

Ref: R.W. Harvey. 1991. Parameters involved in modeling movement of bacteria in groundwater, p. 89-114, In C.J. Hurst (ed.) Modeling the Environmental Fate of Microorganisms, ASM Press, Washington, DC.

Chapter 5

Parameters Involved in Modeling Movement of Bacteria in Groundwater

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Continued widespread contamination of shallow drinking water aquifers by microbial pathogens and chemical wastes has resulted in an increased interest in the factors that control bacterial transport through groundwater. It has been hypothesized that degradation of highly mobile and persistent groundwater contaminants may be facilitated by cotransport of indigenous bacteria that have become acclimated to their degradation (Harvey and Barber, submitted). Recent advances in the application of molecular genetics have enhanced the feasibility of employing genetically engineered microorganisms for aquifer restoration. However, the success of many schemes that employ genetic technology in in situ treatment of organically contaminated aquifers involves, in part, the ability of the engineered organism to reach the contaminant-affected area in the aquifer.

The movement of nonindigenous bacteria through aquifers has long been a public health concern, since contamination of water supply wells by microbial pathogens has contributed significantly to the number of waterborne disease

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outbreaks (Keswick, 1984). A frequent problem relating to the domestic use of untreated groundwater involves transport through the aquifer of disease-causing bacteria from contamination sources upgradient, particularly from septic tanks, domestic waste lagoons, landfills, and on-land disposal facilities for domestic effluents. The reported ability of bacteria to move through limestone (Kingston, 1943) and fractured bedrock (Allen and Morrison, 1973) is not surprising. However, there are also a number of observations suggesting transport of pathogenic and enteric "indicator" bacteria over long distances (400 to 1,000 m, horizontally) through a variety of other aquifers, including pebbles, gravels, sand and gravel, stony silt loam, and even fine sand (Dappert, 1932; Merrell, 1967; Sinton, 1980; Anan'ev and Demin, 1971; Kudryavtseva, 1972; Martin and Noonon, 1977). Often, such observations are difficult to interpret because the source term(s) was ill-defined. Nevertheless, between 1971 and 1982, over 75% of the reported illnesses of known etiology relating to the consumption of inadequately treated or untreated groundwater involved pathogenic bacteria (Craun, 1985).

Unfortunately, the ability to model groundwater flow through the subsurface greatly exceeds our understanding of the factors which affect transport of indigenous and nonindigenous bacteria (Yates and Yates, 1988). Presently, predictive modeling of bacterial transport through contaminated groundwater is problematic and there is a scarcity of field data deriving from controlled, in situ experiments. It is clear that improvements in mathematical models that involve transport of bacteria in groundwater will require a better understanding of the various mechanisms and abiotic and biotic factors involved in bacterial migration through porous media. Transport of bacteria through contaminated groundwater is affected by a number of factors in addition to the geological and hydrological characteristics of the aquifer itself. These biotic and abiotic factors include bacterial growth, predation by eucaryotic microorganisms, parasitism by bacteriophages (bacteria-specific viruses) and predatory bacteria (*Bdellovibrio* sp.), lysis under unfavorable conditions, changes in bacterial size and propensity for attachment to solid surfaces in response to alterations in nutrient conditions, spore formation in the case of some gram-positive species, sorption and biological adhesion to solid surfaces, detachment from surfaces, and straining. This chapter discusses a number of these factors in the context of modeling.

APPROACHES TO MODELING

The modeling of bacterial transport is complicated by a number of factors that can substantively affect bacterial transport behavior. Several of these factors may be interrelated through other processes. For example, the chemical (including nutrient) conditions in the groundwater may influence several processes (e.g., survival, growth, attachment, and detachment) affecting abundance of bacteria traveling through the aquifer. Figure 1 depicts many of these factors and the manner in which they are interrelated. For the purposes of constructing a transport model, it is useful to consider the major processes that substantively change the abundance of unattached bacteria within a unit of aquifer sediment. These factors include interaction with solid surfaces (reversible and irreversible attachment and detachment), predation and parasitism, straining, transport (advective, diffusive, flagellar), and lysis (Fig. 2). A number of these factors (e.g., in situ predation and growth) remain poorly understood for groundwater environments, and how best

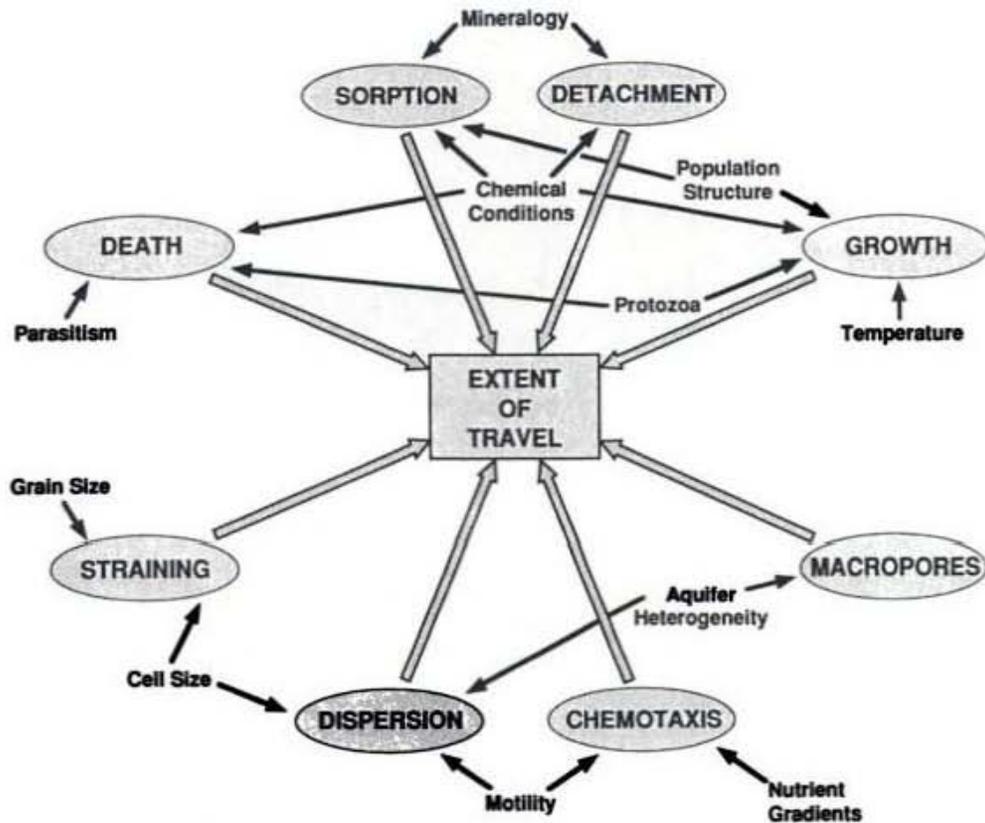


Figure 1. Schematic representation of the interrelationship of parameters and factors involved in the transport of bacteria through porous-medium aquifers.

to describe them mathematically is unclear. However, because of the complexity involved in integrating the various controlling factors into bacterial transport models, the modeling approach may be as important as the mathematical descriptions.

Models for describing subsurface transport of bacteria have employed both conceptual and data-based approaches. Recent theoretical models involving microbial transport through porous media (Corapcioglu and Haridas, 1984, 1985) have incorporated a number of governing processes depicted in Fig. 1 and 2. However, verification of existing theoretical models by field observation is problematic, owing to the complexity of the models and the number of parameters that need to be determined a priori. The predictive value of these models appears to be limited; in some cases, the degree of transport is significantly underpredicted, even when the model parameters are stressed to permissible limits (Germann et al., 1987). Nevertheless, comprehensive theoretical models serve to provide a conceptual framework for the development of more realistic models that are grounded with field observations. More accurate models will undoubtedly be developed as knowledge is gained about the parameters that govern bacterial transport through the aquifer.

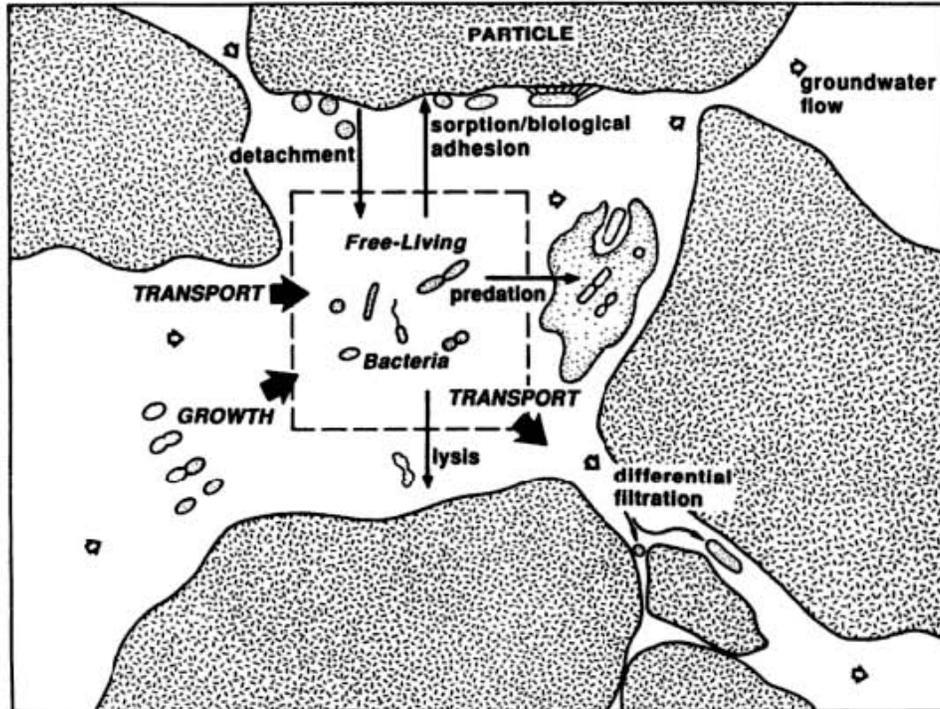


Figure 2. Schematic representation of factors affecting the mass balance of unattached bacteria in a unit of aquifer sediment.

As a result of both laboratory and field transport investigations, important advances have been made toward a better understanding of key processes governing microbial migration through aquifers. Columns packed with subsurface material allow experiments to be conducted with much greater control than is possible in field studies. Such experiments have allowed a clearer delineation of several factors governing microbial transport through the subsurface, including the effects of size-dependent exclusion from small porosity (Bales et al., 1989), survival (Bitton et al., 1979), sorption (Hendricks et al., 1979), and bacterial growth and taxis (Reynolds et al., 1989; Jenneman et al., 1985; Bosma et al., 1988). Column experiments have also proven useful in studying transport behavior of genetically engineered bacteria before they are released into the subsurface (Trevors et al., 1990). However, it has also been shown that transport of microbes through subsurface sediments that have been repacked into columns can differ from that observed in the field, even when flow velocity, porosity, and physicochemical conditions are similar (Harvey, 1988). Therefore, the overall mathematical descriptions of bacterial transport through aquifers may necessitate more information derived from field investigations, since repacking into columns can destroy the original secondary (preferred flowpath) pore structure (Smith et al., 1985).

There have been few reported attempts to model in situ observations of bacterial transport through a portion of an aquifer. One such model was used to simulate breakthrough curves for fluorochrome-labeled indigenous bacteria (whole

population) that had been injected into sandy aquifer sediments 7 m upgradient from an evenly spaced network of multilevel observation wells (Harvey and Garabedian, in press). The instrumented site is part of the U.S. Geological Survey's groundwater contamination study area involving an unconfined glacial outwash aquifer near Otis Air Force Base on Cape Cod, Massachusetts. Construction of the hydrologic portion of the model was facilitated by the coinjection of a conservative, nonreactive tracer with the labeled bacteria. Employment of a solute transport model in the simulation of microbial transport through saturated sediment has also been employed in column experiments with nonsorbing viruses (Grondin and Gerba, 1986). The model described by Harvey and Garabedian (in press) includes terms for storage, advection, dispersion, and reversible and irreversible adsorption, and it handles physical heterogeneity within the bacterial population and the aquifer by superimposition of separate solutions. This deterministic approach was satisfactory over short distances (Harvey and Garabedian, in press) because the length of the layers having similar conductive properties (as delineated by hydraulic conductivity profiles) appears to be on the order of several meters (Hess, 1989). However, the modeling of transport over longer distances or thicknesses of the aquifer would necessitate a stochastic approach because it would be difficult to define aquifer structure deterministically at a larger scale. Both stochastic and deterministic approaches that attempt to account for physical heterogeneity are facilitated by information concerning the variability of hydraulic parameters within the aquifer.

Although the effect of aquifer pore structure upon microbial transport is beyond the scope of this chapter, it is clear that models describing bacterial transport in groundwater must take geohydrology into account. This is particularly important where preferred flow path (macropore) structure significantly influences the microbe's apparent downgradient mobility. In a number of groundwater tracer studies, microorganisms introduced into an aquifer appeared to travel faster than would be predicted from groundwater flow measurements, since their peak arrival at downgradient samplers occurred in advance of the conservative tracer. This phenomenon has been observed for yeasts (Wood and Ehrlich, 1978), for bacteria (Champ and Schroeter, 1988; Harvey et al., 1989), and, in sand-packed columns, for viruses (Grondin and Gerba, 1986). The apparent retardation of conservative tracer relative to bacteria in small-scale tracer tests was most significant in fractured rock (Champ and Schroeter, 1988) and least significant in sand and gravel (Harvey et al., 1989; Harvey and Garabedian, in press; Havemeister et al., 1985). Clearly, a key parameter governing transport of bacteria in groundwater involves the portion of the total porosity that is available for fluid flow, but unavailable for bacterial transport.

MODES OF MACROSCOPIC MIGRATION

Transport of bacteria through groundwater can involve several mechanisms. Three nondiffusive modes are continuous transport, discontinuous (intermittent) transport, and tactic migration (Fig. 3). All three modes of bacterial migration may be common to both indigenous and nonindigenous bacteria in groundwater. However, the contributions of each type are often poorly understood and depend upon the geohydrology, mineralogy, type of bacteria, and physical and chemical

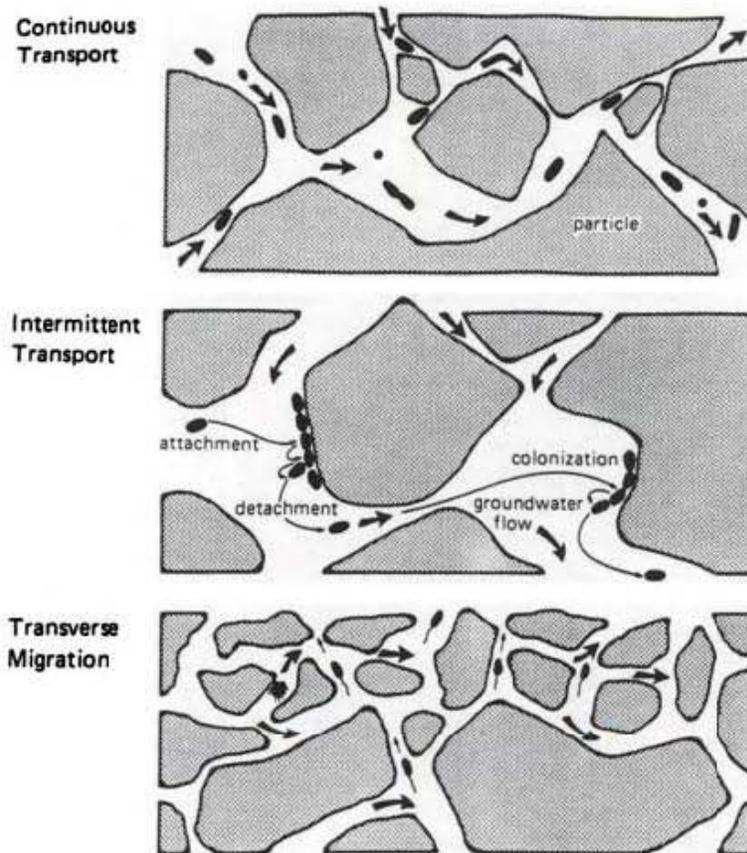


Figure 3. Schematic depiction of three modes of bacterial migration through aquifer sediments (from Harvey, 1989).

conditions of the aquifer. Facilitated migration of bacteria within a nonaqueous phase (e.g., micellar transport) is not considered here.

Continuous (Unretarded) Transport

"Continuous" is used here to refer to the unimpeded, advective movement of unattached bacteria through a section of the aquifer and implies an absence of retardation. Continuous transport is likely to be most significant within large fractures or macropores or in aquifers with high flow rates, elevated concentrations of readily degraded organic carbon, and low amounts of clay, fine sediments, and total dissolved solids. There are many conditions that affect the degree to which continuous transport contributes to the large-scale mobility of groundwater bacteria.

There is some evidence for continuous transport in several small-scale groundwater injection and recovery experiments, where breakthrough curves of bacteria at downgradient samplers were compared with those of conservative tracers (Champ and Schroeter, 1988; Harvey et al., 1989; Harvey and Garabedian, in press;

Havemeister et al., 1985). Because of the high degree of attenuation (immobilization of bacteria at solid surfaces), these experiments have been conducted over limited distances (2 to 30 m). The simultaneous arrival of the peak concentrations of conservative tracer and labeled bacteria at downgradient samplers in forced- and natural-gradient investigations performed at the Cape Cod groundwater study site (Harvey et al., 1989; Harvey and Garabedian, in press) suggests that many of the bacteria traveled through the sandy aquifer sediments in a relatively unimpeded manner. However, the appearance of some of the labeled bacteria after concentrations of the conservative tracer had declined to undetectable levels also suggested that at least some of the bacteria were subject to intermittent (retarded) transport, presumably as a result of interactions with solid surfaces. Furthermore, the concentration histories of the labeled bacteria at samplers downgradient in the latter experiments (Harvey and Garabedian, in press) were successfully modeled assuming both continuous and intermittent transport mechanisms were operative.

Retarded (Intermittent) Transport

Because of sorptive losses of unattached bacteria from moving groundwater to stationary surfaces, retarded transport would be expected to be more significant over longer distances, particularly in acidic or neutral-pH groundwater moving through relatively fine-grained sediments. Intermittent transport refers to the slow, discontinuous migration of bacteria through an aquifer. This can involve travel with groundwater flow, attachment to solid surfaces, and subsequent detachment and travel of at least a portion of the attaching population. However, adherent bacteria may become irreversibly adsorbed by an active process involving the use of highly surface-active, extracellular bridging polymers (exopolymer) (discussed under Sorption, below). Bacteria attached to solid surfaces with exopolymer may not be able to degrade their own exopolymer and are likely to be incapable of further transport. Therefore, detachment and subsequent transport of adherent groundwater bacteria likely involves weakly (reversibly) adsorbed cells or daughter cells in microcolonies that are associated with other surface-attached bacteria, but not directly with the surface.

The role of intermittent transport in the movement of bacteria through aquifers remains poorly understood. This type of transport can be substantially retarded due to long surface residence times. Retardation factors (ratio of groundwater flow velocity to bacterial migration velocity) as high as 10.0 have been reported for bacterial transport through sandy aquifers in Germany (Matthess et al., 1988). Although a much slower process, intermittent transport may be important both in the movement of subsurface bacteria over geological time and as a mechanism by which large areas of contaminated aquifers are eventually seeded with specific types of bacteria from the surface environment.

Tactic and Transverse Migration

Movement of motile groundwater bacteria in response to chemical gradients, herein referred to as "chemotactic migration," can involve movement via flagella toward or away from higher concentrations of a given nutrient or chemical. Many bacteria in aquifers appear to be motile (Harvey and Garabedian, in press) and presumably capable of directional locomotive response to certain chemical stimuli.

Chemotactic migration is proportional to the population density and to the concentration gradient of the attracting chemical. Growth of indigenous bacterial populations in uncontaminated groundwater environments is often limited by a lack of sufficient dissolved organic carbon that can be readily utilized as an energy source. This also appears to be the case for at least some contaminated aquifers such as the one at Cape Cod (Smith and Duff, 1988). The presence of spatial gradients in organic pollutants may be important to the net movement of bacteria through contaminant plumes, particularly in the vertical direction, where there is typically a low degree of dispersivity.

Bacterial movement in porous media due to chemotaxis can be substantially faster than that due to random thermal (Brownian) motion. Jenneman et al. (1985) demonstrated that the rate of bacterial penetration through Berea sandstone cores in the presence of a sucrose gradient was three- to eightfold higher for a motile bacterium, *Enterobacter aerogenes*, than for a nonmotile bacterium, *Klebsiella pneumoniae*. Observed rates of penetration by chemotactic bacteria in the sandstone core experiments ranged up to 0.11 m/day. This rate of movement suggests that motility may be an important mechanism in the movement of bacteria through contaminated, porous rock aquifers with low flow rates. A model which combines mathematical descriptions for chemotactic response with those for microbial growth by Monod kinetics was used by Bosma et al. (1988) to successfully simulate the degradation of 1,2-dichlorobenzene in soil columns inoculated with *Pseudomonas* sp. strain P52. The term used in their model to describe chemotactic movement of the pseudomonads is

$$\frac{\partial X}{\partial t} = -K_c \frac{\partial}{\partial z} \left[X \frac{\partial C}{\partial z} \right] \quad (1)$$

where K_c is the chemotactic coefficient, which has the units of centimeters squared per liter per microgram per hour; X is bacterial abundance; z is the longitudinal space coordinate; C is the concentration of the organic substrate; and t is time. Recently it was reported that penetration of motile strains of *Escherichia coli* through Ottawa sand-packed cores under anaerobic, nutrient-saturated conditions occurred four times faster than for nonmotile mutants (Reynolds et al., 1989). However, motile, nonchemotactic mutants penetrated the cores faster than did the chemotactic parental strain, suggesting that chemotactic response may not be required for enhanced transport of motile bacteria through unconsolidated porous media. In the absence of chemical gradients, taxis would be random and should also have the effect of increasing the apparent dispersion of the bacterial population being transported.

IMMOBILIZATION

In many situations involving transport of bacteria in groundwater, the extent of horizontal migration will be governed largely by the degree to which the bacteria become immobilized at solid surfaces. This is particularly true for indigenous groundwater bacteria that are well adapted to the low-nutrient conditions and, therefore, can survive for long periods of time in the near-absence of organic substrate. The two mechanisms responsible for the permanent immobilization of

unattached bacteria from groundwater moving through porous media are straining and irreversible adsorption. Although the two mechanisms are often treated the same in microbial transport models, they can lead to quite different results. From a physical point of view, straining preferentially removes the larger bacteria, whereas the opposite is true for sorption. Also, the manner in which the capture efficiency of the sediment for bacteria, via straining and sorption, changes over time as the aquifer sediment "filter" develops can be substantially different. Therefore, it is desirable to treat straining and sorption separately in modeling transport of bacteria through porous media. This may require a priori knowledge of the grain and bacterial size distributions and the degree of physical heterogeneity of the aquifer.

Straining

Straining refers to the immobilization of suspended bacteria that occurs when they get caught in pore openings smaller than their limiting dimensions. It has been observed in porous media column experiments that strained particles can be dislodged and resuspended by flow reversal, but not by increases in flow velocity (Sakthivadivel, 1966). Straining is thought to be an important mechanism of immobilization in porous media when the average diameter of the free-living bacterial population is greater than 5% of that for the stationary sediment particles. This is based, in part, upon results of flow-through column experiments in which the effect of grain diameter upon removal of fine suspensions was assessed (Herzig et al., 1970). For ratios of media grain diameters to suspended particle diameters (d_m/d_p) of 20 and 50, it was observed that only 0.53% and 0.053%, respectively, of the bed volume would be filled by strained particles.

In highly heterogeneous media, such as aquifer sediments, criteria for straining that are based solely upon average grain and colloid diameters can be inaccurate, since sediments are typically characterized by a wide distribution of intergranular pore sizes. It has been suggested that for bacterial transport through sediments, the ratio of the bacterial diameter (d_p) to the critical pore size in sediments must be greater than 1.5 for straining to occur (Matthess and Pekdeger, 1985), i.e.:

$$\frac{d_p}{F_s d_K} \geq 1.5 \quad (2)$$

where F_s is the empirical transit factor for suffusion and is related to the heterogeneity of the porous media, and d_K is the hydraulic equivalent diameter of pore canals. The latter parameter is equivalent to $0.455 U^{1/6} e d_{17}$, where U is the uniformity coefficient (calculated as d_{60}/d_{10}) and e is the ratio of void to solid volume in the medium. The parameters d_{17} , d_{60} , and d_{10} are the grain diameters at which 17, 60, and 10%, respectively, of the particulate mass is of a smaller size. For sediments with uniform grain diameters, straining of most bacteria would not be predicted even for coarse silt (0.02- to 0.06-mm grain size), since the critical pore size may be estimated to be $\sim 7 \mu\text{m}$. However, it is estimated that over 10% of the pores in some heterogeneous sands are small enough to interfere with bacterial transport (Matthess and Pekdeger, 1985).

In field experiments, the contribution of straining in immobilization of bacteria

traveling through heterogeneous, sandy aquifer sediments is often difficult to ascertain. In small-scale (2 to 7 m), forced- and natural-gradient tracer experiments (Harvey et al., 1989), involving various sizes of bacteria-sized microspheres moving through sandy aquifer sediments (0.59 mm, median grain size), straining did not appear to be a significant determinant of immobilization. The observed increase in the average diameter of the polydispersed suspension of microspheres during transport downgradient could be accurately simulated by sorptive-filtration theory (Harvey and Garabedian, in press) and was inconsistent with what would be expected with straining. In contrast, straining is likely to be an important cause of immobilization of bacteria traveling through silt (2 to 50 μm) and, in the absence of fractures, reasonably precludes bacterial transport through clayey layers in an aquifer. However, straining of bacteria traveling through heterogeneous, highly stratified sandy aquifers cannot be overlooked, particularly if longer travel distances (e.g., hundreds of meters) are considered. This is because bacteria may encounter a number of conductive layers, some of which can contain substantial amounts of silt. Straining may also be a factor in wastewater injection, since buildup of biofilm may restrict pore sizes in porous media adjacent to the well screen. A thorough discussion of straining and clogging is given by McDowell-Boyer et al. (1986).

Sorption

Mechanistic modeling of bacterial sorption that occurs during transport through an aquifer is complicated by the existence of different mechanisms for attachment, changes in bacterial surface properties and size in response to alterations in environmental conditions, changes in the nature of attachment due to biological processes, and the heterogeneous and complex nature of both bacterial and aquifer surfaces.

Surface properties

A primary determinant of the degree of bacterial attachment during transport through sandy or gravelly aquifers involves the characteristics of the surfaces involved. Several studies involving bacterial attachment to synthetic material have focused upon the surface characteristics of the abiotic solid. The two parameters of the solid phase which appear to be the more important controls of bacterial attachment are hydrophobicity (which involves the unfavorable reorientation of water molecules around nonpolar molecules) and surface charge. Fletcher and Loeb (1979) demonstrated that both hydrophobic and electrostatic interactions were involved in bacterial attachment. However, the degree of substratum hydrophobicity was the more important determinant of sorption onto hydrophobic surfaces, whereas the surface charge appeared to control sorption onto hydrophilic surfaces. Paul and Jeffrey (1985) suggested separate mechanisms governing attachment of *Vibrio proteolytica* onto hydrophilic and onto hydrophobic surfaces.

Many recent investigations involving the controls of bacterial sorption in aquatic environments have focused on the role of surface properties characterizing both microbe and abiotic solid. Bacterial cell hydrophobicity has been observed to affect bacterial attachment to a variety of materials, including glass (Kjelleberg and Hermansson, 1984), polystyrene (McEldowney and Fletcher, 1986; van Loosdrecht et al., 1987b; Rosenberg, 1981), mineral particles (quartz, albite, feldspar, and

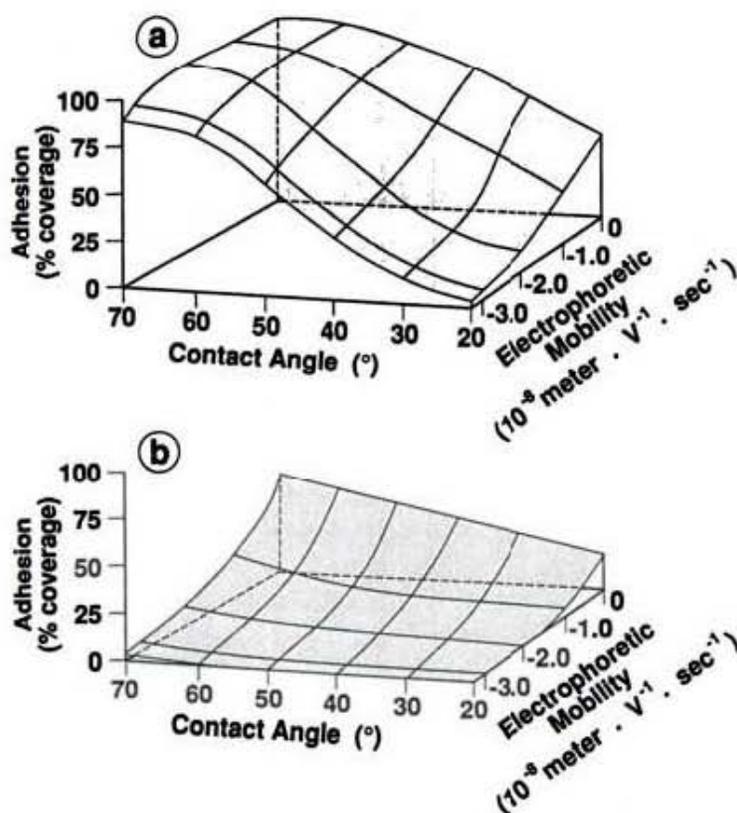


Figure 4. Relationship between bacterial adhesion to (a) sulfated polystyrene (hydrophobic) or (b) glass (hydrophilic) and bacterial surface characteristics as determined by contact angle measurement and electrophoretic mobility (reproduced with permission from van Loosdrecht et al., 1990b).

magnetite) (Stenstrom, 1989; Stenstrom and Kjelleberg, 1985), and whole sediments (van Loosdrecht et al., 1990b). Cell hydrophobicity may also have a role in determining the kinetics of initial bacterial attachment onto some surfaces. In one study involving sorption of 15 different isolates of *Pseudomonas aeruginosa* onto stainless steel, cell hydrophobicity correlated strongly with the sorption rate constant (Vanhaecke et al., 1990). A number of bacterial surface components are thought to contribute to cell hydrophobicity, including fimbriae, proteins A and M, prodigiosin (a pigment), core oligosaccharides, or outer membrane lipids. Bacterial surface hydrophobic components (hydrophobins) and the role of hydrophobic interactions in bacterial adhesion are reviewed in detail by Rosenberg and Kjelleberg (1986).

Several studies have demonstrated correlations between the degree of bacterial attachment to solid surfaces and the magnitude of charge on the bacterial surface (Feldner et al., 1983; van Loosdrecht et al., 1987b). However, only recently has the combined effect of bacterial cell hydrophobicity (which can be inferred from water drop contact angle measurement) and surface charge (which can be estimated from electrophoretic mobility data) upon the propensity of bacteria for attachment to

both hydrophobic and hydrophilic surfaces been put into a proper perspective (van Loosdrecht et al., 1987a, 1987b; van Loosdrecht et al., 1990b). The relationship between the combined effects of a bacterium's electrophoretic mobility and hydrophobicity upon fractional adsorption is depicted in Fig. 4 for hydrophobic polystyrene (Fig. 4a) and hydrophilic glass (Fig. 4b). It is clear that the relative importance of hydrophobicity and surface charge with regard to bacterial sorption is different for hydrophobic and hydrophilic surfaces. However, maximal sorption onto both types of surfaces in the investigations of van Loosdrecht et al. (1990b) was evident for uncharged, highly hydrophobic cells.

There is a dearth of information relating bacterial and grain surface characteristics to bacterial sorption behavior in the aquifer. Most surfaces in the aquifer would be expected to be hydrophilic or only slightly hydrophobic. Scholl et al. (in press) reported that, in the absence of organic carbon, the surface charge of various minerals (quartz, muscovite, limestone, and iron hydroxide-coated quartz and muscovite) was the primary determinant of sorption for a slightly hydrophilic (contact angle measurement of 30°) bacterium (Lula-D) isolated from a sandy aquifer in Oklahoma. However, bacterial hydrophobicity may have a significant role in the initial attachment of bacteria moving through aquifer sediments, particularly where organic matter is present on mineral surfaces. Indeed, both hydrophobic attraction and electrostatic repulsion were necessary to explain the sorptive tendencies of a variety of bacteria in the presence of Rhine River sediment (0.05% organic carbon) (van Loosdrecht et al., 1990b).

There is growing evidence suggesting that extracellular appendages (fimbriae, flagella, and holdfasts) can facilitate sorption of bacteria, sometimes under conditions that would otherwise be energetically unfavorable. Adhesion of *Salmonella typhimurium* to a variety of mineral surfaces (albite, biotite, feldspar, magnetite, and quartz) was observed to be markedly enhanced by the presence of fimbriae (Stenstrom and Kjelleberg, 1985), and Rosenberg et al. (1982) demonstrated that thin fimbriae on *Acinetobacter calcoaceticus* enhanced its attachment to polystyrene. The role of fimbriae in the adhesion of bacteria to surfaces is thought to involve localized patches of positive charge and the apparent ability of the fimbriae to penetrate the electrostatic energy barrier between the bacterium and a like-charged surface (Isaacson, 1985). Fimbriae are found almost exclusively among the gram-negative bacteria and may be common features of many indigenous and "pollutant" bacteria found in contaminated aquifers. Flagella also appear to facilitate sorption of bacteria to at least some inorganic surfaces (Fletcher, 1979). Although the mechanism is not clear, flagella-induced motion (motility) appears to be insufficient to overcome the electrostatic barrier adjacent to a like-charged surface (Marshall et al., 1971), but flagella, like fimbriae, can apparently directly sorb to surfaces (Doetsch and Sjoblad, 1980). The relevance of morphologically distinct attachment structures called holdfasts to bacterial attachment in the groundwater environment is not known. However, a few species known to have holdfast-type structures are found in the subsurface. A review of the older (pre-1980) literature involving the role of microbial surface structures in attachment to surfaces is provided by Corpe (1980).

The role of extracellular polymers (exopolymer) in the initial attachment of bacteria is somewhat unclear. Allison and Sutherland (1987) reported that an exopolymer-producing bacterial isolate attached to glass to the same degree as a non-polymer-producing mutant. In contrast, exopolymer produced by a marine *Pseudomonas* sp. measurably affected its degree of adhesion to hydrophobic surfaces

(Wrangstadh et al., 1986). Undoubtedly, the role of exopolymer in the initial attachment of bacteria to solid surfaces depends upon the nature of the exopolymer, both surfaces, and the physicochemical conditions. However, it is clear that exopolymer has a key role in the time-dependent irreversible adhesion of bacteria to surfaces, which will be discussed in more detail in a subsequent section.

Models for bacterial attachment

An important control of bacterial immobilization occurring in porous aquifers involves the rate at which bacteria encounter stationary surfaces. For this reason, a number of transport models involving subsurface transport of microbes have incorporated filtration theory into descriptions of immobilization. The colloid filtration model of Yao et al. (1971), often employed to describe removal of colloidal particles during deep-bed filtration in water treatment applications, was used to account for immobilization in theoretical models describing bacterial transport through porous media (Corapcioglu and Haridas, 1984; Matthess and Pekdeger, 1981) and, more recently, in in situ bacteria transport experiments involving a contaminated, sandy aquifer (Harvey and Garabedian, in press). The colloid filtration model has the advantage of being relatively simple, accounting for the abiotic mechanisms by which bacteria contact stationary solid surfaces, and reasonably predicting the effect of cell size upon the rate of bacterial deposition. However, there is some uncertainty involving buoyant densities of bacteria in the aquifer, the effect of bacterial motility, and how to mathematically treat some of the physical differences between situations of groundwater flow through sandy aquifers and rapid, packed-bed filtration for which the model was developed. In addition, filtration theory does not account for differences in surface characteristics among bacteria. However, filtration theory appears to be useful in a multicomponent description of immobilization in transport models involving bacteria in sandy aquifers (Harvey and Garabedian, in press).

The filtration model considers three mechanisms by which suspended particles come into contact with stationary solid surfaces, i.e., Brownian motion (diffusion), sedimentation due to gravity, and physical interception resulting from the sizes of the stationary and suspended particles. An important parameter is the single collector efficiency, η , which is the rate at which suspended particles strike a single porous medium grain divided by the rate at which they approach the surface. The collector efficiency represents the physical factors determining microbial contact with grain surfaces and is the algebraic sum of collector efficiency caused by diffusion, interception, and settling (Fig. 5). If close-approach effects are neglected, collector efficiency may be estimated by the equation of Yao et al. (1971):

$$\eta = \eta_D + \eta_I + \eta_G = 0.9 \left[\frac{kT}{\mu d_p v} \right]^{2/3} + 1.5 (d_p/d)^2 + \frac{(\rho_p - \rho)gd_p^2}{18 \mu v} \quad (3)$$

where η_D is the colloid-collector collision caused by Brownian motion; η_I is the colloid-collector collision caused by interception; η_G is the colloid-collector collision caused by settling; k is the Boltzman constant; T is the solute temperature; μ is the fluid viscosity; d_p is the bacterial diameter; ρ is the fluid density; ρ_p is the bacterial density (specific gravity of bacterial biomass); v is the fluid velocity; and g is the gravitational constant. An equation describing η that utilizes a similar trajectory

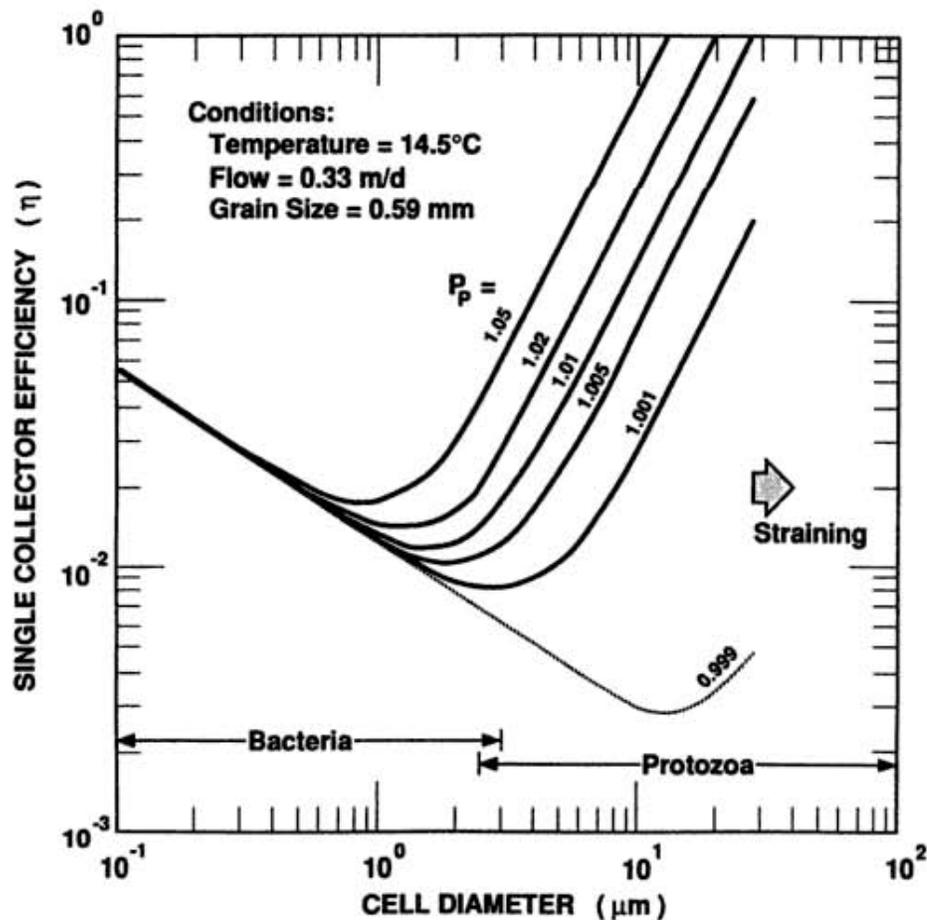


Figure 5. Theoretical estimates for collector efficiency as a function of cell diameter for microbes traveling through Cape Cod aquifer sediments. Different curves represent different cell buoyant densities (reproduced with permission from Harvey and Garabedian, in press).

approach, but includes hydrodynamic retardation and London-van der Waals interaction forces, is provided by Rajagopalan and Tien (1976).

For mixed bacterial populations, η must be determined separately for each size class. The effect of buoyant density and cell size upon collection efficiency for bacteria moving through sandy aquifer sediments is depicted in Fig. 5. As indicated by the minima in the η -versus-size function in Fig. 5, the optimal size for transport of bacteria with near-neutral buoyancy through the sandy sediments of the Cape Cod aquifer is likely 2 to 3 μm (diameter). In general, diffusion predominates for the 0.2- to 1- μm size classes of bacteria typically found in groundwater environments. The model predicts that smaller bacteria within this size range will be immobilized on solid surfaces at faster rates than will larger bacteria. This prediction is consistent with results of a recent groundwater injection and recovery experiment in which 0.2-, 0.7-, and 1.3- μm (diameter) microspheres were employed (Harvey et al., 1989).

Collector efficiency, η , is related to the flux of bacteria that become irreversibly sorbed in the porous media they are moving through by the so-called collision efficiency, α . α , which represents the physicochemical factors determining bacterial attachment to grain surfaces, is generally determined experimentally based upon observed immobilization rates of the suspended particles and theoretical values of η (Tobiason and O'Melia, 1988). For a pulse injection of bacteria and conservative tracer into a sandy or gravelly aquifer where the longitudinal dispersivity is small relative to the travel distance, α may be estimated by the following (Harvey and Garabedian, in press):

$$\alpha = \frac{-d \ln (RB)}{1.5(1 - \theta) \eta x_1} \quad (4)$$

where d is the median grain diameter; θ is the porosity; x_1 is the travel distance; and RB is the ratio of relative (injectate-normalized) masses of bacteria to conservative tracer appearing at the sampling point (Harvey et al., 1989). However, α is probably not constant and may vary spatially within the aquifer. This is because α depends, in part, on the aquifer characteristics, which may have substantial spatial variability. Although the value of α might be handled stochastically, the use of experimentally determined data obtained from small-scale in situ transport experiments would be helpful.

Models used to describe the initial attachment of bacteria to solid surfaces in aqueous systems have generally invoked either a surface free energy (Absolom et al., 1983; Busscher et al., 1984) or a colloid stability theory (Marshall et al., 1971; Rutter and Vincent, 1984; van Loosdrecht et al., 1989) approach. Although generally considered to be fundamentally different, there are important interrelationships between the two models (Pethica, 1980), and some recent progress has been made toward unifying the two approaches (van Loosdrecht et al., 1990b). However, the surface free energy approach, which involves "short-range" interactions (i.e., interfacial tensions), assumes that the bacteria are in direct contact with the solid surface, whereas the colloid stability theory is best used where there is a separation distance of 1 nm or greater between bacterium and surface (van Loosdrecht et al., 1989). The separation assumption is necessary because the colloid stability model is based upon so-called "long-range" (electrostatic and van der Waals) interactions. At shorter distances, the colloid stability model is complicated by the existence of other shorter-range interactions (e.g., steric effects, hydrogen bonding).

For direct bacterial contact on the solid surface, the extent of attachment is controlled by the surface properties of all three phases (liquid, solid, and bacteria). The surface thermodynamic approach employed by Absolom et al. (1983) involves a balance of surface tensions when a new phase boundary (bacterium-solid) is created because of attachment, i.e.:

$$\Delta F^{\text{adh}} = \gamma_{\text{BS}} - \gamma_{\text{BL}} - \gamma_{\text{SL}} \quad (5)$$

where ΔF^{adh} is the free energy of adhesion and γ_{BS} , γ_{BL} , and γ_{SL} are the interfacial tensions between bacterium and solid, bacterium and liquid, and solid and liquid, respectively. The difficulty with this approach involves obtaining accurate values for the various interfacial tensions. From theoretical considerations, bacterial

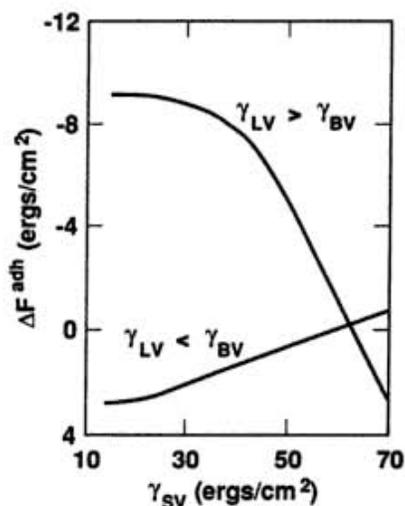


Figure 6. Free energy of adhesion calculated for *E. coli* 2617 as a function of substratum surface tension for liquid surface tension of 72.8 millinewton/m (upper curve) and 64.0 millinewton/m (reproduced with permission from Absolom et al., 1983).

adhesion increases with increasing surface tension of the solid (γ_{sv}) if the bacterium-vapor surface tension is greater than the liquid-vapor surface tension, i.e.: $\gamma_{LV} < \gamma_{BV}$. If, however, $\gamma_{LV} > \gamma_{BV}$, then bacterial adhesion would be expected to decrease with increasing surface tensions of the solid (higher-energy surfaces) (Absolom et al., 1983). The manner in which free energy of adhesion for *E. coli* varied as a function of the solid-vapor surface tension in the Absolom et al. study is depicted in Fig. 6. A potential problem in using this approach to predict the degree of bacterial sorption in a freshwater aquifer is that most bacteria attaching to a like charge surface would be held at a finite distance from the surface due to electrostatic repulsion.

The interactions between bacteria and particles have most often been explained in terms of long-range interactions by employing the theories of Derjaguin and Landau and Verwey and Overbeek, known collectively as the DLVO theory of colloid stability. In the absence of steric effects, the total energy of interaction as a function of separation distance between bacterium and a like-charged surface may be estimated as the algebraic sum of the repulsive (electrostatic) and attractive (van der Waals) forces. Although there are complications in applying the DLVO theory to sorption of bacteria (Pethica, 1980), it seems to explain the initial phase of bacterial adhesion in a number of studies (Marshall, 1980, 1985; van Loosdrecht et al., 1989, 1990a, 1990b).

An important parameter in the DLVO model for bacteria-surface interaction is the concentration of electrolyte. This is because the magnitude of electrostatic repulsion as a function of separation distance is controlled by the thickness of the diffuse double layer (electrical double layer) of counterions in solution, which in turn is a function of ionic strength (Shaw, 1976). The manner in which the total energy of interaction between bacteria and a negatively charged surface varies with distance at different concentrations of electrolytes is depicted in Fig. 7.

In highly saline aquifers, the electrical double layer is compressed to the point where the electrostatic energy barrier near the surface no longer exists and, therefore, the London-van der Waals attractive force dominates the overall bacteria-surface interaction energies at all separation distances (Fig. 7A). This may allow for

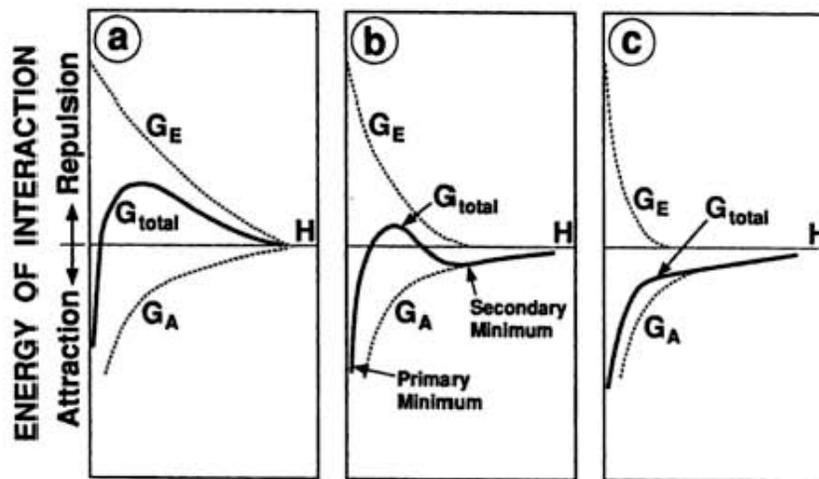


Figure 7. Schematic representation of Gibbs energy of interaction between a colloid-sized sphere and a like-charged surface as function of separation distance (H) for low (a), intermediate (b), and high (c) concentrations of electrolyte. G_{total} , Total energy of interaction; G_E , electrostatic interaction; G_A , van der Waals interaction. (Reproduced with permission from van Loosdrecht et al., 1990a.)

direct bacteria-surface contact. Since the interaction energy between a bacterium and a like-charged surface is at a minimum right at the surface, bacteria in direct contact with the surface are considered to be irreversibly adsorbed. In drinking water aquifers, the low ionic strength allows for a substantial electrical double layer (on the scale of nanometers in thickness), and electrostatic repulsion dominates such that an energy barrier separates approaching bacteria from the surface. At extremely low ionic strength (e.g., 10^{-4} M), there is a net repulsion that extends for a considerable distance from the surface (Fig. 7C). At intermediate ionic strengths (e.g., 10^{-2} M), the repulsive and attractive forces interact in a manner which allows for a secondary minimum of interaction energy at a finite distance (several nanometers) from the surface (Fig. 7B). Although there is a "primary" energy minimum right at the surface, approaching bacteria lack sufficient kinetic energy to penetrate the repulsive barrier. Bacteria which become trapped at the secondary minimum are reversibly sorbed and can be easily dislodged by their own motility or by changes in flow (Marshall, 1985).

In natural systems, application of the DLVO model can be complicated by presence of exopolymers. The presence of polymer on the solid surface as well as the bacteria can lead to additional interactions (steric and bridging) as well as alterations of van der Waals and electrostatic forces (van Loosdrecht et al., 1990b). Although entrapment in the secondary minimum, as predicted by the DLVO theory, is generally invoked to explain the ability of sorbing bacteria to exhibit a variety of movement at short distances from the surface (e.g., Marshall et al., 1971), there is at least some evidence to the contrary. Abbott et al. (1983) observed that about half of the *Streptococcus mutans* organisms sorbing onto clean and protein-coated glass oscillated a short distance from the surface, but apparently were not held in a secondary minimum. These authors suggested that the bacteria were

sorbing to the surfaces by means of exopolymers, which can occur over a much longer distance than is possible with either van der Waals or electrostatic interactions (Rutter, 1980).

Reversibility of attachment

Irreversible sorption can be the most significant control of the extent of bacterial transport. This is particularly true for indigenous bacteria that are capable of surviving for long periods of time under typical groundwater conditions. Since reversible sorption is what controls retardation (slowing down of bacterial transport relative to groundwater flow), it is important to differentiate the two types of adsorption in transport models. However, such differentiation would be difficult in the absence of a priori information deriving from sorption experiments with core material and the bacteria of interest. Also, although bacteria that are reversibly sorbed can be removed by the shearing effects of a water jet or by sharp changes in chemical conditions (ionic strength, pH, or competing ligand concentrations), their "surface residence times" on aquifer solids may be quite long. This is because drastic changes in chemistry or in hydraulic conditions in the groundwater environment are rare. Therefore, it may be useful to incorporate a time frame into criteria used to differentiate reversibly sorbed from irreversibly sorbed bacteria in an aquifer.

Irreversible sorption or adhesion of bacteria onto solid surfaces has been defined as a time-dependent process in which bacteria are no longer subject to Brownian motion and cannot be recovered by washing (Marshall et al., 1971). Irreversible sorption requires some sort of bacterium-surface contact. Since bacteria in freshwater aquifers would be unable to contact most surfaces because of the formidable energy barrier created by the overlapping of bacterial and mineral electrical double layers, the initial adhesion would be largely reversible. Exopolymer is thought to be the primary mechanism responsible for permanent adhesion, and its role in the "cementing" of aquatic bacteria to solid surfaces is well documented (Marshall et al., 1971; Corpe, 1974). Although carbon limited, aquifer bacteria appear to channel a disproportionately large amount of their assimilated carbon into the production of extracellular and storage polymers. Adherent aquifer bacteria are often observed under electron microscopy to be anchored by large amounts of exopolymer (Ghiorse and Wilson, 1988).

Reversible sorption of bacteria to negatively charged surfaces involves the instantaneous attraction by so-called long-range forces near, but not directly at, the surface. Bacteria which are reversibly sorbed continue to exhibit Brownian motion and can be easily dislodged (Marshall, 1985). In general, the initial sorption of bacteria in a freshwater aquifer at low ionic strength is, by definition, reversible and can be an important determinant of bacterial transport. However, it is not always clear how best to represent reversible sorption in models. In the Cape Cod study, it was found that the breakthrough curves could be accurately simulated by using either of two approaches to adsorption (Harvey and Garabedian, in press). The first assumed that adsorption is instantaneous and linear, but that the propensity of the bacteria for solid surfaces differs among segments of the population. The second employed a kinetic approach that assumed that all the bacteria are equally capable of interacting with solid surfaces, but that the rates of adsorption and desorption are different. It is likely that sorption of a bacterial population in heterogeneous aquifer sediment involves both a kinetic limitation for adsorption and a difference

among cells in their surface activity. It is clear that detailed studies are needed to more accurately describe sorption of bacteria onto aquifer material.

GROWTH

Growth of many pathogens and displaced bacteria in groundwater environments is likely to be negligible, since copiotrophic bacteria typically shift their metabolic capabilities from growth to starvation survival in response to low-nutrient conditions (Kurath and Morita, 1983). However, growth rates of indigenous bacteria moving through aquifers can be significant and must be accounted for in describing the population's movement downgradient (Harvey and George, 1987). Several models involving transport of bacteria or biodegradable contaminants have employed Monod kinetics to describe bacterial growth dynamics and utilization of organic carbon (Corapcioglu and Haridas, 1985, 1984; Molz et al., 1986). The Monod relationship is a hyperbolic function in which growth rate is related to the concentration of a limiting substrate and to two kinetic "constants," i.e., an asymptotic value defined as the maximum growth rate and the saturation constant. The latter is equivalent to the substrate concentration that will yield half the maximum growth rate. Monod kinetics were developed using experimental data derived from laboratory batch systems in which pure bacterial cultures and well-defined substrates were used. Although Monod kinetics have been successfully used to describe organic substrate utilization and bacterial growth in aquifer-derived laboratory microcosms (Godsy et al., 1990), their applicability to groundwater habitats has not been demonstrated.

The Monod relationship is used in the description of growth in subsurface transport models because it generally works well for bacterial populations having low saturation constants for organic substrates, as would be the case in most aquifers. However, a complication in this approach is that the kinetic "constants" in the Monod equation can be subject to change in response to changes in temperature, the nature of the substrate, and other factors (Gaudy and Gaudy, 1980). Therefore, caution should be used in the application of Monod growth kinetics to problems involving bacterial transport in contaminated groundwater, particularly where there are temporal and spatial changes in nutrient and physical conditions or in the bacterial populations themselves.

Growth of heterotrophic bacteria in the aquifer typically results from utilization of a variety of organic substrates. Therefore, in accounting for bacterial growth in transport models, it may be necessary to employ an aggregate measure of biodegradable organic material, such as chemical oxygen demand or dissolved organic carbon, instead of concentrations of an individual substrate. Rittmann et al. (1980) suggested this approach to account for changes in the nature of the growth-limiting carbon source as preferred substrates in a complex contaminant mixture are used up. However, the "aggregate approach" could benefit from the development of better methods for quantifying low levels of readily degraded dissolved organic carbon, since spatial changes in the nature of this substrate often occur in zones of contaminated groundwater (Harvey and Barber, submitted).

Before bacterial growth can be accurately accounted for in models involving bacterial transport in groundwater, more information on the growth characteristics of groundwater bacteria is needed. Accurate estimates of in situ growth rates for aquifer bacteria can be difficult to obtain owing to problems in obtaining uncon-

taminated samples, in the methods (frequency of dividing cells, tritiated thymidine uptake, closed-bottle incubations) themselves, and in using a number of inherent assumptions that may not be applicable to the aquifer (Harvey and George, 1987). Nevertheless, it appears that growth rates of bacteria in uncontaminated aquifer sediments are at least several orders of magnitude lower than those observed for surface sediments (Thorn and Ventullo, 1988). However, bacterial growth in oligotrophic aquifers can still affect the extent of bacterial transport over long periods of time, since groundwater flow can be quite slow (<1 m/day) and bacterial movement by intermittent transport can be even slower. Bacterial growth in organically contaminated groundwater can be quite rapid and approach that of at least some productive surface waters (Harvey and George, 1987). The contribution of growth to the overall down-gradient movement of indigenous bacteria in some contaminant plumes would appear to be significant (Harvey and Barber, submitted).

SURVIVAL

Since the initial attachment of bacteria sorbing to solid surfaces in freshwater systems is generally reversible (Marshall, 1985), the ultimate limitation of bacterial pathogen migration in groundwater may involve the duration of viability. For some bacterial pathogens, mortality in the groundwater environment can be quite significant. Survival of displaced bacteria in the aquifer can involve a number of factors, including the bacterial species involved; temperature; the presence of microbial competitors, predators, and parasites; groundwater chemistry; organic matter; and the degree of attachment. Generally, there is an initial period of relatively constant abundance. This is followed by a period in which temporal changes in abundance may be modeled by exponential decline. The initial period before exponential decline may vary from days in highly contaminated water to months under oligotrophic conditions (Matthess et al., 1988). For some displaced bacteria, die-off times in groundwater appear to be slow and a 1-order-of-magnitude reduction in abundance can involve several weeks or longer (Crane and Moore, 1984; Bitton et al., 1983). A review of the factors involved in survival of displaced bacterial populations in the subsurface is provided by Yates and Yates (1988).

Lysis of bacteria in the groundwater environment can result from unfavorable conditions, such as a lack of sufficient nutrients needed for cell maintenance or significant changes in salinity, or from parasites (e.g., bacteriophages or *Bdellovibrio* sp.). Bacteria that are indigenous to groundwater environments seem to be well adapted to low-nutrient conditions. Therefore, oligotrophic populations are likely to survive for long periods of time and even proliferate under conditions of severe nutrient limitation found in most contaminated groundwater. The extent of mortality due to parasitism in populations of indigenous groundwater bacteria is not known, but could be substantial in aquifers that are highly contaminated with organic material. The ability of genetically engineered bacteria to survive in the aquifer is also unclear and promises to be an important topic of future research.

Until recently, it was assumed that bacterivorous protozoa were likely to be absent in most aquifers, since eucaryotic microorganisms were typically not detected in direct microscopic examinations of aquifer core material (Ghiorse and Balkwill, 1983; Wilson et al., 1983; Harvey et al., 1984). Also, bacterial abundances

in groundwater appeared to be too low to support growth of a protozoan population. However, it now appears that this assumption is incorrect, at least for a number of aquifers. Sinclair and Ghiorse (1987) recently reported substantial numbers of protozoa in uncontaminated aquifer sediments that could be detected by using a most-probable-number technique, and Kinner et al. (1990), using both culturing and direct counting methods, have detected protozoa in contaminated aquifer sediments. Where present, protozoa are likely to constitute a significant sink for bacteria being transported in groundwater and, consequently, should be accounted for in transport models.

GROUNDWATER CONDITIONS

Groundwater conditions can have a significant effect upon the extent and reversibility of bacterial sorption onto solid surfaces in the aquifer. In general, bacterial attachment to stationary surfaces in an aquifer can be predicted to increase with increasing ionic strength. Increasing propensity for bacterial attachment with increasing ionic strength has been demonstrated for a variety of surfaces, e.g., muscovite (Scholl et al., in press), hydroxyapatite (Gordon and Millero, 1984), quartz sand (Sharma et al., 1985), and glass (Marshall et al., 1971). A decrease in ionic strength to the point where bacteria are repelled from the surfaces causes release of those cells which were reversibly sorbed (e.g., Marshall et al., 1971). Ionic composition is also important in bacterial attachment; calcium and magnesium ions appear to be particularly important and have been shown to be a requirement for irreversible sorption of a *Pseudomonas* sp. onto glass (Marshall et al., 1971).

The pH of the groundwater can have a significant effect upon bacterial attachment. In general, bacteria seem to sorb more readily to most surfaces under slightly acidic conditions, although exceptions to this rule have been reported. Scholl et al. (in press) reported greater sorption of a groundwater isolate onto quartz at pH 5 than at pH 7. In contrast, maximal adhesion of *P. aeruginosa* onto stainless steel occurred under near-neutral conditions. Abbott et al. (1983) reported that sorption of *S. mutans* onto clean and protein-coated glass surfaces was relatively insensitive to pH. Therefore, the role of pH upon bacterial sorption behavior may depend not only upon the chemistry of the groundwater, but the nature of the surfaces involved.

Dissolved organic matter can affect bacterial transport in groundwater by sorbing to abiotic surfaces. By sorbing to mineral surfaces in the aquifer, organics can substantively alter their sorptive characteristics. Sorbed proteins have generally been observed to have an inhibitory effect upon bacterial attachment (Pratt-Terpstra et al., 1987; Pringle and Fletcher, 1986). Decreased sorption of bacteria onto hydrophobic surfaces has been shown to occur with surfactants (Goldberg et al., 1990), and fulvic acid has been shown to decrease bacteriophage sorption onto soils (Bixby and O'Brien, 1979).

In addition to the effect upon bacteria growth rate, dissolved organic matter may also affect a bacterium's transport behavior in groundwater by causing alteration in cell size and hydrophobicity. Starvation is known to cause dwarfing in a number of bacteria, particularly in copiotrophic species (Kjelleberg, 1984; Humphrey et al., 1983). Because smaller bacteria exhibit greater Brownian motion, they would have a greater tendency to become immobilized in sandy aquifer sediments than do larger bacteria possessing similar surface characteristics (see Immobiliza-

tion, above). Furthermore, it has been observed that starvation conditions can also cause an increase in cell hydrophobicity and, consequently, an increased propensity for attachment, at least in some species (Kjelleberg, 1984; Dawson et al., 1981). However, the relationship between starvation conditions and bacterial tendency for attachment is not entirely clear. There is at least one report suggesting that starvation conditions can lead to a decrease in hydrophobicity (Wrangstadh et al., 1986). In recent investigations involving organically contaminated aquifers, it has been observed that bacterial partitioning to the solid phase increases with increasing distance from the source of contamination (Godsy et al., submitted; Harvey and George, 1986). This suggests that unattached bacteria that are further away from their major source of organic substrate may have a greater tendency for attachment. However, it is clear that the role of organic matter in bacterial transport in groundwater needs further study.

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