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Movement of Bacteria in the Subsurface

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CONTENTS

KEY WORDS: *attachment, adhesion, detachment, retention, retardation, advection, dispersion, filtration, hydrophobicity, chemotaxis, motility, hydraulic heterogeneity, porous media, models, transport.*

13.1 Introduction

Research of the past 10 years has demonstrated that there is a subsurface microbial community that is widely distributed, highly diverse, and often quite active. The discovery of the unexpectedly abundant biota at great depths has prompted the obvious question of how the microbes got there. In some cases it may be that the community has persisted through centuries or even millennia after being buried in surficial sediments. In fractured igneous or metamorphic rock it seems unlikely that a diverse community could arise from survivors of the original melt due to the high temperature of deposition. In both the sedimentary and igneous/metamorphic locations, postdepositional transport of microbes is often postulated.

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For subsurface habitats close to the surface and well connected with the surface, there can be little doubt that organisms are transported from the surface or from injection sites below the surface to become widely dispersed in both saturated and unsaturated zones of the subsurface. The simple fact that sewage indicator organisms are not infrequently found downgradient from septic fields is conclusive evidence that microbes can be and are transported through subsurface environments (e.g., Gerba and Bitton, 1984; Yates and Yates, 1988).

Given the importance of microbes as mineralizers of organic carbon, and given that microbes have been demonstrated to be effective agents of biorestoration of a variety of contaminated sites, much interest is focused on the role of microorganisms as potential agents of aquifer restoration (e.g., Thomas and Ward, 1989; National Research Council, 1993). Bioremediation is already a working solution for some contaminated aquifers. Intrinsic bioremediation, in which indigenous communities degrade contaminants, and biostimulation, in which indigenous communities are stimulated to degrade a contaminant by addition of organic or inorganic nutrients, have been shown to be successful in sites including both surface and subsurface contamination of aqueous and solid phase components. Bioaugmentation, in which competent nonnative organisms are inoculated into a contaminated site, has not as yet been shown to be an effective means of reducing levels of contaminants, although competent organisms are often easily isolated and cultured, or constructed in the laboratory through genetic engineering approaches. Experience with injection of microbes in microbially enhanced oil recovery studies and studies concerning the growth and activity of microbes in porous media suggests that growth may result in clogging of the media near the injection point. Concern about adhesion of the microbial cells to the grain surfaces also suggests that transport and dispersal of bacterial cells in porous media will have a profound effect on the ability of introduced organisms to penetrate the zone of contamination to the extent that gowth of the microbes will result in remediaton of the contaminated aquifer.

Other facets of subsurface microbiology are similarly strongly influenced by bacterial transport considerations. The simple fact that abundant microorganisms have been found at great depths in both saturated and unsaturated media begs the question of the origin of the assemblage. In depositional environments, the cells could be descendents of microbes laid down during the original depositional event, or they could be of more modern origin and have been moved through the sedimentary material rapidly or slowly, to produce the observed distribution. The presence of actively metabolizing microbes at depth in fractured rock and deep unsaturated deposits suggests a transport-related origin, since depositional conditions of these materials would not seem conducive for survival of a diverse assemblage.

In near-surface environments, transport of microbes has been considered for some time as a primary means of contamination of water supplies by indicator organisms and pathogens from failed septic fields. In all of these cases, fundamental aspects of microbial transport should be similar, and application of enhanced understanding of those aspects may improve wastewater treatment and contaminant reduction in the subsurface.

13.2 Factors Affecting Transport of Microorganisms in Porous Geologic Media

The factors which control the rate and extent of bacterial transport in porous media are usually divided into two major categories: hydrogeological and biological. With a few exceptions which can be specified, transport is considered to be advective, with the major controlling factors being those related to removal of microbial cells from suspension. Some of the controls are strictly physical; clogging of narrow pore throats, for example. Others are related to the interaction of cells with the mineral grain surfaces. Properties of the cells which influence these controls will be discussed as biological phenomena, while properties of the mineral grains that influence the interactions will be included as geochemical properties.

Harvey (1991) listed the important processes and factors that form the parameters for bacterial transport through porous media. The variables included straining (pore clogging), dispersion, sorption and detachment, growth and death (grazing by bacteriovores was included in the latter), motility, and chemotaxis. Other factors which influence these particular processes, such as the mineralogy and pore water composition, pore size distribution, etc., were considered separately, as it was the modeling parameters that Harvey sought to identify. These parameters and factors have not been replaced as the primary influences on bacterial transport, rather they have been refined and examined in more detail by a number of investigators in the period since Harvey's review.

Several terms regarding the transport of microbes that are based on terms used in flow and transport of dissolved and particulate substances have come into common use. **Advection** is the movement of the bulk pore fluid and its dissolved and suspended constituents. In advective transport, nonreactive substances will be transported at a rate equal to the average linear velocity of the fluid. The term **convection** is often used in place of advection. Hydrodynamic **dispersion** is the phenomenon by which the dissolved and suspended constituents spread both longitudinally and laterally from the path expected on the basis of hydraulics alone. Dispersion is comprised of two components, molecular diffusion (or Brownian movement in the case of particles) and mechanical dispersion which results from mixing in the pore throats and from the varying distances (lengths of tortuous paths) that any individual molecule or particle may follow. **Retention** refers to the removal of cells from the pore fluid so that they do not return to suspension, i.e., in a column experiment, retained cells never emerge in the effluent. **Retardation** refers to the interaction of the cells with the porous material in such a way that the cells may be delayed in their passage past a given point in the flow path (e.g., emergence in the eluant of a column). **Adhesion** refers to the association of the cells with the surfaces of the porous medium. This term is often used synonymously with adsorption, although adsorption is really one of the mechanisms of adhesion. Adhesion can be reversible (as in equilibrium or nonequilibrium sorption) in which case it contributes to retardation, or it can be irreversible (as when a cell actually cements itself to a solid surface by secretion of a polymer) and in which case it contributes to retention. Other terms are also used frequently, and they will be defined as they are introduced.

13.2.1 Hydrogeological Controls on Bacterial Transport

13.2.1.1 Hydraulic Heterogeneity: Transport in Preferred Flow Paths vs. Matrix Transport

Many of the experiments associated with particle transport in porous media, including transport of bacteria, have been performed in columns containing a uniformly sized medium. Glass beads, plastic spheres, and even carefully sieved sands have been used to develop and test theory about hydraulic properties of porous media and the effect of those properties on bacterial transport. The resultant theories are simplistic in their view; even the more complicated multidimensional models usually treat the porous medium as a bed of uniformly sized spheres. Heterogeneity is recognized as being important, because experimental results in heterogeneous systems differ substantially from those obtained in hydraulically homogeneous media. (For more detail concerning heterogeneity, see Chapter 6.)

Porous media are hydraulically heterogeneous at all scales. Even if all the grains were perfectly uniform spheres of an exact diameter, the inability to pack the spheres in completely open or closed arrangement leads to heterogeneity in the distribution of pore sizes. If the pores are all substantially larger than the bacterial cells, this effect will be minimized, but the closer the size of the grains (and their interspersed pores) to the size of the bacterial cells the greater the effect on the individual cells will be. In media with a wide range of grain diameters, a high probability of large- and small-diameter flow paths exists. Bacteria will preferentially travel through the larger of the paths, and the cells in those paths will be least affected by interactions with porous medium grains. As a result, those cells will be the first to break through at some point downgradient of the inception of transport. Cells which move into the areas with the finest pores (often referred to as the matrix) will be most susceptible to straining and adhesion on grain surfaces, and thus will be delayed (retarded) in their breakthrough. Pore blockage and irreversible adhesion lead to permanent retention of the cells in the porous matrix, or at least they are retained until they are dislodged or until growth of a biofilm on the mineral grains sloughs some cells into the suspension. It can be concluded, therefore, that rapid transport of bacteria in porous media occurs primarily through preferred flow paths, but longer-term transport can occur through finer-textured matrixes, and the matrixes can also serve as sources of cells for continued inoculation of the more permeable zones. These statements should be generally true for all porous media from unconsolidated sands and gravels to fractured rock. Rapid transport of bacteria through preferred flowpaths has been demonstrated experimentally in the laboratory by Toran and Palumbo (1992), Story et al. (1996), Fontes et al. (1991), and Morley (1995). In the latter two cases, large peaks of bacterial cells eluted from columns of quartz sand containing a single vein of coarser-textured grains well in advance of the first pore volume of eluant (Figure 13.1). A second peak of bacteria containing many fewer cells than the first then followed. The first peak was thought to represent transport through the preferred flow path while the second peak represented those cells moving through the finer-textured annular matrix. Models fit to the data to test this hypothesis gave results that were consistent with the two-domain mechanism. Harvey and Garabedian (1991) attributed early initial breakthrough of bacterial cells and microspheres followed by a series of smaller peaks to the presence of multiple preferred flow paths in their field experiments in the sands and gravels on Cape Cod.

13.2.1.2 Reactive Surfaces

That bacteria attach preferentially to different mineral substrates has been known for some time. Mills and Maubrey (1981) demonstrated that bacterial attachment to limestone was faster than to sandstone in both lotic and lentic environments. Scholl et al. (1990) showed that media of different mineralogies retained different proportions of bacteria in packed column studies. In general, bacteria tended to adhere more to minerals with a greater proportion of leachable ions than to more inert substances. Scholl et al. (1990) also showed that differences in base mineralogy were less important than the nature of coatings on the mineral grains. In particular, the presence of surface coatings of iron sesquioxide dramatically increased the adhesion of bacteria to chips of limestone and sandstone suspended in bacterial suspensions over uncoated chips,

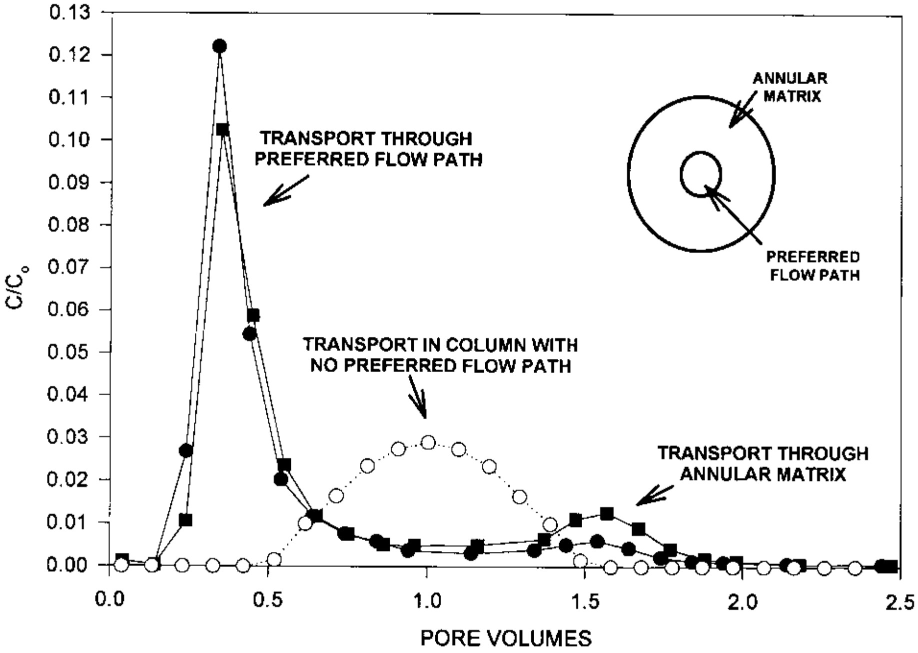

FIGURE 13.1

The effect of physical (hydraulic) heterogeneity on bacterial transport in porous media. Open circles are the results of a breakthrough experiment with bacteria in a column homogeneously packed with quartz sand sieved to a uniform size. The solid circles represent a pair of columns packed to contain a preferred flow path of coarse-textured sand surrounded by an annulus of the same sand as in the homogeneous column. For the homogeneous column, the peak breakthrough was observed at one pore volume. For the heterogeneous column, two peaks were observed. The first represents transport through the preferred flow path and the second smaller peak represents transport throughout the annulus. For both column types, the breakthrough curves are similar to those observed for a conservative tracer except for the amount of material recovered. Recovery of the conservative tracer was complete, whereas the recovery of the bacteria in the homogeneous case was very small. In both cases, bacterial breakthrough represents retention without retardation.

and also increased the retention of bacteria in packed columns over columns packed with clean quartz sand.

Mills et al. (1994) further showed that the presence of iron coatings (and by analogy, Al and Mn coatings) on clean quartz sand caused the sorption of bacteria to the sand in batch experiments to change from that demonstrating a typical equilibrium isotherm to a mode in which all added bacteria were apparently irreversibly sorbed to the coated sand grains until a saturation point was reached beyond which no additional sorption was observed. Using the observed behavior, Mills et al. (1994) offered a model which predicted the adhesion behavior of bacteria in mixtures of iron-coated and clean quartz sand, and experimental results were consistent with the predictions.

Other surface coatings can also affect the retention of bacteria on mineral surfaces. Adhesion of bacterial cells to surfaces can be inhibited by the presence of proteins on the surface or in solution (Fletcher, 1977; Feldner et al., 1983), enhanced by oxyhydroxide coatings on the surface (Scholl et al., 1990; Scholl and Harvey, 1992; Mills et al., 1994), and/or affected by nutrient conditions (McEldowney and Fletcher, 1986a; Kjellberg and Hermansson, 1984). Richardson (1994) examined bacterial attachment to iron-coated sand in the presence of humic acid and observed a high retention of cells in the presence of humic acid, but the number of cells adsorbed to the iron-coated sand in the presence of adsorbed humic-coated sand was significantly lower than adsorbed to the iron-coated sand in the absence of the humic acid. Richardson (1994) concluded that the humic acid competed with the bacteria for available sites on the iron-coated surfaces of the sand. Many of these experiments were done in batch systems, and some caution is necessary in extrapolating such results to flowing situations. For example, adhesion was found to be consistently greater in dynamic columns as compared to static batch system, even for short residence times (Rijnaarts et al., 1993). Furthermore, questions of kinetic controls on adhesion make predictions of dynamic situations (e.g., as in column experiments) with parameter estimates obtained from batch experiments unreliable, unless specifically tested for a particular situation. More information on kinetic control of sorption is offered below.

13.2.2 Biological Controls on Bacterial Transport

13.2.2.1 Cell Surface Characteristics

A substantial effort has been directed at identifying the characteristics of bacterial cells that affect their transport and quantifying their effect on the transport process. The characteristics affecting transport seem to be related either to the interaction of the bacterial cell surface with the mineral grain surface and the gas-liquid interface in the case of unsaturated media, or to the behavior of the cells as buoyant particles aside from their cell surface characteristics.

There is a fairly rich literature that discusses the roles of cell surface properties and particle surface properties on bacterial adhesion. The tendency for bacterial cells to adhere to mineral grain surfaces appears to be most strongly influenced by the surface charge on the cell, and the cell surface hydrophobicity. Hydrophobicity is quantitatively defined on the basis of water contact angle (the angle formed by a drop of water in contact with a surface). Water contacting a highly hydrophobic surface tends to "bead up", yielding a steep angle at the contact point, whereas on less hydrophobic surfaces the water tends to spread out, forming a smaller contact angle. A number of other methods for describing hydrophobicity have been used; they include partitioning of cells into nonaqueous solvents (BATH — bacterial attachment to hydrocarbons), and hydrophobic exclusion chromatography (Rosenberg and Doyle, 1990). The methods tend to be internally consistent, but do not always yield results that are consistent across methods (Figure 13.2).

Surface charges associated with colloidal particles are usually described by the zeta potential, which is derived from the migration of particles in an electric field (electrophoretic mobility). Both the sign (positive or negative) and the magnitude (distance traveled) can be expressed by this measure. Surface charges can also be determined by potentiometric titrations (Harden and Harris, 1953), but this technique is much less satisfactory for bacteria than the determination of electrophoretic mobility.

Bacterial adhesion to materials such as glass, polystyrene, sand, stainless steel, and even meat has been shown to decrease with increasing zeta potential of the bacterium or substrate (increasing zeta potential describes an increasing negative charge) (Fletcher and Loeb, 1979; Abbott et al., 1983; Van Loosdrecht et al., 1987b; Dickson and Koohmaraie, 1989). Furthermore, adhesion also tends to increase with increasing solution ionic strength, due to compression of the double layer as predicted by DLVO theory (Abbott et al., 1983; Fontes et al., 1991; Mills et al., 1994; Zita and Hermansson, 1994). Adhesion also tends to increase with increasing hydrophobicity of the surfaces (Fletcher and Loeb, 1979; Van Loosdrecht et al., 1987a; Stenstrom, 1989; Dickson and Koohmaraie, 1989; Vanhaecke et al., 1990; Sorongon et al., 1991). Bacterial adhesion to

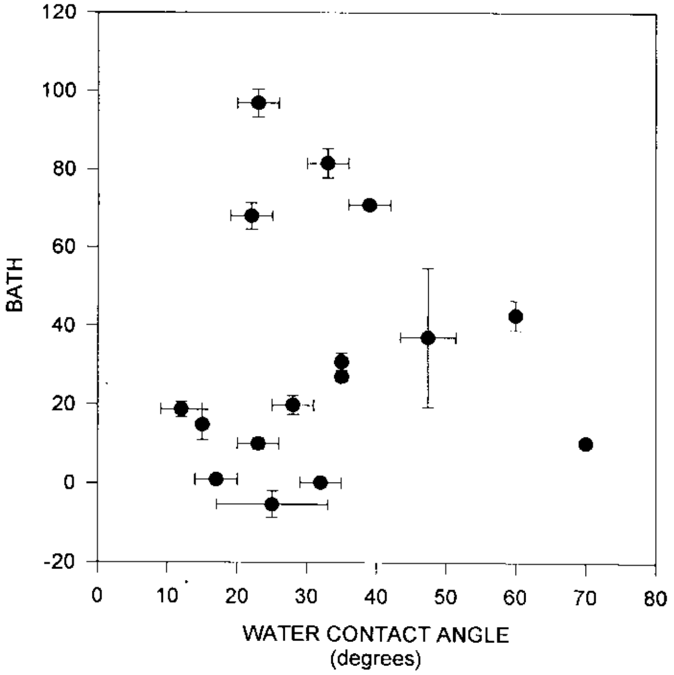

FIGURE 13.2
Comparison of cell surface hydrophobicity determined on several bacterial isolates using both the bacterial attachment to hydrocarbon (BATH) and water contact angle methods. Although there is a generally positive relationship, the weakness of the relationship ($r^2 = 0.01$) contraindicates any interchangeability of values or comparison of the values across strains. (From Richardson, R.L., M.S. Thesis, University of Virginia, Charlottesville, 1994.)

glass and Teflon® was also shown to be related to the cell surface hydrophobicity of several coryneform bacteria and that correlation extended to the chain length of mycolic acids on the bacterial surfaces (Bendinger et al., 1993).

In general, then, bacterial adhesion tends to increase with increasing hydrophobicity of the bacterial cell and substratum, and decreases as the cell or substratum becomes more negatively charged. However, hydrophobicity is the dominant factor with regard to adhesion to uncoated glass, as highly hydrophobic cells demonstrated high levels of adhesion, regardless of their surface charge (Van Loosdrecht et al., 1987b). In contrast to the majority of the literature, Mafu et al. (1991) found that bacterial hydrophobicity and zeta potential were not correlated with adhesion to polypropylene, rubber, glass, or steel, while McEldowney and Fletcher (1986b) noted that bacterial adhesion to polystyrene was not correlated with ionic strength of the bulk solution. In general, then, it would be expected that the more hydrophobic bacteria would tend to be transported less readily than hydrophilic cells (Figure 13.3). Experimental data from complex systems do not always support this hypothesis, but often, multiple confounding variables may mask the effect (e.g., Gannon et al., 1991a,b).

13.2.2.2 Other Cell Properties

Cell properties other than those measured as surface chemical characteristics have also been implicated in affecting the transport of bacteria. Some are related to cell behavior, e.g., motility and chemotaxis, whereas others are related to cell properties that might not be included in the surface chemistry category. Examples of the latter

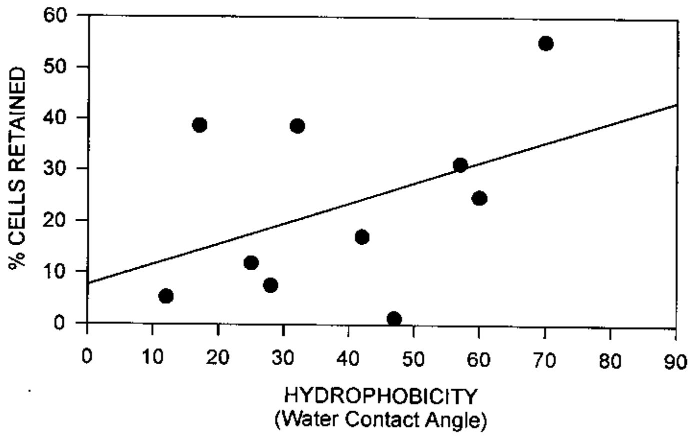

FIGURE 13.3
Relationship of cell surface hydrophobicity and the proportion of bacterial cells retained in columns of clean quartz sand. Although the relationship is generally weak ($r^2 = 0.2$) the general observation that increased hydrophobicity leads to decreased ease of transport is visible. The weakness of the relationship suggests that factors other than hydrophobicity contribute to controlling bacterial transport in porous media.

include the observation or the fact that there is a correlation between adhesion and the presence of protruding cell surface features (Uyen et al., 1988; Busscher and Weerkamp, 1987). Obviously, surface structures that can actively adhere to surfaces, or that can penetrate the electrostatic repulsive layers, will enhance attachment and thereby inhibit or at least retard transport in porous media.

13.2.2.2.1 Size and Shape

The transport of bacteria has been investigated quantitatively using colloid filtration theory (Harvey and Garabedian, 1991; Russel et al., 1989; see below). Aside from advection and dispersion in the flowing pore waters, the processes that affect the transport of bacteria are the kinetically controlled rate at which bacteria are deposited on the mineral grains of aquifer material and the kinetically controlled rate at which bacteria detach from the grain surfaces. These processes are affected by the size of cells and suggest that cells of about 1 μm in diameter may be optimal for transport through sand-sized media (Yao et al., 1971). In practice, filtration theory assumes all particles are spheres (Harvey and Garabedian, 1991; Yao et al., 1971; Hornberger et al., 1992).

While fluid dynamic forces will influence colloid particle-grain interactions, a quantitative treatment of phenomena related to particle morphology is not available; the only approach that has been used is based on empirical extrapolation from theoretical results for spherical particles (Hornberger et al., 1992). Particles with non-spherical morphologies are typically represented by an "equivalent diameter" equal to the average of the major diameters of the particle (McDowell-Boyer et al., 1986). Bacteria found in geological formations are known to have a variety of shapes, including filamentous forms, spirals, rods, and ellipsoid, ovoid, and coccoid shapes (Balkwill et al., 1989; Hirsch and Rades-Rohkohl, 1990; Ghiorse and Wilson, 1988) even though the majority of bacteria removed from sampling wells in unconsolidated aquifer materials are small cocci or coccoid rods (Balkwill et al., 1989; Ghiorse and Balkwill, 1983). To what extent different cell shapes influence bacterial transport through aquifers and how shape, size, and cell surface properties interact, is currently unknown.

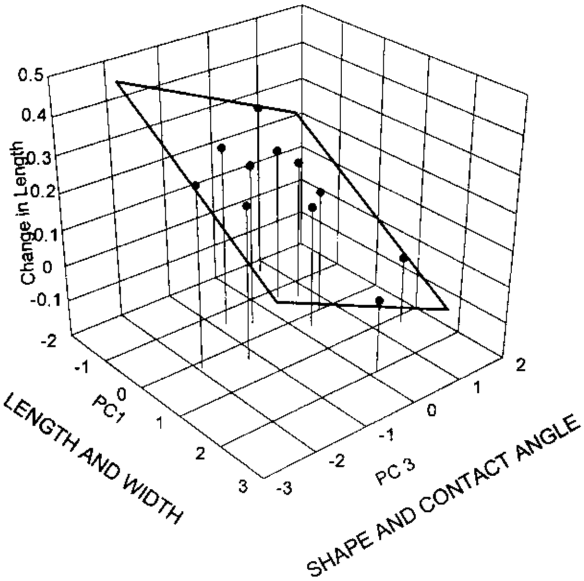

FIGURE 13.4
Effect of size, shape, and cell surface hydrophobicity on the "chromatographic" selection of shorter rounder cells as a cell suspension passes through a column of sand. Data axes were generated by a principal components analysis of the data. Each data point represents the data for a single bacterial isolate. The plane is the least squares regression surface for the data. (Reprinted from Weiss, Th.H., Mills, A.L., Herman, J.S., and Hornberger, G.M., *Environ. Sci. Technol.*, 29, 1737, 1995. With permission.)

Weiss et al. (1995) examined the effect of cell shape on the transport of bacteria in columns packed with clean quartz sand. Although a chromatographic cell-size effect on the transport of bacteria through a sand aquifer was noted by Harvey and Garabedian (1991), Weiss et al. (1995) observed a chromatographic effect for cell shape as well as size. When 14 strains of bacteria with differing size (as indicated by both cell length [L] and cell width [W]), shape (W/L), and cell surface hydrophobicity were eluted in pulses through short columns packed with clean, quartz sand of about 0.75 mm grain diameter, eluted cells for 12 of the 14 strains were more spherical (greater median cell shape indexes) than cells in the influent suspension. This observation suggests that transport models may have to be adjusted to account for cell shape.

Bacterial cells are thought to attach to mineral surfaces due to surface-surface interactions, either electrostatic or hydrophobic in nature (e.g., Harvey, 1991; Van Loosdrecht et al., 1989; McEldowney and Fletcher, 1986a,b). Although it is not clear why these attachment mechanisms would lead to preferential removal of long, rod-shaped cells, some reports suggest that cell attachment to solid surfaces indeed may be greater for elongated cells than for spherical cells (Van Loosdrecht et al., 1989; Feldner et al., 1983). Examination of a fitted-response surface for data that had been reduced by principal components analysis shows that short rods with low contact angles generally underwent the greatest decreases in cell length (Figure 13.4). There is no currently available theoretical explanation for these observed interactions, and the current theory is unlikely to advance in the absence of further experimental work.

To attain a more complete understanding of the transport behavior of bacteria in porous media, all of the important factors that can affect transport, including (but probably not limited to) size, shape, hydrophobicity, electrostatic charge, and as suggested by our results, the interactions among the factors, must be considered. The interactions, however, are not well understood. The role of particle shape has not been included in most studies thus far. Mackie and Bai (1992) recognized that particle size distributions change as the particles travel through the column and, as we do here, encouraged caution when using filtration models assuming uniform particle size. Although the major axis dimension is the most commonly selected descriptor of size properties employed in predictive transport models, it is considered in those models to be a parameter that does not evolve during the transport process. The chromatographic effect reported by Weiss et al. (1995) calls this assumption into question.

Experiments with bacterial transport in the laboratory are usually conducted at high flow rates; e.g., Fontes et al. (1991) used rates of about 14 cm h^{-1} in their column experiments. Some experiments are run at slower rates, but continuous-flow experiments in the laboratory rarely approximate the rates found in many aquifers that can be expressed in terms of a few meters per year or less. At typical laboratory flow rates, processes such as motility or settling are not likely to be demonstrated as important. In many natural situations, however, motility or settling might produce movement of cells that could approach, or even exceed, the rate of movement due to advective transport.

13.2.2.2.2 *Sedimentation*

Most considerations of bacterial transport have considered bacterial cells to be colloids and have therefore ignored sedimentation of cells as an important factor in bacterial transport. Colloid filtration theory includes a consideration of particle sedimentation as a means of moving the particles to the surface of the porous medium grains, but advective processes are usually thought to overwhelm any significant effect of sedimentation. The assumption of neutral or near-neutral buoyancy is a reasonable approximation for high flow rates and short time scales such as experienced in many column studies and probably even in forced-gradient field experiments (Hornberger et al., 1992). Wan et al. (1995) recently reported median free sedimentation rates (rates in liquid suspension) of 7 and 42 mm d^{-1} for two strains of groundwater bacteria. While they accepted the fact that actual settling velocities would be slower in the presence of particles and flowing water (measured values were 7 and 17 mm d^{-1}), short-column experiments yielded significant breakthrough of bacteria through saturated sand that was as high as 90% of the free sedimentation rate. The specific gravity measured (1.117 and 1.074) for each of the two strains used was in the middle of the range of values obtained for 25 bacterial strains (1.040 to 1.121, with a mean of 1.0882 for the 25 strains tested.). The sedimentation rates of the two strains tested were inversely related to the buoyant density, suggesting that density alone does not dictate the sedimentation rate, a conclusion consistent with the importance of the role of shape and size claimed by Weiss et al. (1995). Sedimentation of bacterial cells may indeed play a role in transport of bacteria in very slow-moving ground water over long periods of time.

13.2.2.2.3 *Motility and Chemotaxis*

Bacterial motility and chemotaxis present interesting concerns in bacterial transport. Experimental results suggest that motility may enhance the movement of bacteria in porous media, but the conclusion is by no means universal. Similarly, chemotaxis (with its implicit assumption of motility) seems to enhance movement of bacteria in porous media in some but not all cases. Motile bacteria *(Enterobacter aerogenes)* were able to penetrate saturated sandstone cores under static conditions three to eight times faster than a nonmotile strain of *Klebsiella pneumoniae* (Jenneman et al., 1985). However, because the motile strain also grew faster than the non-motile strain, the growth rate differences may have been partly responsible for the differences in penetration. In packed sand cores under static conditions, motile *Escherichia coli* penetrated the medium more quickly than the nonmotile mutants of the strain (Reynolds et al., 1989). The motile chemotactic parent strain used by Reynolds et al. (1989) moved through the cores more slowly than did the nonchemotactic mutant. It was also observed that the growth rates of the nonchemotactic mutants exceeded that of the chemotactic parent, and that the relative order of penetration was related to the growth rate of each strain. Thus, Reynolds et al. concluded that the controlling factors in the experimental system used were growth and random motility — chemotaxis was not a contributing factor. Similarly, experiments with a chemotactic strain of *Pseudomonas putida* were unable to demonstrate a significant effect of the chemotaxis in transport of the cells when compared in the presence or absence of a chemical attractant (Barton and Ford, 1995). While motility and chemotaxis must play a role in movement of microbes in some instances, those situations have not yet been defined adequately. A reasonable probability exists that these processes will be important in the movement of competent cells into contaminated areas of low permeability, where advective transport is minimal compared with adjacent preferred flow paths. Additional research is clearly required in this area.

13.2.3 Transport in Unsaturated Media

A potentially important although rarely considered aspect controlling the movement of bacteria in porous media is the role that gas-water interfaces may play in microbial transport. Wan and Wilson (1994) used glass micromodels (consisting of tiny channels between two glass plates) and flowing water to observe polystyrene beads, clay particles, and bacterial cells adhering to gas-water interfaces. The sorption to the interface appeared to increase with increasing particle hydrophobicity, solution ionic strength, and decreasing positive charge on the particles. These observations suggested an initial adsorption due to van der Waals' and electrostatic interactions, followed by an essentially irreversible adhesion due to capillary force. Wan and Wilson predicted that for a relatively hydrophobic strain of bacterium even small amounts of residual gas could dramatically reduce advective transport. An additional observation by Pitt et al. (1993) showed moving gas bubbles swept glass and polymer surfaces free of adsorbed bacteria.

Although moving gas-water interfaces could move adherent bacteria along with them, the presence of gas-water interfaces has usually been found to reduce the transport of microbes. This may be due to the tendency of bubbles to remain in place relative to water moving through nearly saturated pores, but some other explanations may also be important. Powelson et al. (1990) found that MS2 bacteriophage was not removed during passage through 1 m of saturated soil, but was 95% removed in unsaturated soil. The authors suggested that the partially hydrophobic virus adsorbed to air-water interfaces in the unsaturated soil and were degraded by physical disruption of viral structure. Poletika et al. (1995) attempted to predict retardation of MS2 bacteriophage transport in unsaturated soil from equilibrium adsorption isotherms in batch experiments with soil slurries. The adsorption experiments indicated that the virus did not attach to the soil particles. Modeling of the virus breakthrough, however, yielded a retardation factor of 254, indicating strong adsorption. These authors suggested that the difference in the slurry and unsaturated experiments may have been due to the interaction of viral particles with air-water interfaces in the unsaturated soil. Tan et al. (1992), however, were able to predict transport of bacteria through unsaturated sand columns from batch adsorption to the solids without considering additional adsorption due to air-water interfaces. Such success is not common (Mills et al., 1994), and may have been a result of the use of a weakly adsorbing bacterial strain by Tan et al. (1992). Huysman and Verstraete (1993a,b) attributed greater adsorption of bacteria in drier soil conditions to slower water flow rates, rather than interaction with air-water interfaces. Clearly, additional work on air-water interfaces is necessary before their role in bacterial transport can be defined and used in prediction.

13.3 Models of Bacterial Transport

In an effort to provide quantitative estimates of bacterial transport in porous media, scientists and engineers have turned to mathematical models to assist with prediction of transport in applied situations, and to test hypotheses of potential mechanisms of reaction and transport of bacteria in porous media. It is far beyond the scope of this chapter to review modeling of bacterial transport in detail. An excellent document specifically dealing with modeling the fate and transport of microorganisms in the environment was edited by Hurst (1991), and the reader is referred to that volume for the details of fundamental modeling of transport phenomena.

Two basic approaches have been followed to model bacterial transport in porous media. There are important interconnections between the approaches; both attempt to quantify the same processes and recognize the important factors that control transport on a microscopic scale. But there are differences that make each approach more or less useful for different applications. Filtration models are derived from theories developed for particles passing through porous media, while advection-dispersion models were developed initially to describe the transport of dissolved species.

13.3.1 Filtration Models

The fundamental concept behind filtration theory is that particles are removed from a suspension as it passes through a porous medium (Elimelech and O'Melia, 1990). The processes that govern the rate and extent of particle removal are related to the relative sizes of the suspended particles and the filter medium, and the potential for reaction of the suspended particles with the surfaces of the medium. Specifically, straining (the removal of particles too large to fit through a pore) is considered as is sorption (reversible or irreversible) of the particles to the medium surfaces. In operation, filtration models generally lump the processes together to generate a filtration coefficient. The general filtration equation has the form:

$$\frac{\partial C}{\partial x} = -\lambda C \tag{13.1}$$

where C is the concentration of particles in the suspension, and λ is the "filter coefficient". This form of the equation suggests that particle concentration should decrease in some exponential fashion as depth in the filter increases, and most data support that idea. Another term that is often used to describe filtration is the "collection efficiency". The collection efficiency explicitly incorporates the concept "sorption" or "adhesion" into its theory. In this formulation, the medium grains are considered to be "collectors" that trap particles in proportion to the collector's cross-sectional area.

A single grain is the fundamental unit, and a term "single collector efficiency" is frequently employed. The collection efficiency, η, is defined mathematically as:

$$\eta = \lambda A_c N \tag{13.2}$$

where A_c is the projected cross-sectional area of a single collector (mineral grain) and N is the total number of collectors in the filter. The term λ is referred to as the filter coefficient. This very simple model describes the filtration phenomenon reasonably well. There is difficulty, however, in relating the filter coefficient to measurable quantities associated with physical-chemical processes.

A careful analysis of the interactions between suspended particles and the collectors indicates that for viscous flow (exemplified by most ground water situations), the interactions result in trapping of particles by sedimentation, attraction, and Brownian diffusion. These processes cause the particles to cross stream lines and contact the mineral grain surface where they are retained. A number of authors have reported good results in estimating the collector efficiency by taking these processes into account. Filtration theory does not permit release of particles back to the pore fluid (entrainment), but Hornberger et al. (1992) demonstrated that use of a deposition coefficient and an entrainment coefficient accurately predicted the transport of particles in sand columns, and further suggested that the ratio of the theoretical coefficients approximated a collector efficiency. Also, simple filtration models were not formulated for situations in which the total particle mass can change, as when bacteria grow. Model terms can be placed in the equations, however, that will adjust the concentration of cells in the filter incrementally to account for changes due to growth and death.

13.3.2 Advection-Dispersion Models

Advection-dispersion (A/D) models represent transport as a combination of flow and dispersion. They are derived from fundamental equations that describe the flow of water in a porous medium. When a nonreactive tracer is added at a point in a flowing fluid, the point tends to spread as it proceeds through the porous medium. The spreading is due to molecular diffusion and to mixing processes that are caused by small-scale turbulence within pores, and by the large number of potential flow paths that exist within a porous medium. A/D-based models describe the movement of conservative (nonreactive) substances well. They were originally formulated to describe the transport of dissolved material, but can be used for colloidal or particulate material in many cases. A simple A/D equation is represented as:

$$\frac{\partial C}{\partial t} = -A\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} \tag{13.3}$$

where C represents the concentration of bacteria (in this case), x represents the distance through a section of porous medium, and A and D are constants that represent the advection (average linear velocity) and dispersion, respectively. This formulation is for a completely nonreactive substance. Equations for reactive materials often include a term (frequently denoted as R) that represents reactions that can remove dissolved or suspended material from the moving pore fluid:

$$\frac{\partial C}{\partial t} = -A\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - R \tag{13.4}$$

where the reaction term, R, can have a variety of forms that describe the processes whereby material is removed from (or enters) the mobile fluid.

In an effort to provide a complete description of bacterial transport, Corapcioglu and Haridas (1984) presented an A/D formulation that included all of the processes thought to control bacterial transport. Effectively, the term R was expanded significantly to take all the processes into account. Terms were included to account for cell growth and cell death (both in suspension and on the particle surface), pore clogging and declogging, gravitational settling, and motility. The formulation of Hornberger et al. (1992) used the basic approach of Corapcioglu and Haridas (1984) and ignored growth, death, differential settling, and motility, but expanded the clogging-declogging concept to include all forms of deposition and entrainment.

A distinct advantage of the A/D approach as opposed to the simple filtration approach is that the former can include considerations of hydrologic and geochemical heterogeneities in expanded formulations.

In modeling the transport of dissolved substances, the concept of retardation is often used. Retardation describes the delay in breakthrough of a transported material due to interaction with the surfaces of the porous medium. Retardation theory was developed on the basis of equilibrium sorption, and a common calculation for the retardation coefficient is based on K_D, the equilibrium isotherm:

$$R_D = 1 + K_D(1-n)\frac{\rho_B}{n} \tag{13.5}$$

where n = the porosity of the porous medium and ρ_B = the bulk density of the mineral grains. The retardation coefficient is then applied to the advection-dispersion equation as:

$$R_D\frac{\partial C}{\partial t} = -A\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} \tag{13.6}$$

Breakthrough curves that display retardation appear similar to those in Figure 13.5. In laboratory column experiments on bacterial transport, breakthrough curves for bacteria often display little retardation, even though substantial retention of cells in the column is observed. Two factors may be responsible for this phenomenon. First, much of the rapid transport of microbes in porous media occurs through preferred flow paths — macropores or zones of higher permeability. Given the assumption that the pore diameters in preferred flow paths are larger than in the surrounding matrix, it could be speculated that rapid (unretarded) breakthrough of bacterial cells represents those organisms that never contact a solid surface. They are neither strained nor adsorbed from suspension. There is support for this contention in the observations of Harvey et al. (1989) and Harvey and Garabedian (1991) that bacteria tended to break through heterogeneous media before chemical tracers, suggesting that rapidly transported bacteria travel through the largest pores, while the tracers are moving through the large and fine pores. Fontes et al. (1991) also observed similar transport in preferred flow paths in laboratory simulations using constructed heterogeneities in sand columns. Thus, a large proportion of the cells that travel through these preferred flow paths are neither retained nor retarded.

Even in cases where bacterial transport is rapid, a phenomenon referred to as tailing is often observed. Tailing refers to the tendency for slow elution of bacterial cells for prolonged periods after the primary breakthrough peak has passed. Tailing is observed in all of the studies of bacterial transport in laboratory columns, but it often

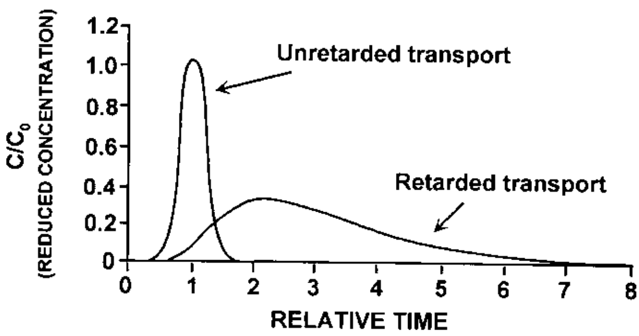

FIGURE 13.5
Retardation of transport in a porous medium. The unretarded case is expected for so-called "conservative" tracers that do not interact with the grains of the medium. The retarded case represents the effect of interaction on the breakthrough. The retarded case is often seen when equilibrium sorption processes are operative, although other mechanisms can produce similar curves. The reduced concentration (C/C_0) is the concentration at the end of the flow path divided by the initial concentration at the beginning of the flow path. The curve for retarded transport was drawn by application of Equation 6 to some idealized data.

does not show in the data because the numbers of cells eluting from the column are several orders of magnitude below that of the primary peak. When data are plotted on semilogarithmic axes, the tailing is plainly visible. In the model formulation reported by Hornberger et al. (1992), tailing could be approximated by appropriate selection of the deposition and entrainment coefficients.

An additional argument for the lack of bacterial retardation (although retention is not explained), deals with the kinetics of sorption and desorption (deposition and entrainment). Jury and Roth (1990) presented explanations for the lack of retardation of dissolved substances in laboratory studies as an artifact of the kinetics of some sorption processes. In short columns with rapid flow rates, the contact time of the sorbing material (in our case, bacterial cells) is too short to permit equilibration. While some sorption is probably occurring, it is too little to be readily observed in the breakthrough curves. Jury and Roth (1990) suggested that longer flow paths and slower flow rates should allow adequate equilibration for retardation to be observed (Figure 13.6). This argument is sometimes invoked to justify the use of the "local equilibrium" assumption for field situations, even when equilibrium is not observed in laboratory simulations. Use of deposition and entrainment coefficients as suggested by Hornberger et al. (1992) seems to provide a reasonable approach to approximation of the retardation behavior observed in laboratory simulations. Such an approach does not imply a kinetic control, and the models have not been tested for large-scale, slow-flow, field systems.

13.4 Summary

Nearly all of the data thus far collected point to the fact that microorganisms are transported through the subsurface and that dispersal of bacteria is an ongoing process. The factors which control dispersal may vary, however, depending on the spatial and temporal scales of the system being examined. For short-distance (tens to hundreds of meters) rapid transport, the hydrogeochemical and cell-associated biological factors probably dominate the dispersal process. The presence and distribution of preferred flow paths (which serve as the conduits for rapid bacterial transport) and the

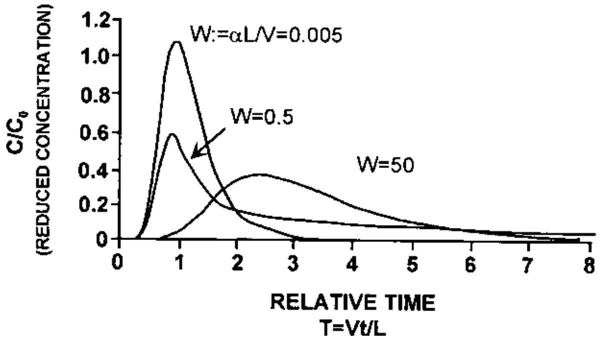

FIGURE 13.6
The effect of increasing the length of an experimental column and simultaneously decreasing the flow rate. The value W is the ratio of the column length (L) to the flow velocity (V) (α is a proportionality constant for consistency in units). Note that for large values of V and small values of L (i.e., W = 0.005) there is little retardation. Retardation is evident, however, when the ratio of L to V is increased. (Redrawn from Jury, W.A. and Roth, K., *Transfer Functions and Solute Movement Through Soil: Theory and Applications*, Birkhauser, Boston, 1990. With permission.)

distribution of geochemical phenomena (such as mineral grain coatings in combination with the cell properties of the bacterial strain of interest) will control the rate and extent of the transport of bacteria.

For regional transport that may occur over distances of kilometers and tens to hundreds or even thousands of year, survival of the microbe in the subsurface is necessarily the most important of all factors. Given adequate time, surviving microbes will permeate the subsurface environment as they have the surface; no data reported thus far contradict this hypothesis. The old adage of "everything is everywhere and the environment selects" is as true for the subsurface as it is for surface and near-surface environments.

Thus, in applied situations, for example where introduced microorganisms are considered as a part of a redemption design for a contaminated aquifer, attention should be paid to the specific cell characteristics that will enhance transport through the contaminant plume in the specific hydrogeological setting containing the contamination. For considerations of long-range, long-term transport, only persistence of viable organisms is of any real importance.

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