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Project Summary

Movement of Bacteria Through Soil and Aquifer Sand

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The transport of microorganisms in soils is of major importance for bioremediation of subsurface polluted zones. A procedure for evaluating the relative mobility and recovery of bacteria in the soil matrix was developed. Nineteen bacterial strains were selected that differed in their ability to be transported through soils. Measurements were made of sorption partition coefficient, hydrophobicity, net surface electrostatic charge, zeta potential, cell size, encapsulation, and flagellation of the cells. Only sorption and cell length were correlated with transport of the bacteria through soil. The breakthrough curves for *Pseudomonas* sp. KL2 moving through a column packed with a sandy aquifer material were determined. Ionic strength of the inflowing solution, bacterial density, and velocity of water flow were found to have an effect on breakthrough.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Approximately 50% of the United States population depends upon ground water as a source of drinking water. Consequently, the contamination of ground water by organic chemicals is widely recognized as a critical environmental problem. The transport and mobility of bacteria in soil and subsurface materials has been a subject of interest for the past few decades because of the environmental importance of these organisms. The bioremediation of underground waste-disposal sites by the use of introduced bacteria requires that the microorganisms move from the point of their introduction to the site of contamination. Such inoculation is necessary if microorganisms degrading the chemical contaminants are not present in the hazardous waste site or adjacent ground water.

Many toxic organic chemicals persist at underground hazardous waste sites despite being readily biodegradable under laboratory conditions. When this occurs, introduced bacteria selected for their capacity to degrade target contaminants, and capable of surviving and proliferating after injection in the aquifer, might be used to promote biodegradation. The introduction of bacteria to degrade toxic wastes in soil and subsurface has generated renewed interest in the transport and mobility of bacteria. However, the mobility of the added bacteria may determine their effectiveness for *in situ* bioremediation. If bacteria capable of degrading a contaminant are not present at the site of contamination, then bioremediation will only be effective if the added bacteria have both the capacity to reach the contaminated zone and to move through porous materials with the contaminant plume.

Considerable attention has been given to the mobility of bacteria and other microorganisms in soil and subsurface materials. These studies were conducted primarily because of concern with the dissemination

of pathogens from land spreading operations, ground-water recharge, or the disposal of manure or municipal sludge. Several studies have shown poor mobility of the investigated species of bacteria through soil. However, considerable movement of some bacteria was observed in field studies.

It is unclear whether the movement of bacteria that has been observed occurred through the soil matrix or through the macropores or channels that afford the organisms a relatively unhindered passage. It is, however, generally acknowledged that the movement of bacteria in a homogeneous porous medium is poor because of both adsorption and mechanical filtration of bacterial cells, which have been suggested as mechanisms for their retention in soils. In a study by various investigators, streptomycete conidia and a bacterium were displaced through a sand column; only 0.2% of the bacterial cells and 6% of the conidia were recovered in the effluent after passage of four pore volumes of water, but over 90% of the organisms were recovered in the sand within 3 cm of the inlet surface. When bacteria were injected into a sandy aquifer using a forced gradient, the relative breakthrough of cells into a sampling well located 1.7 m from the injection well was less than 1%. Further studies found nearly a 16-fold greater breakthrough of *Escherichia coli* through intact cores compared to disturbed cores of a sandy loam. It was concluded that soil structure and the velocity of water flow are critical in determining movement of bacteria through soil.

Scientists reviewed a number of mathematical models describing the concurrent growth of bacteria and transport of biodegradable substrates in saturated porous media; these models assume that bacteria form either continuous biofilms or discrete microcolonies on surfaces of solid particles and hence are immobile. However, many environmental factors greatly affect bacterial transport. Ionic strength is particularly important. The literature showed that infiltrating solutions with low ionic strength decrease retention of bacteria in sand. In acid-treated sand, the efficiency of coliform retention was higher when the bacteria were suspended in tap water than in distilled water, and no retention occurred when the bacteria were suspended in triple-distilled water. On the basis of the electrical double layer developing in the vicinity of charged surfaces, it was concluded that the number of bacteria attracted to surfaces lessened with decreased electrolyte concentrations, whereas adsorption of cells to surfaces increased with higher electrolyte concentrations. Researchers reported that the application of rain water or distilled water will result in the desorption of viruses from soil particles. Additional studies reported that complete blockage of pores due to soil dispersion does not occur in sodic soils leached with water of low electrolyte content, and that dispersed clay may be transported considerable distances before deposition. Despite these findings on the effects of ionic strength, recent models for bacterial transport in porous media do not include ionic strength as a factor in determining the movement of cells.

The effect of ionic strength on movement of bacteria through subsurface earth materials seems to have been largely ignored in terms of promoting bioremediation with introduced bacteria.

The objectives of this study were to develop a reproducible procedure that would yield consistent measurements of relative mobility of bacteria in soil by avoiding uncontrolled variations in bacterial behavior and to relate transport to efficiency of recovery and adsorption of the cells. In the procedure that was developed, flow through macropores did not occur. In addition, a study was conducted to determine the influence of certain properties of bacterial cells on their movement through soil. The traits investigated were net surface electrostatic charge, hydrophobicity, cell size, and presence of capsules. Disturbed columns of aquifer sand were used to study the breakthrough of bacteria and of a chloride tracer. The effect of variations in the ionic strength of an inflowing solution, bacterial cell density, and flow velocity on the transport of bacteria through the earth materials was evaluated.

Discussion

Macropore flow may be a major mechanism of bacterial transport in soils. Therefore, the use of undisturbed soil columns might have provided data on bacterial transport that would have particular relevance to circumstances prevalent in the field. However, columns of homogeneous soil were used to avoid uncontrolled, preferential movement of bacteria through macropores and thus to permit a definition of the factors that control the movement of bacteria through the soil matrix itself. For the development of the procedure for determinations of mobility, a loamy soil was selected to avoid the extremes of limited bacterial sorption and nearly free movement in sandy soils on the one hand and the restricted movement resulting from mechanical filtration and increased sorption in fine structured soils on the other hand. Had these more extreme conditions been imposed, the ability of the procedure to detect small differences in bacterial mobilities might not have been evaluated.

Saturated soil with a constant head of water was used to mimic bacterial movement under conditions of saturated flow. This permitted the occurrence of mass transport of the cells in the sufficiently large pores of the homogeneous soil. Researchers showed that movement of bacteria through soil columns stopped when the water content was at or below field capacity, and other investigators demonstrated that movement of bacteria through soil was not detectable in the absence of a transporting agent such as water.

The procedure described here has several advantages for testing bacterial mobility in the soil matrix. Spurious data on mobility resulting from bacteria moving at the interface between the soil and the column wall are avoided through the use of a relatively wide column and the coating of the column walls with petrolatum to bind soil particles to the walls. Channels through which bacteria could move preferentially were eliminated by grinding and sieving the soil prior to preparing the column and, to ensure reproducible measurements of transport, by uniform packing to a fixed bulk density. Bacterial death from predation or parasitism was avoided because such predators and parasites were killed by irradiating the soil. Increases in cell numbers arising from growth and decreases associated with starvation were prevented by performing the tests of transport at 2 to 5°C. Furthermore, the marked differences in mobility among the isolates suggest that the proposed procedure does indeed distinguish among bacteria with different capacities for movement.

For inoculation of the soil surface with bacteria that can degrade organic pollutants at some underground site, some of the added cells must move through the soil to the zone of contamination at depth. Evidence exists, however, that introduced organisms may fail because they are not transported to sites containing the chemical. In this context, it is worth noting that many of the carefully controlled experiments in which inoculation resulted in biodegradation required transport to soil depths of only 10 cm. Measurements such as those described in the present study will enable extrapolation of the potential penetrability of the bacteria to considerably greater depths.

The capacity to degrade a chemical in culture is a necessary but not sufficient requisite for successful biodegradation in the field because, in addition to other traits the inoculum strain must possess the trait

that enable it to move through soil, subsoil, or aquifer materials to reach the area of chemical contamination. The ability of bacteria to be transported to subsurface contaminated zones may be evaluated from bacterial properties such as cell size and their susceptibility to adsorption. The correlation between these properties and mobility can be determined by the procedure here proposed. Such a correlation may enable the classification of bacteria according to their potential mobility. The susceptibility of a bacterium to predation or parasitism in the matrix through which it must pass must also be determined before its ability to reach the zone of contamination can be predicted.

It was possible to estimate an upper and lower boundary on the soil to water distribution coefficient of the bacteria (K_d). The data show a significant inverse correlation between the lower-bound K_d values and the fraction of cells transported through the column. The values for recovered cells transported varied from nearly zero to 25% at lower-bound K_d <2, whereas the values never exceeded 8% at lower-bound K_d >2. Adsorption, when sufficiently strong, can effectively retard the bacteria. If adsorption is weak, the transport of cells through the soil matrix may be controlled by mechanical filtration. The correlation between K_dl and K_dh was good enough to make their use in many cases interchangeable.

The dimensions of the bacterial cells affected their mobility. As the cell length increased, the highest value for recovered cells transported among bacteria of a given length decreased. Thus, the longer the cell, the less its likelihood of passing through the soil matrix. Cell length was not significantly correlated with the recovery, higher-bound K_d or lower-bound K_d.

Cell hydrophobicity, net surface electric charge, and the presence of capsular polysaccharides were evaluated because they are properties of bacterial cells that appear to be involved in adsorption of bacteria to solid surfaces. The sizes of the cells were tested because larger cells may be more readily removed by filtration than smaller cells. The presence of flagella might also impede movement, so their occurrence on the test strains was investigated. Varying degrees of hydrophobicity were observed among the bacteria, but the results of two different assays for hydrophobicity did not agree. The nonuniformity of the bacterial surface may cause a bacterium to be hydrophilic in one assay and hydrophobic in another. Moreover, hydrophobicity is not a definitive characteristic but varies with the hydrocarbon used for the assay.

Measurements of zeta potential revealed that all of the test bacteria had net negative surface charges. However, bacteria with positive charges on nonuniform cell surfaces may adhere to negatively charged particles of soil. No pattern was observed between the extent of transport and bacterial surface charges.

Adherence of bacteria to solid surfaces may result from their having extracellular polysaccharides. Of the strains for which more than 1.0% of the cells passed through the soil, six had capsules, indicating that capsules do not necessarily hinder transport. Measurements were made of cell size because large bacteria presumably are less likely to pass through soil pores than small cells, and a statistical relationship between size and transport of bacteria through soil was evident. Although cell appendages could impede mobility, a relationship was not observed between flagellation and transport. Motility was not considered important in the present study because transport was tested at low temperatures.

The present findings suggest that it should be possible to obtain bacteria that have both the capacity to biodegrade unwanted organic compounds and the ability to move through earth materials to sites containing these chemicals, as indicated by the observation that two benzene degraders and one chlorobenzene-utilizing bacterium moved through soil in appreciable numbers.

The movement of bacteria through homogeneous sand increased when the inflowing solution had a low ionic strength. When deionized water was used, the bacteria moved readily through the sand column after an initial period of retention.

A 0.01 M solution of NaCl dramatically reduced bacterial breakthrough. The total bacterial breakthrough was low because the cells were removed from solution by interactions with the sand matrix. The replacement of the NaCl solution with deionized water resulted in a second peak of bacterial breakthrough. The retention caused by the adsorption mechanisms appeared to be reversible when ionic strength was changed.

Differences in the transport of bacteria noted in experiments with 0.01 M NaCl and deionized water were more marked at 1.0×10^8 than 1.0×10^9 cells per mL. An increase in flow velocity somewhat reduced bacterial retention, a decrease that may be related to the reduction in bacterial residence or "reaction" time at higher flow velocity.

Conclusions

The experimental results suggest that adsorption significantly contributed to the retention of bacteria and that bacterial movement through aquifer sand was enhanced by reducing the ionic strength of the inflowing solution. Cell density and flow velocity also influenced bacterial movement. For bioremediation of contaminated sandy aquifers, manipulation of ionic strength may thus be a means to facilitate movement of bacteria to the site of organic contamination.

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The complete report, entitled "Movement of Bacteria Through Soil and Aquifer Sand," (Order No. PB91-164277/AS; Cost: $15.00, subject to change) will be available only from:

National Technical Information Service
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