SYNOPSIS

Project Number: 05
Start: 06/01/94
End: 05/31/95

Title: The Effect Of Inhibitory Organic Compounds On The Transport And Distribution Of Bacteria In The Subsurface

Investigator(s): Clifford R. Lange
Department of Civil Engineering
College of Engineering
Auburn University

Focus Categories: GW, TS, ST

Congressional District: Third

Descriptors: Groundwater Modeling, Bacteria, Toxic Substances, Soil Microbiology
SYNOPSIS

The Effect of Inhibitory Organic Compounds on the Transport and Distribution of Bacteria in the Subsurface

Clifford R. Lange
Department of Civil Engineering
Auburn University, AL

A. Problem and Research Objectives

The transport of bacteria in the subsurface and the distribution of bacteria between porewater and the solid matrix are two related biological factors which are important to both modeling and remediation efforts. Both transport and attachment phenomena can be impacted by the presence of toxic organic compounds. Currently, modeling efforts assume that attached bacteria are most important with respect to contaminant biodegradation. If a contaminant leads to decreases in bacterial attachment, most existing models will underestimate the extent of biodegradation. For successful bioremediation, bacteria must be transported to the contaminated zone, attach to the matrix, and grow. If transport and attachment are altered by the presence of the contaminant, the rate and extent of bioremediation may be affected. This would have significant implications when the addition of genetically engineered bacteria to achieve greater biodegradation (Bioaugmentation) is attempted.

The focus of this investigation was to assess the effects of inhibitory organic compounds on the distribution and transport of bacteria in the subsurface. To accomplish this goal, a number of tasks were performed as follows:

1. The distribution of bacteria between porewater and the solid matrix before and after exposure to inhibitory organic compounds was determined.

2. The effect of inhibitory organic compounds on the transport of bacteria through sand columns was assessed.

3. The effect of acclimation to the inhibitory compound on transport and distribution was determined.

The results of these tasks are summarized in this document.

B. Methodology

Pure cultures of three bacterial species (Pseudomonas putida, Flavomonas sp., and Bacillus sp.) were grown in chemostats to provide consistent inocula for experimental investigations. Glass chromatography columns, measuring 0.5 meters long and 2.5 centimeters in diameter, were packed with Ottawa sand and saturated with water. After
the column was saturated, fluorescein dye was introduced as a tracer to assess the hydraulic characteristics of the column. Upon completion of the tracer testing, the column was flushed with buffer solution. To initiate a test run, a buffer solution containing bacteria was pumped through the column at a velocity of 5 meters/day. Bacteria passing through the column were quantified using epifluorescence microscopy and standard plate counting techniques. Data was collected over time until constant numbers of bacteria in the column effluent were measured for at least six hours. Next, the bacterial solution was exchanged for a solution containing the inhibitory test compound and the bacterial counts in the column effluent were quantified over time until a constant level was achieved. Finally, the column was dissected, and the distribution of bacteria between the porewater and matrix was determined as a function of column height using direct microscopic counts and viable plate counts.

Testing was conducted using toluene and pentachlorophenol over a range of organic concentrations from zero to saturation. The effects of the inhibitory compound on bacterial metabolism was quantified by Specific Oxygen Uptake Rate (SOUR) testing. To assess the effect of acclimation on transport, a second round of experiments were performed using bacteria cultured in the presence of the targeted inhibitory compound.

C. Principal Findings

Based on the results of the experimental study a number of interesting results were observed. Sorption of bacteria onto the sand was strongly a function of bacterial species. Flavomonas sp. demonstrated the highest amount of sorption without the presence of an inhibitory compound. Only two percent of the bacteria applied to the columns were retained on the matrix. This implies that bacteria injected into an aquifer would largely pass through the aquifer. For all three bacterial species tested, less than 20 percent of the retained bacteria were associated with the matrix and the majority were found in the porewater. In higher organic soils, it is likely that the conditions would be more favorable for adsorption onto the solid matrix, and a higher fraction would be associated with the matrix. However, this low fraction of matrix associated bacteria would skew models which assume the majority of bacteria exist as a biofilm.

The addition of inhibitory organic compounds catalyzed the desorption of many of the sorbed bacteria. This effect was demonstrated by a spike of bacteria occurring one pore volume after the inhibitory compound was pumped through the column, a decrease in the number of bacteria associated with the matrix, and an increase in bacteria in the porewater. This finding is significant since most groundwater fate and transport models assume the bacteria to be non-mobile. This desorption would cause the bacteria to move with the contaminant front.

Acclimation of the bacteria, by culturing the bacteria in the presence of the inhibitory organic compound did not change the degree of sorption onto the matrix. However, the desorption of bacteria following a pulse of the organic compound was significantly diminished. Thus, it was concluded that acclimation of bacteria to the organic compound may play a significant role in maintaining the organisms on the matrix. The
acclimated, compound biodegrading bacteria, may not move with the contaminant front and may exist as an attached film.

The findings indicate that the common assumption that attached organisms are the most important for subsurface biodegradation may not be true. In this research, a majority of the bacteria were associated with the porewater. When a spike of toxic organic compound passed through the column (as would occur as a contaminant front migrates through the subsurface) many of the sorbed bacteria were desorbed and left the column. Consequently, the bacteria would likely move with the contaminant front. This has great implications for the bioremediation of groundwater contamination.

D. Publications/Presentations


Manuscript under preparation for submission to Groundwater

E. Students Supported By Project

Jeanae C. Blackwell, Master of Science, Civil Engineering, August 31, 1996.
Angela Fernandez, Master of Science, Civil Engineering, April, 1997 (expected).
Katherine A. Call, Bachelor of Science, Environmental Science, June, 1996.