for subsequent CT degradation studies. Bottles were first equilibrated with air and resealed, followed by injecting the same amount of fresh gaseous C sources as mentioned above, and spiked with CT (0.26 μmoles). CT and component gaseous C sources were analyzed by GC method after 4 months incubation. Three bottles that did not show any C removal from Hole 2 were also included in this study to determine if sediment initially negative for gaseous C removal could become positive with a double enrichment. Autoclaved sediments served as sterile controls. Those with CT concentrations at least 30% lower (2x standard deviation of the CT concentration in sterile controls), were scored as positive CT degradation. Incubation is ongoing and we plan to carry out a final 10 month sampling in August 2003.

Table 1. Sediments Used In This Study

Hole 1: well 299-W15-95, north side of Z-9 trench			Hole 2: well 299-W15-84, west side of Z-9 trench		
depth, ft	% moisture	formation	depth, ft	% moisture	formation
102.5	6.0	Hanford	112	10.5	Hanford
105	13.0	Hanford	114	15.0	Hanford
109	21.2	Plio-Pleistocene	118	20.7	Plio-Pleistocene
110.5	17.9	Plio-Pleistocene	122	17.8	Plio-Pleistocene
113	16.1	Ringold	125	4.0	Ringold
117	3.1	Ringold	128	3.9	Ringold
122	2.4	Ringold	131	2.6	Ringold
125	3.4	Ringold	142	4.9	Ringold
132	5.7	Ringold	152	6.9	Ringold
146	2.0	Ringold	162	2.6	Ringold
157	3.0	Ringold	172	2.3	Ringold
186	4.3	Ringold	194	2.1	Ringold

Description of formation:

Hanford: sand and silty sand derived from catastrophic floods

Plio-Pleistocene: ancient and buried soils composed of sandy silt above caliche w/gravel

Ringold: riverbank deposits composed of sandy gravel and sands

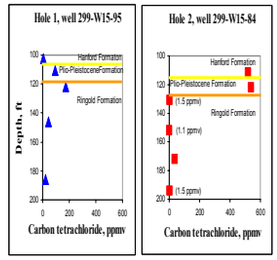

Figure 1. Measured Concentration of Carbon Tetrachloride in Borehole Gas Samples

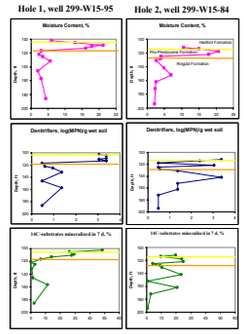

Figure 2. Relationship Between Sediment Moisture and Microbial Activities. Microbial activities in the deep vadose zone are low and in general are greater in sediments with higher moisture content. Population of denitrifying bacteria ranged from 3.5 log units per gram wet soil, in the upper depths and decreased to below detection limit (0.5 log unit) in the lower depths. The same trend was observed for microbial heterotrophic activities as measured by soil microorganism's ability to mineralize glucose and acetate. However, microbial distribution in these environments is patchy and heterogeneous as evidenced by the occurrence of several high activity zones in the lower depths (e.g. in Hole 2 at 140-160 ft depths).

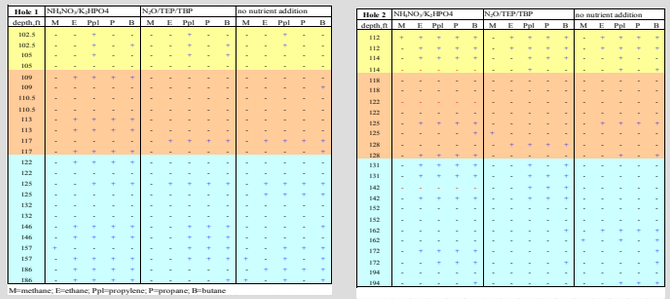

Table 2B. Summary of Table 2A. # and % of Bottles Using Individual Carbon Sources

	methane		ethane		propylene		propane		butane	
Treatments	hole 1	hole 2	hole 1	hole 2	hole 1	hole 2	hole 1	hole 2	hole 1	hole 2
NH_4NO_3/K_2HPO_4	1	1	10	9	14	10	11	10	12	12
N_2O/TEP/TBP	0	1	2	3	9	9	6	7	9	10
no nutrient	2	1	4	4	9	9	5	5	10	10
total # of positives--->	6		32		60		44		63	
% positives--->	4		22		42		31		44	

Table 2A. Microbial Utilization of Gaseous Carbon Substrates. After 6 months incubation, approximately 50% of the samples showed removal of at least one gaseous C source. The most commonly used gaseous C sources are butane (44%), followed by propylene (42%), propane (31%), ethane (22%), and methane (4%) (Table 2B). Removal of gaseous C was enhanced by amendment with traditional N and P (NH_4NO_3 and K_2HPO_4). However, addition of gaseous N and gaseous P (N_2O/TEP/TBP) did not stimulate gaseous C removal compared to the no N and P addition treatment.

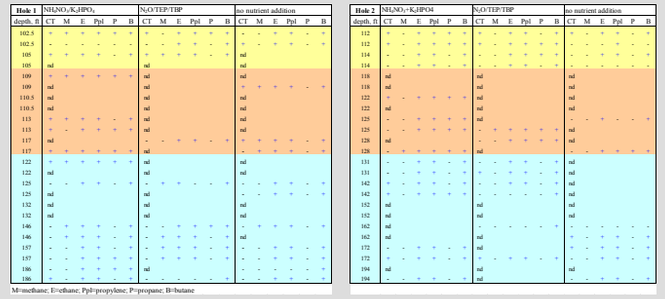

Table 3B. Summary of Table 3A. # and % of Bottles Degrading Carbon Tetrachloride

	Hole 1		Hole 2	
Treatments	# bottles tested	CT degraded	# bottles tested	CT degraded
NH_4NO_3/K_2HPO_4	15	8	15	6
N_2O/TEP/TBP	10	2	12	4
no nutrient	12	3	11	3
total # bottles--->	37	13	38	13
% positives--->	35		34	

Table 3A. Microbial Degradation of Carbon Tetrachloride in Presence of Gaseous Carbon Sources. When CT and gaseous C were spiked to those bottles showing initial removal of at least one gaseous C source, gaseous C removal was again observed, and CT degradation occurred in about 35% from both Hole 1 and Hole 2 (Table 3B).

Conclusions

• Microbial denitrifying and heterotrophic activities in deep vadose zone are low, and decrease with depth and sediment moisture at this site.

• Subsurface microorganisms in these low biomass sediments have the ability to utilize gaseous hydrocarbons. Of the 5 gaseous carbon sources tested, butane and propylene were most commonly used, propane and ethane were used to a lesser extent, and methane was not a viable carbon source in these deep vadose zone sediments.

• Gaseous N and gaseous P did not stimulate removal of gaseous C sources compared to no addition of N and P.

• About 1/3 of the sediments that initially degraded gaseous carbon sources were able to degrade CT in presence of gaseous carbon sources. With a double enrichment, two of the three bottles initially negative for gaseous C removal became positive for both gaseous C removal and CT degradation.

• Our results indicate there is potential for microbial stimulation in arid-climate deep vadose zones by gaseous hydrocarbons, particularly butane and propylene. The quantity of gaseous C removal indicates growth of microbes, which in turn, were able to degrade CT.

Acknowledgements

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