Chapter 15

Microbial Transport in the Subsurface

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Microbial transport through the subsurface is of interest not only from the standpoint of introducing microorganisms for beneficial purposes, but also in the removal of waterborne-disease-causing microorganisms from wastewater. Beneficial applications include introduction of bacteria that may enhance biodegradation of organic contaminants; remediation of metal-contaminated sites; improvement of soil structure; increased crop production; and biological control of plant pathogens. However, to obtain such benefits, the introduced microbe must be able to be transported through the soil and vadose zone. In the case of pathogens, the goal is to limit their transport and use the soil and vadose zone to improve the microbial quality of groundwater. Almost 40 million people in the United States depend on subsurface disposal of sewage wastes via septic tanks, a practice which can potentially contaminate drinking water wells or surface waters. In addition, soils are used to enhance the removal of enteric pathogens from wastewater or contaminated river water (riverbank filtration). Almost half of the waterborne outbreaks reported every year in the United States are due to contaminated groundwater and viral contamination of groundwater is estimated to occur in at least 27% of drinking water wells (Bradbury *et al.*, 2013). Understanding transport of pathogens allows us to determine safe distances from septic tank drainfields and wells used for drinking water.

In this chapter we examine the factors that determine the transport of microorganisms and nucleic acids through soil and the subsurface. These include microbial adhesion to and detachment from solid surfaces, which are governed by the physical–chemical properties of both the porous medium surfaces and the surrounding solution; the surface properties of the microbe; and the impact of water saturation and flow on movement. We also examine microbial survival and activity during transport, approaches to facilitating transport and mathematical models that describe and predict microbial transport.

15.1 FACTORS AFFECTING MICROBIAL TRANSPORT

Transport of microorganisms is governed by a variety of factors including: adhesion processes, filtration effects,

I.L. Pepper, C.P. Gerba, T.J. Gentry: Environmental Microbiology, Third edition. DOI: http://dx.doi.org/10.1016/B978-0-12-394626-3.00015-6

physiological state of the cells, porous medium characteristics, water flow rates, predation and intrinsic mobility of the cells.

Understanding each of these factors separately is the first step toward a holistic assessment of microbial or nucleic acid transport. With such an understanding it becomes possible to assess exposure that results from the transport of pathogens, or the feasibility of delivering beneficial microbes to a target site.

15.1.1 Microbial Filtration

Transport of microbes and other contaminants occurs within the pore spaces of a soil or subsurface material. One mechanism by which microbial transport is limited is physical straining or filtration of cells by small pores. Filtration of bacterial cells has been shown to be statistically correlated with bacterial size (Gannon *et al.*, 1991). Filtration becomes an important mechanism when the limiting dimension of the microbe is greater than 5% of the mean diameter of the soil particles (Herzig *et al.*, 1970). Thus, for a sandy soil with particle diameters of 0.05 to 2.0 mm, filtration will have a relatively small impact on the retention of bacteria of diameter approximately 0.3 to 2 μm. However, in a soil containing a significant portion of silt or clay particles (particle diameters range from 0.2 to 50 μm), filtration will be a major mechanism of bacterial cell removal. In contrast, studies of factors affecting the movement of particles less than 50 nm in diameter, such as viral particles, have shown that filtration has little effect on movement (Gerba *et al.*, 1991). An example of typical pore sizes found in a sandy loam is shown in Figure 4.8.

Cell shape, defined as the ratio of cell width to cell length, has also been shown to influence bacterial transport through a porous medium. Weiss *et al.* (1995) examined the transport of 14 strains of bacteria suspended in artificial groundwater through columns packed with quartz sand. A comparison of the distributions of size and shape of cells in the effluent with those in the influent suspensions revealed that cells in the effluent were smaller and rounder.

One consequence of microbial filtration is micropore exclusion, which states that bacteria may be excluded from the microporous domain of structured (e.g., aggregated, macroporous) porous media (see Figure 4.4). Most bacteria range from 0.3 to 2 μm in diameter, and micropores or pore throats located in the microporous domain of structured media can be much smaller in size. As a result, bacterial cells are physically excluded from the micropores (Figure 15.1). Thus, the location and rate of microbial activities can vary over a relatively small scale within a porous medium. In other words, microbial activity within the micropores that exclude microbes can be expected to be nonexistent, whereas an immediately adjacent site that is colonized may have an extremely high rate of activity.

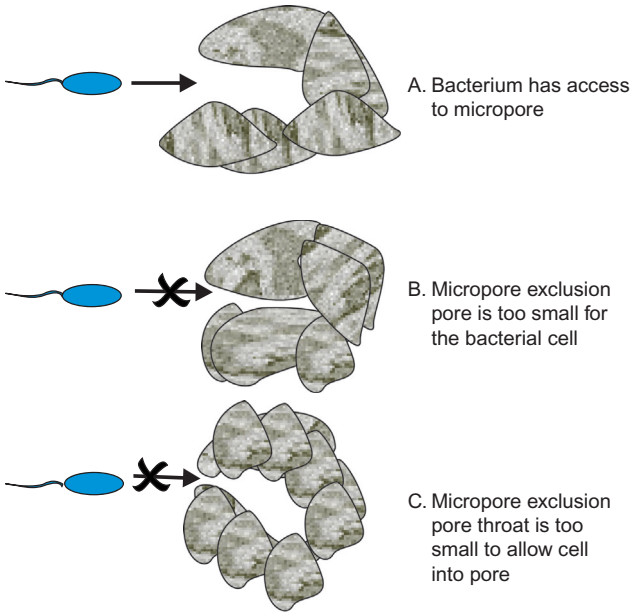

FIGURE 15.1 Exclusion of a bacterial cell from microporous domains in structured porous media.

Micropore exclusion of bacteria can have negative impacts. For example, when contaminants diffuse into micropores that exclude bacteria, they become unavailable for biodegradation. Because diffusion occurs slowly, this is a problem that normally worsens as the contact time between the contaminant and the porous medium increases. This process, known as contaminant aging, results in slower rates of contaminant degradation.

15.1.2 Physiological State

A variety of factors influence the size of a microbe and thus its transport potential. The physiological state of microbial cells is one such factor. When nutrients are not limiting, most cells produce exopolymers that form a capsule on the outer surface of the cell. Exopolymers increase the effective diameter of a cell; help the cell adhere to surfaces; and when released from the cell may modify solid surfaces to promote attachment. All of these, in addition to cell proliferation, may lead to pore clogging. Pore clogging can severely limit transport of bacteria and can lead to poor septic tank performance related to clogging of the drain field, clogging of nutrient injection wellheads used for *in situ* bioremediation and reduced rates of groundwater infiltration in recharge basins.

Under starvation conditions, bacteria tend to decrease in size (0.3 μm or even smaller), round up and shed their exopolymer capsule (Young, 2006). These so called ultramicrobacteria may have increased transport potential,

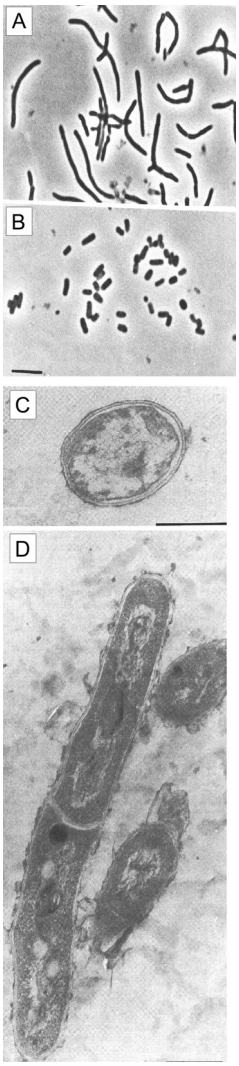

FIGURE 15.2 (A) Phase-contrast micrograph of an isolate grown on *p*-nitrophenol as the sole carbon source. (B) Phase-contrast micrograph of the *p*-nitrophenol degrader after 10 weeks of starvation in phosphate-buffered saline. (C) Electron micrograph of the starved *p*-nitrophenol-degrading isolate. (D) Electron micrograph of the starved *p*-nitrophenol-degrading isolate after resuscitation on *p*-nitrophenol. Modified with permission from Herman and Costerton (1993).

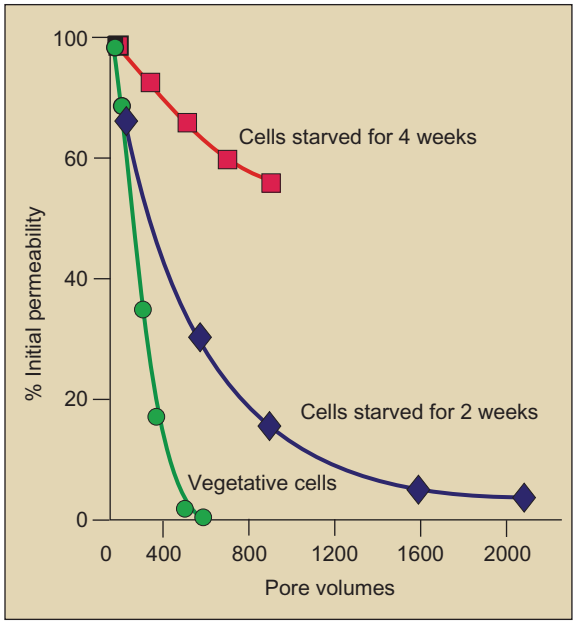

FIGURE 15.3 Permeability reduction profiles of fused glass bead cores injected with *K. pneumoniae* cells either in a vegetative state or starved for a period of time in phosphate-buffered saline. Modified with permission from MacLeod *et al*. (1988).

because both cell size and surface properties are changed. Herman and Costerton (1993) subjected a *p*-nitrophenol degrader isolated from a waste lagoon to starvation by placing it in phosphate-buffered saline for 10 weeks. The difference in cell size and morphology before and after starvation is clearly shown in Figure 15.2A and B. The starved cells were then resuscitated by adding *p*-nitrophenol to the medium (Figure 15.2C and D).

To demonstrate the difference in transport of ultramicrobacteria and normal cells, MacLeod *et al*. (1988) examined the movement of starved and normal *Klebsiella pneumoniae* cells through glass bead columns. The starved cells not only were smaller, but also demonstrated a reduction in capsule production as compared with the vegetative cells. As expected, the starved cells penetrated further into the column than did the vegetative cells. Figure 15.3 depicts the observed reduction in column permeability as *K. pneumoniae* cells (10^8 ml^{-1}) in different metabolic states were injected into the column. The ability of the cells to cause a reduction in permeability within the column was shown to be dependent on the length of starvation prior to inoculation and on the volume of cells at a given concentration injected through the core. Cell distribution within the columns also differed depending on nutrient status. Starved cells were evenly distributed throughout the column, whereas the vegetative cells were found in much higher numbers near the inlet end of the column (Figure 15.4). Upon nutrient stimulation, the starved cells were found to enter a state of growth accompanied by increased exopolymer production. This work demonstrates that inoculation with starved cells followed by nutrient stimulation has potential for increasing bioaugmentation efforts. For example, a starved cell that migrates farther through the terrestrial profile has an increased likelihood of reaching a target contaminated site. Once at the site, the contaminant can serve as a nutrient source, inducing the microbe to enter a metabolically active state.

15.1.3 Microbial Adhesion—The Influence of Cell Surface Properties

The adhesion of microbes to soil particles and vadose zone materials requires an initial interaction between the

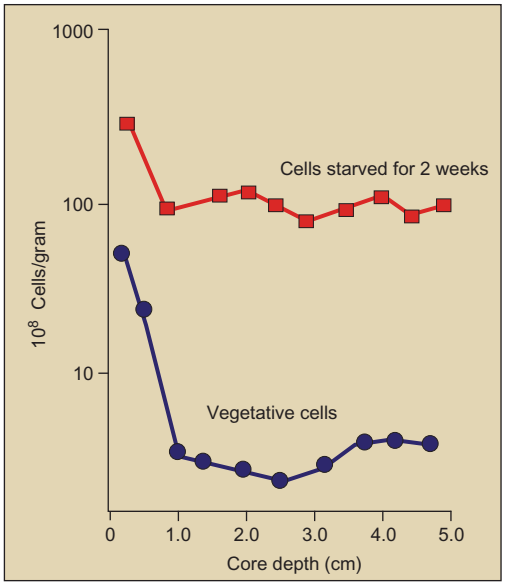

FIGURE 15.4 Differences in the DNA-derived cell distribution in cores injected with either a vegetative cell culture of *K. pneumoniae* or a cell suspension that was starved in phosphate-buffered saline for 2 weeks. Modified with permission from MacLeod *et al.* (1988).

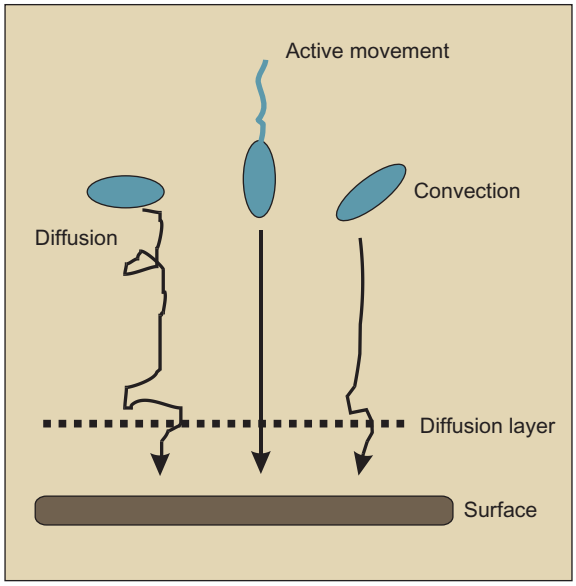

FIGURE 15.5 Different ways in which a cell can approach a solid surface. Modified with permission from van Loosdrecht *et al.* (1990).

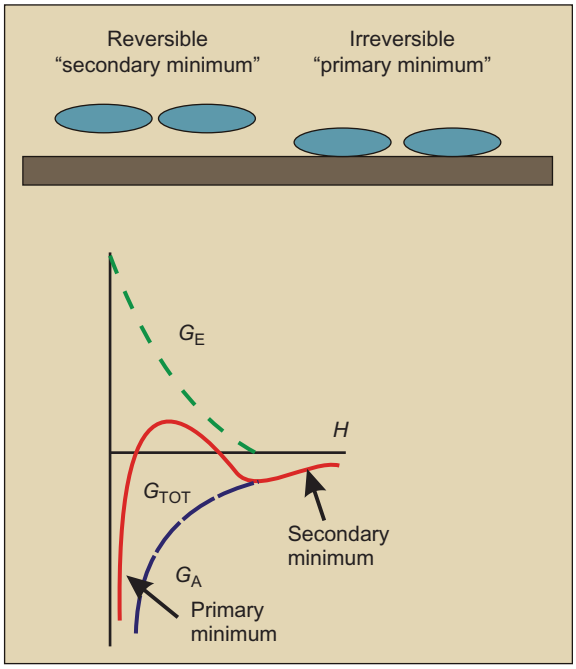

FIGURE 15.6 Gibbs energy of interaction between a sphere (in this case a bacterial cell) and a flat surface having the same charge, according to DLVO theory. G_E, electrostatic interaction; G_A, van der Waals interaction; G_{TOT}, total interaction; H, shortest separation distance between the two surfaces. Modified with permission from van Loosdrecht *et al.* (1990).

cell and a particle surface (van Loosdrecht *et al.*, 1990). Once a cell is in the vicinity of a surface, this initial interaction can occur in one of three ways: diffusion, active movement or advective transport. Diffusion is a result of Brownian motion and allows random interactions of cells with surfaces. The effective rate of diffusion is small; on the order of 40 μm/h. Motile cells may also come in contact with the surface through active movement in response to a chemotactic chemical gradient or in some cases simply by chance. Active movement also occurs on a micrometer scale. Finally, advective transport is due primarily to water movement and can move many orders of magnitude faster than diffusive or active transport (Figure 15.5).

Once contact between the cell and a particle surface has been made, adhesion can take place. Adhesion is a physicochemical process, and depending on the mechanisms involved, can be reversible or irreversible. Reversible adhesion, often thought of as initial adhesion, is controlled primarily by the balance of the following interactions: electrostatic interactions, hydrophobic interactions and van der Waals forces. These are explained in detail in the following sections.

In general, electrostatic interactions are repulsive because both cell and particle surfaces are negatively charged. In contrast, hydrophobic interactions and van der Waals forces tend to be attractive. Initial adhesion occurs when attractive forces overcome repulsive forces. Porous medium properties in conjunction with cell surface properties determine the relative importance of each of these interactions. Figure 15.6 illustrates how the interaction of electrostatic and van der Waals forces governs reversible adhesion at various distances between the cell and particle surfaces. As can be seen from this figure, when a cell surface is in actual contact with, or very close to, a particle surface, the attractive forces are very strong, creating a primary minimum. The forces governing the primary

FIGURE 15.7 Irreversible attachment is mediated by physical attachment of cells to a surface, which can occur via production of exopolymers or special cell surface structures such as fibrils. Modified with permission from van Loosdrecht *et al*. (1990).

minimum are short-range forces such as hydrogen bonding and ion pair formation. As the two surfaces are separated slightly, e.g., by several nanometers, repulsive forces grow quickly and prohibit adhesion. At slightly longer distances, another shallower minimum exists called the secondary minimum. It is the secondary minimum that is responsible for the initial reversible adhesion of microbes. As shown in Figure 15.6, the cell and particle surfaces are not in actual contact at the secondary minimum. As a result, cells can be removed from the surface easily, for example, by increasing the water flow velocity or by changing the chemistry of the porous medium solution, e.g., ionic strength.

After initial adhesion, cells can become irreversibly attached to a particle surface. Irreversible attachment is a time-dependent process that occurs as a result of the interaction of cell surface structures such as fimbriae with the solid surface or as a result of the production of exopolymers that cement the microbial cell to the surface (Figure 15.7). The role of reversible and irreversible attachment in biofilm formation is discussed further in Chapter 6.

15.1.3.1 Electrostatic Interactions

Electrostatic interactions occur between charged particles. In terms of microbial transport, repulsion is the dominant electrostatic interaction because both the porous medium including organic matter and mineral surfaces are generally negatively charged. As already mentioned, microbial cell surfaces are also generally negatively charged. The negative charge comes primarily from lipoteichoic acids on the surface of Gram-positive bacteria and lipopolysaccharides on the surface of Gram-negative bacteria. Viral protein coats are also predominantly negatively charged. The overall charge on a microbe can be measured by electrostatic interaction chromatography or by electrophoretic mobility. Surface charge varies between types and species of microbes and can be affected by the pH of the matrix solution.

15.1.3.2 van der Waals Forces

Interactions between neutral molecules generally result from van der Waals forces. Van der Waals forces occur because while neutral molecules have no net charge or

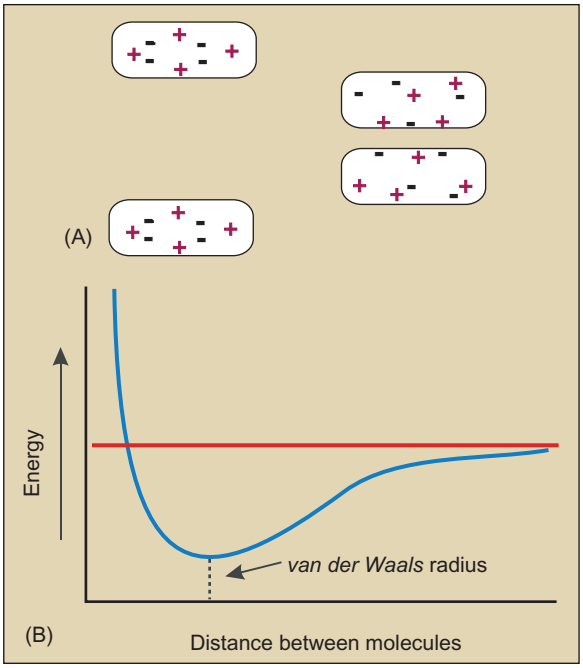

FIGURE 15.8 (A) For a neutral molecule the charge distribution in a molecule can vary to produce a net electrostatic attraction, allowing the molecules to approach very closely. This is a very weak attraction called the van der Waals force. Van der Waals forces can become strong if they are numerous enough. (B) As two molecules approach each other, the van der Waals attractive force increases to a maximum, then decreases and becomes repulsive.

permanent dipole moment, they do have a dynamic distribution of charge. As two molecules approach, this charge distribution can become favorable for interaction between the two molecules (Figure 15.8A). What actually occurs as two molecules approach is that the van der Waals attractive forces increase to a maximum, then decrease and become repulsive (Figure 15.8B). The van der Waals radius is defined as one half the distance between two equivalent atoms at the point of the energy minimum (where attractive forces are at a maximum). Van der Waals radii range from one to several angstroms in length, so these forces are effective only over short distances. Although individual van der Waals interactions are weak, the total attraction between two particles is equal to the sum of all attractive forces between every atom of one particle and every atom of the other particle. Thus, total van der Waals interactions can be quite strong.

15.1.3.3 Hydrophobic Interactions

Hydrophobic interactions refer to the tendency of nonpolar groups to associate in an aqueous environment. Hydrophobicity can be measured in a variety of ways, including contact angle determination, which is done by examining the shape of a drop of water that is placed on a layer of bacterial cells (Figure 15.9). Other methods

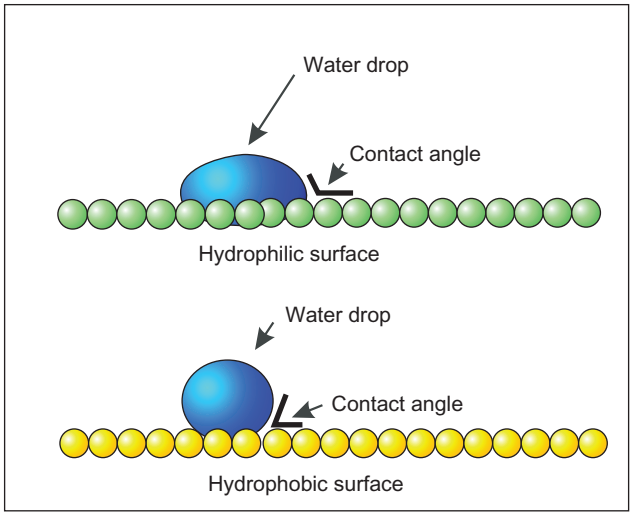

FIGURE 15.9 Water, which is a polar material, spreads out on a hydrophilic or polar surface but forms a round bead on a hydrophobic or nonpolar surface. The angle that describes the interaction of a water droplet with a surface is called the contact angle.

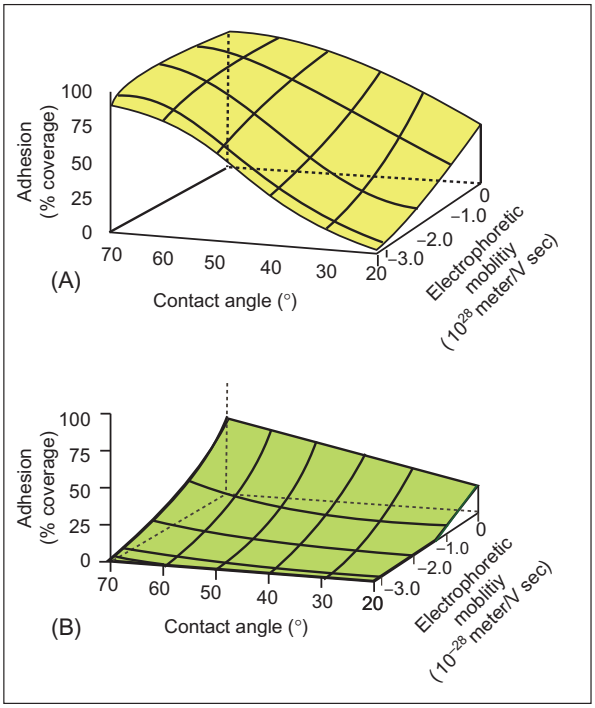

FIGURE 15.10 Relationship between bacterial adhesion to (A) sulfated polystyrene (hydrophobic) and (B) glass (hydrophilic) and bacterial surface characteristics as determined by contact angle measurement and electrophoretic mobility. Modified with permission from van Loosdrecht *et al.* (1990).

commonly used to assess hydrophobicity include phase partitioning (BATH test) and hydrophobic interaction chromatography. The BATH (bacterial adherence to hydrocarbon) test is a relatively simple test that measures the partitioning of microbial cells between a water phase and an organic phase.

As a result of hydrophobic forces, cells tend to partition from the aqueous phase and accumulate at the solid–water interface, resulting in decreased transport potential. Van Loosdrecht *et al.* (1990) examined adhesion of a variety of bacteria with different cell surface properties to two surfaces, one hydrophobic (polystyrene) and one hydrophilic (glass). Both cell surface charge and cell surface hydrophobicity were considered in this series of experiments. As shown in Figure 15.10A, cell surface hydrophobicity was the dominant force in determining adhesion to the hydrophobic polystyrene surface. In summary, two trends in cell adhesion can be inferred from this study: (1) adhesion typically decreases with decreasing hydrophobicity of either the solid surface or the cell surface; and (2) adhesion generally increases with decreasing cell surface charge. Knowledge of the combined effects of hydrophobicity and cell surface charge can be used to predict initial microbial adhesion of a particular microbe.

Sanin *et al.* (2003) examined the effect of starvation on the adhesive properties of three cyanuric acid-degrading bacteria. Microorganisms were independently starved for carbon and nitrogen. Surface hydrophobicities of all three strains remained fairly constant during carbon starvation, but decreased significantly when starved for nitrogen with a concomitant decline in attachment. Understanding starvation responses could have significant impacts on bacterial transport.

Similarly, hydrophobic effects and electrostatic repulsion govern the sorption of viruses depending on viral and subsurface characteristics. Zhuang and Jin (2003) examined the influence of natural organic matter on the retention and transport of two bacteriophages (MS-2 and ΦX174) through sand. MS-2 is an icosahedral phage of diameter 24 to 26 nm and a pI of 3.9, while ΦX174 is 25 to 27 nm in diameter, has a pI of 6.6 and is less hydrophobic than MS-2. In the sand alone the retention was high for MS-2 (99.2%) and much lower for ΦX174 (30%). When the sand was coated with organic matter, the retention of MS-2 decreased to 29% while retention of ΦX174 remained essentially the same (23%). In this case, coating the sand surface with organic matter blocked MS-2 interaction with charged sorption sites. Torkzaban *et al.* (2006) studied the transport of these two viruses under differing water saturation conditions. Their results show that both viruses are retained more strongly under unsaturated conditions and that retention is further increased at lower pH and higher ionic strength.

In natural systems, dissolved organic matter, most often present in the form of polymers, can also influence microbial adhesion by adsorbing to the microbe and/or solid surfaces (Dexter, 1979). The polymeric coating may affect adhesion by changing the electrostatic, the van der Waals and/or the hydrophobic interactions between the microbe and the solid surface. When polymers adsorb and

coat both bacteria and the solid surface completely, adhesion is reduced because of an extra repulsive interaction due to steric hindrance (Fletcher, 1976). However, if only one of the surfaces is covered with polymers, or if both surfaces are partly covered with polymers, one polymer molecule may attach to both surfaces, thus forming a "bridge" between the two surfaces. This reduces the Gibbs free energy of adhesion (Figure 15.6) and results in a strong bond (Dexter, 1979).

15.1.4 Impact of pH on Microbial Transport

The pH of the matrix solution within a porous medium does not seem to have a large effect on bacterial transport. However, viral transport can vary greatly depending on the pH of the porous medium solution. The difference in impact of pH on the transport of bacteria and viruses can be attributed to a variety of factors. Remember that the primary interaction limiting bacterial transport is filtration, not adsorption as it is for viruses. In addition, in contrast to viruses, bacteria have very chemically diverse surfaces, and thus a change in pH would not be expected to alter the net surface charge to the same extent as for the more homogeneous viral surface. Finally, the overall charges on the surfaces of bacteria and viruses differ and will be affected differently by pH changes. This can be expressed in terms of the isoelectric point (pI). The pI is the pH at which the net charge on a particle of interest is zero. For bacteria, the pI usually ranges from 2.5 to 3.5, so the majority of cells are negatively charged at neutral pH. At pH values more acidic than the isoelectric point, a microbe becomes positively charged. This will reduce its transport potential because of increased sorption. This will not happen often with bacterial cells in environmental matrices, given their low pI values; the pH would have to decrease to 2.5 or lower to alter cell surface charge significantly. However, viruses display a wider range of isoelectric points (see Table 15.1) making their net surface charge much more dependent on changes in pH.

A study evaluated the influence of viral isoelectric point and size on virus adsorption and transport, and it was concluded that the isoelectric point was an important factor controlling viral adsorption and transport. However, when virus particles were greater than 60 nm in diameter, viral size became the overriding factor (Dowd *et al.*, 1998).

TABLE 15.1 Isoelectric Points of Selected Viruses

Virus	pI
Reovirus 3 (Dearing)	3.9
Rhinovirus 2	6.4
Polio 1 (Bruenders)	7.4, 3.8
Polio 1 (Mahoney)	8.2
Polio 1 (Chat)	7.5, 4.5
Polio 1 (Brunhilde)	4.5, 7.0
Polio 1 (LSc)	6.6
Polio 2 (Sabin T2)	6.5, 4.5
Echo 1 (V239)	5.3
Echo 1 (V248)	5.0
Echo 1 (V212)	6.4
Echo 1 (R115)	6.2
Echo 1 (4CH-1)	5.5
Echo 1 (Farouk)	5.1
Coxsackie A21	6.1, 4.8
Hepatitis A	2.8
Parvovirus AA4. X14	2.6
Noro	6.0, 5.0
Smallpox (Harvey)	5.9, 3.3
Influenza A (PRS)	5.3
Encephalomyocarditis (mengo M)	8.4, 4.4
T2 bacteriophage	4.2
T4 bacteriophage	4–5
MS-2 bacteriophage	3.9
PRD-1	3–4
Qβ	5.3
ΦX174	6.6
PM2	7.3

Data compiled from Gerba (1984); Ackermann and Michael (1987); Michen and Graule (2010).

15.1.5 Impact of Ionic Strength on Transport

It has already been established that frequently the net charge on both particle and cell surfaces is negative, which causes electrostatic repulsion between these two surfaces. The concentration of anions and cations in solution is referred to as the ionic strength of the medium. Soil solution ionic strength influences transport primarily through two mechanisms—by altering the size of the diffuse double layer and by influencing soil structure (see Section 4.2).

The negative charges present on mineral particles electrostatically attract cations in the solution. Thus, in the immediate vicinity of the negatively charged surface, there is an excess of cations and a deficit of anions. Further from the particle surface, the cation concentration

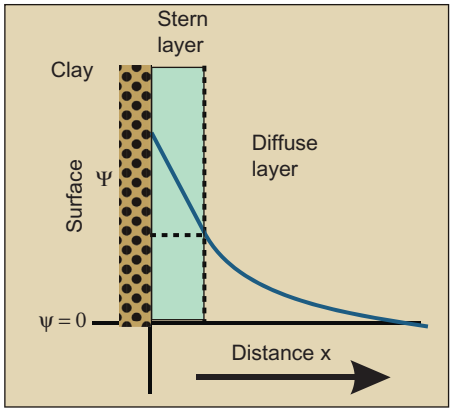

FIGURE 15.11 Illustration of the diffuse double layer. The diffuse double layer is a combination of the charge layer on the surface and the charge in solution. The first monolayer of ions in contact with the surface, the Stern layer, is held tightly to the surface. The second layer, the diffuse layer, responds to the remaining charge on the surface but is held more loosely than the Stern layer ions. This figure shows the energy (ψ) required to bring an ion from the bulk solution to the surface as a function of distance from the surface. Immediately next to the surface the decrease in potential is linearly related to increasing distance from the surface (the Stern layer). As the distance from the surface is increased further, the potential decreases exponentially. Modified with permission from Tan (1993), p. 198, courtesy Marcel Dekker, Inc.

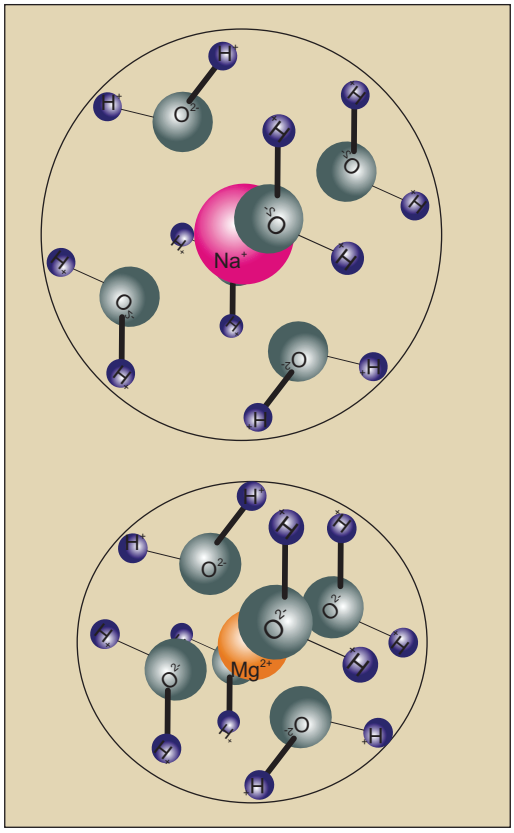

FIGURE 15.12 The radius of hydration of a cation in soil depends on the charge density of the atom. In the example shown, magnesium has a higher charge density than sodium and thus attracts water molecules more strongly resulting in a larger radius of hydration.

decreases until it reaches that of the bulk solution. Thus, the porous medium solution is often thought of as a double layer as depicted in Figure 15.11. The impact of ion concentration on this diffuse double layer will ultimately play a critical role in the transport of microbes. As the ionic strength of the bulk soil solution increases, the difference in cation concentration between the cation-rich layer and the bulk layer is reduced, and thus there is a tendency for cations to diffuse away from particle or cell surfaces. This causes a general compression of the diffuse double layer because the interacting cations and anions neutralize one another. The result is a decreased electric potential, which increases the likelihood of attachment of cells to the surfaces.

In addition to the overall ionic strength, the type of ion contributing to ionic strength is important. This is because the hydration radius of a cation in the soil solution affects the extent of the diffuse double layer and thus the soil structure. The radius of hydration of a particular cation is a function of surface charge density and refers to the radius of the cation and its complexed water molecules (Figure 15.12). In general, monovalent cations have lower surface charge densities and thus larger radii of hydration than divalent cations. Thus, in the presence of high concentrations of monovalent cations such as Na^+, clays tend to be dispersed. Dispersed clays create puddled soils, which are sticky when wet and hard when dry. As a dispersed soil dries, compaction may occur, which reduces pore spaces, inhibiting soil aeration and reducing the capacity for water flow. This adversely affects the transport potential of microbes. On the other hand, the presence of divalent cations such as Ca^{2+} and Mg^{2+}, with smaller radii of hydration, leads to flocculated soils, which have increased pore space and thus favor transport.

The impact of ionic strength on microbial transport is demonstrated by the following examples. Bai *et al.* (1997) found that fewer cells were recovered in a column study when 2 mM NaCl was used as the percolating solution as compared with the use of artificial groundwater with a lower ionic strength. This observation can be explained in terms of cation concentration and associated electrostatic interactions. Viruses either do not readily adsorb or are released from soil particle surfaces suspended in low-ionic-strength solutions (Landry *et al.*, 1979; Goyal and Gerba, 1979).

How can ionic strength vary in a porous medium? One example is following a rainfall event. When it rains, the added water will generally lower the ionic strength of the soil solution. In addition, a rainfall event results in increased rates of water flow. Both decreased ionic strength and increased flow rate will promote microbial transport. This can result in peaks of microbial contamination in groundwater from the release of bacteria and viruses previously adsorbed.

15.1.6 Cellular Appendages

Bacteria may have a variety of appendages such as pili, flagella or fimbriae. Flagella are responsible for bacterial motility, while fimbriae and pili are involved in attachment. These appendages may all play a role in microbial transport through the terrestrial profile. The influence of bacterial motility on overall transport is generally minimal because extensive continuous water films would be needed to support microbial movement and because motility typically occurs on a micrometer scale. However, in nonflowing systems where no advective transport occurs, motility can increase transport potential over a very small scale. For example, Reynolds *et al.* (1989) evaluated bacteria movement through nutrient-saturated sand-packed cores under static conditions. In this study, transport through the column was four times faster with motile strains of *Escherichia coli* than with nonmotile mutants defective only in flagellar synthesis. Thus, the presence of cellular appendages involved in motility (flagella) can lead to measurable increases in microbial transport under certain circumstances.

Movement caused by flagella is usually a result of chemotaxis. Chemotaxis is the movement of microbes toward beneficial substances or away from inhibitory substances. This type of movement is dependent on the presence of a chemical gradient within continuous films of soil solution. The ability on move in this manner may confer survival advantages on the microbe. For example, chemotaxis is thought to play a role in the movement toward and subsequent infection of legume roots by *Rhizobium*, a nitrogen-fixing bacterium.

In contrast to flagella, the presence of cellular appendages involved in attachment (pili and fimbriae) can reduce microbial transport potential. It is thought that cellular appendages can penetrate the electrostatic barrier thereby facilitating attachment at greater distances from the surface. Functional groups (hydrophobic groups or positive charge sites) on the appendages may facilitate interaction with surfaces leading to increased adsorption. Thus, the presence of appendages may actually decrease microbial transport in some cases.

15.1.7 Hydrogeological Factors

Soil texture and structure, porosity, water content and potential, and water movement through the profile are key hydrogeological factors influencing microbial transport (see Chapter 4). The specific soil and vadose zone layers within a site serve as protective or attenuating zones with regard to contamination of groundwater by microbes (or chemical pollutants) via a variety of mechanisms, including filtration and adhesion (e.g., hydrophobic interactions with the air–water interface). In addition to the site-specific makeup of the porous medium, the distance between the soil surface and the vadose–groundwater interface is often a critical factor for determining pollution potential: the greater the distance, the less likely it is that groundwater contamination will occur.

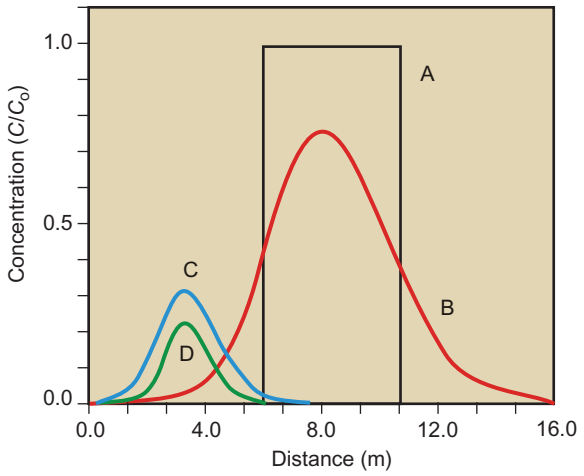

FIGURE 15.13 Effects of various processes on contaminant transport. This figure shows the theoretical distribution of a short pulse of microbes added to a saturated soil column 16 m long. The ordinate represents the relative concentration where C = the microbial concentration in the solution phase at a given point in the column, and C_0 = the influent concentration of microbes. The abscissa represents distance along the column from 0 to 16 m. Pulse A represents microbes that have moved through the column influenced only by advection. Pulse B represents the combined influence of advection and dispersion on microbial distribution. Note that no microbes are lost from the solution phase in either pulse A or B. Pulse C represents addition of adsorption to advective and dispersive processes. In this case microbes are lost to the solid phase and the resulting pulse is smaller and retarded. Finally, pulse D represents the addition of decay to the other three processes, which further removes microbes from the solution phase. Modified with permission from Yates and Yates (1991).

Terms used to describe the flow of water and the transport of dissolved and particulate substances are commonly applied to describe the transport of microbes (Figure 15.13). Advection, the movement of the bulk pore fluid and its dissolved and suspended constituents, is primarily responsible for microbial transport. Dispersion is the combined result of mechanical mixing and molecular diffusion. Mechanical mixing results from the path tortuosity and velocity differences within the pore that depend on pore size and location of the microbe as depicted in Figure 15.14. Spreading due to molecular diffusion, the random movement of very small particles suspended in a fluid, results from the presence of a concentration gradient. It is generally considered negligible with regard to bacterial transport, but can be significant in the transport of smaller particles ($<1\ \mu m$) such as viruses. Finally, adsorption represents the removal of microbes from the bulk solution by reversible and irreversible adhesion.

Because microbes are transported along with the soil solution primarily through advection, the flow rate and degree of saturation of the soil can play significant roles

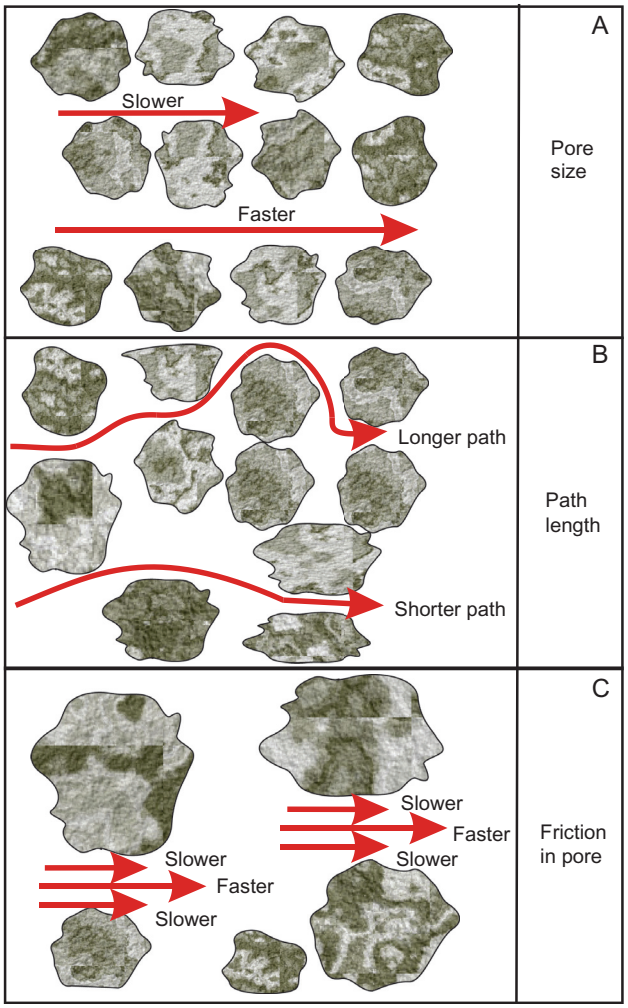

FIGURE 15.14 Factors causing mechanical dispersion at the scale of individual pores. (A) Microbes are transported through small pores more slowly than through large pores; (B) depending on pore sizes and shapes, path lengths can vary considerably; (C) flow rates are slower near the edges of the pore than in the middle. Modified with permission from Fetter (1993), © MacMillan Magazines Limited.

in determining transport potential. In general, higher water content and greater flow velocities result in increased transport. For example, virus penetration through columns packed with loamy sand soil under unsaturated flow was 40 cm, compared with a penetration depth of 160 cm during saturated flow (Lance and Gerba, 1984). The reduced penetration is because under unsaturated conditions water is present as a discontinuous film on soil surfaces and, in addition, under unsaturated conditions there is increased interaction of the viruses with soil surfaces thereby increasing the potential for adsorption to soil and the air–water interface.

The flow rate of water through a saturated soil can be calculated using Darcy's law:

$$Q = K\frac{\Delta H\, At}{z} \qquad \text{(Eq. 15.1)}$$

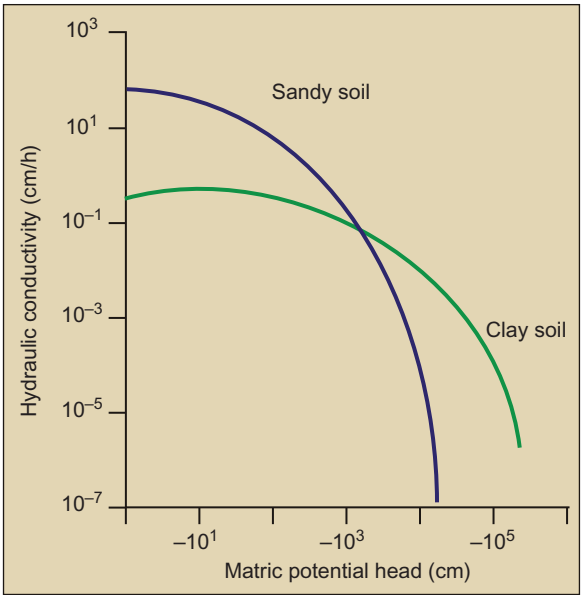

FIGURE 15.15 The hydraulic conductivity of a soil is dependent on the texture and the moisture content of the soil. This figure compares the hydraulic conductivity of a sand and a clay soil as a function of moisture content. Modified with permission from Soil Physics, Jury *et al.* (1991), © John Wiley & Sons, Inc.

where:

Q is the volume of water moving through the column (m^3)
A is the cross-sectional area of the column (m^2)
t is time (days)
ΔH is the hydraulic head difference between inlet and outlet (m)
K is the hydraulic conductivity constant (m/day), and
z is the length of the column (m).

Hydraulic conductivity can be defined as the ease with which water moves through soil. A hydraulic conductivity greater than 4 cm/hour is considered large, whereas a value less than 0.4 is low. For saturated soils, a coarse-textured material such as sand always has a higher conductivity than a clay soil because it contains larger pores, which hold water less tightly and allow for easier flow.

Darcy's law may also be applied to unsaturated soils; however, in this case, the hydraulic conductivity in Eq. 15.1 is no longer constant. This is because the unsaturated hydraulic conductivity of a soil, K(h), is a nonlinear function of the matric potential, which in turn is related to the water content. Figure 15.15 shows typical K(h) values for a coarse-textured soil (sand) and a fine-textured soil (clay). At saturation (low matric potential), the pores are filled with water. Thus, the coarse-textured soil has a higher conductivity because it contains greater numbers of large pores, where the water is held less tightly. When

water is no longer added to the system, these large pores drain first and fairly rapidly, resulting in a pronounced decrease in hydraulic conductivity. As water continues to drain, a point will be reached when the sand and clay soils have similar hydraulic conductivity ($K(\mathrm{h}) = -5 \times 10^3$) because the smaller pores in the clay retain water more strongly. From this point on (at higher matric potentials) the clay soil will have higher $K(\mathrm{h})$ because water remains in the smaller pores. As a result, considerably more water is present in clay soils at high matric potential and there is an increased probability of a continuous water film remaining to facilitate microbial transport.

Darcy's law was developed for steady flow, where Q is constant. However, in the subsurface, conditions are dynamic and thus Q is constant only over short periods of time. To account for changing flow, the flow equation is written in differential form to yield the Darcy flux:

$$q = -K\frac{\partial H}{\partial z} \qquad \text{(Eq. 15.2)}$$

where $q = Q/At$ (m/day) and $\partial H/\partial z$ is the hydraulic gradient (m/m). By definition, q is the volume of water moving through a 1-m^2 face area per unit time. However, because water moves only through pore space and not through solids, the actual velocity of water moving through soil is considerably higher than q, the Darcy velocity. The pore water velocity is proportional to pore size; however, the average pore water velocity is generally defined as:

$$v = \frac{q}{\theta_w} \qquad \text{(Eq. 15.3)}$$

where:

v is the pore water velocity
q is the flow rate per unit area determined for Darcy's law, and
θ_w is the water-filled porosity.

In saturated soils, θ_w is equal to the total porosity, so the pore water velocity is approximated well by the Darcy velocity. However, in unsaturated soils there is a marked increase in pore water velocity over Darcy velocity.

Another factor to consider is hydrologic heterogeneity arising as a function of soil structure. Variations in structure such as cracks, fissures and channels can greatly affect flow rates by creating preferred flow paths, with increased flow velocities. This phenomenon is termed preferential flow. Such structural inconsistencies can greatly increase microbial transport.

15.1.8 Persistence and Activity of Introduced Microbes

Persistence and activity are key transport considerations because, ignoring for the moment the possibility of genetic exchange, it is the movement of live/intact microbes that is of concern to environmental and health considerations. Microbes introduced into a soil environment typically decline rapidly in number through cell death or viral inactivation and the average activity per cell of the surviving microbes is often reduced. Microbial adhesion to particle surfaces tends to provide some degree of protection from adverse factors. Access to and utilization of solid phase-associated nutrients (i.e., electrostatically held cations (NH_4^+, Na^+, K^+, Mg^{2+}) and nutrients released from sorbed organic material) may account, in part, for the increased survival of adsorbed microorganisms. Moisture content plays a multifaceted role in microbial survival. Too little water leads to desiccation, while moisture contents above a certain optimal level may lead to decreased microbial numbers, potentially due to oxygen depletion in saturated pores. In addition, microbial predators such as protozoa tend to be more active at higher soil moisture contents. Microbial sorption to particles and within small pores is thought to provide protection from protozoa, which are typically larger and thus may be excluded from certain pores or bacterial sorption sites. Indeed, in the presence of protozoa, higher percentages of particle-associated bacteria have been observed (Postma *et al.*, 1990).

Immobilization of bacterial cells in a carrier material such as polyurethane or alginate has been investigated as an improved inoculation technique leading to increased survival and degradation capabilities of the inoculum (van Elsas *et al.*, 1992; Hu *et al.*, 1994). For introduction into the soil environment, the carrier material confers on the inoculum some protection against harmful physicochemical and biological factors.

Although the soil environment is often detrimental to introduced organisms and thus their transport, certain biotic components of the terrestrial profile can increase movement of added microbes. For instance, channels formed by earthworms have been shown to increase transport by creating regions of preferential flow (Thorpe *et al.*, 1996). Similarly, bacterial transport has been shown to be stimulated by root growth (Hekman *et al.*, 1995). Water movement through channels formed by root growth and/or in films along the root surfaces contributes to the increased bacterial dispersion.

15.2 FACTORS AFFECTING TRANSPORT OF DNA

The survival and transport potential of introduced microbes are both issues of concern. However, it is important to realize that a dead or inactivated microbe usually breaks open, releasing its genetic material to the environment. Upon lysis, there is potential for the genetic material to be transported or sorbed to colloids where it can remain protected from degradation (Ogram *et al.*, 1988). Free or

desorbed nucleic acids may be reincorporated into other microbes via transformation. This can result in the expression of genes encoded by these nucleic acids or in the potential transport within the intact recipient cell.

Sorption of free DNA depends on several factors, including the mineralogy of the matrix material, ionic strength and pH of the soil solution, and length of the DNA polymer. DNA has a pK_a of approximately 5. At pH values equal to the pK_a the DNA is neutral, and at lower pH values it is positively charged. In either of these states the DNA is subject to adsorption to colloids and to intercalation into certain minerals, such as montmorillonite. This is enhanced by the fact that the pH of the microenvironment surrounding a soil particle may be as much as two or three units below the pH of the bulk solution. However, at higher pH values the DNA is negatively charged and is repelled from the negatively charged surfaces. Ogram *et al*. (1988) determined that the surface pH of some natural soils and sediments may be near the pK_a of DNA, and thus significant amounts of DNA could remain nonsorbed and be present in the aqueous phase. The same group also found that higher molecular weight DNA was sorbed more rapidly and to a greater extent than lower molecular weight DNA. Depending on the specific conditions and soil sample, DNA sorption can be highly variable (Ogram *et al*., 1988).

15.3 NOVEL APPROACHES TO FACILITATE MICROBIAL TRANSPORT

For a number of applications including bioremediation and oil recovery, delivery of viable microbes is critical to the success of the application. As a result, strategies have been developed to attempt to optimize the "natural conditions" that favor transport. Several novel approaches designed to facilitate microbial transport through the terrestrial profile are being investigated. Formation of ultramicrobacteria and biosurfactants and gene transfer are among those that show potential.

15.3.1 Ultramicrobacteria

Marine bacteria react to starvation by dividing and shrinking to one-third their normal size. Such bacteria are referred to as ultramicrobacteria (UMB). Similarly, isolates obtained from soil can be placed in a nutrient-deprived medium such as phosphate-buffered saline and form UMB. After several weeks of starvation, a distinct morphological change takes place in these cells. As shown in Figure 15.2, the cells shrink to approximately 0.3 μm in size and become rounder. They also lose their capsule layer, thereby becoming less sticky. These bacteria can then be resuscitated by providing a carbon source. They recover both morphologically and physiologically. Such UMB have been shown to penetrate farther into sandstone cores than their vegetative counterparts. For example, Ross *et al*. (2001) demonstrated in a bench-scale experiment that the permeability of a limestone fracture was reduced by 99% in 22 days following inoculation with an indigenous groundwater community and then flushing with a molasses solution at a carbon loading rate of 1.08×10^{-2} mg carbon per ml/per minute.

Interest in UMB first centered on their potential for use in oil recovery. The ability to control flow through the subsurface has potential use for containment or biotreatment of contaminated sites or to improve oil recovery. For oil recovery, after oil is initially flushed from a geologic formation, removal of further oil residuals becomes more difficult because flow paths have become established. At this point, ultramicrobacteria injected into the formation move relatively easily through established flow paths. They can then be resuscitated by nutrient injection, grow and divide, thereby plugging pores and forcing flow through other regions of the geologic formation. For example, Bossolan *et al*. (2005) examined the response of a *Klebsiella pneumoniae* isolated from an oil well to starvation and resuscitation and determined that this strain was a viable option for transport and growth of microorganisms inside porous media, with possible applications to microbially enhanced oil recovery (MEOR).

15.3.2 Surfactants

Another approach involves the use of a chemical additive, specifically a surfactant, to increase the transport potential of microbes. Bai *et al*. (1997) investigated the influence of an anionic monorhamnolipid biosurfactant on the transport of three *Pseudomonas* strains with various hydrophobicities through soil under saturated conditions. Columns packed with sterile sand were saturated with sterile artificial groundwater, and then three pore volumes of ^{3}H-labeled bacterial suspensions with various rhamnolipid (RL) concentrations were pumped through the column. Four additional pore volumes of rhamnolipid solution were then applied. Rhamnolipid enhanced the transport of all cell types tested but to varying degrees. Recovery of the most hydrophilic strain increased from 22.5 to 56.3%, recovery of the intermediate strain increased from 36.8 to 49.4% and recovery of the most hydrophobic strain increased from 17.7 to 40.5%. Figure 15.16 shows the breakthrough curves for the most hydrophilic strain at different rhamnolipid concentrations.

In this experiment it was found that the surface charge density of the bacteria did not change in the presence of the rhamnolipid, but the negative surface charge density of the porous medium increased. Thus, reduced bacterial sorption may be due to one of several factors including

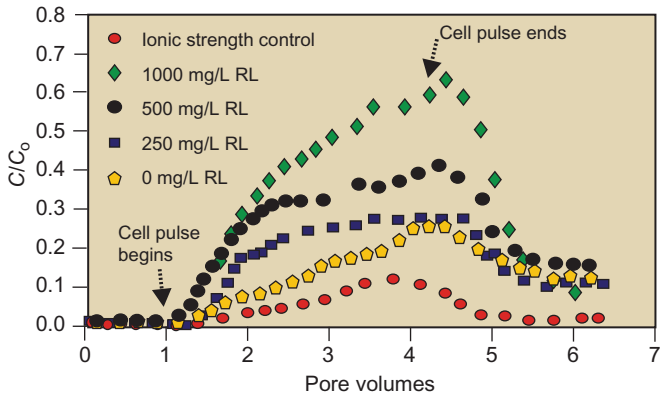

FIGURE 15.16 The effect of a rhamnolipid (RL) biosurfactant on transport of a *Pseudomonas* sp. where C_0 is the CFU/ml in the influent solution, and C is the CFU/ml in the effluent solution. A pore volume is the amount of liquid it takes to fill all of the soil pores in the column. Modified with permission from Bai *et al.* (1997).

an increase in surface charge density caused by rhamnolipid adsorption; solubilization of extracellular polymeric glue; or reduced availability of sorption sites on porous surfaces. The advection–dispersion transport model used to interpret these results suggests that the predominant effect of rhamnolipid was to prevent irreversible adsorption of cells.

Streger *et al.* (2002) investigated the use of surfactants to enhance transport of *Hydrogenophaga flava* ENV735, a bacterium capable of degrading the gasoline oxygenate methyl tert-butyl ether (MTBE), a widespread groundwater contaminant. While several tested surfactants were toxic to this bacterium, one nonionic surfactant, Tween 20, was not toxic and enhanced transport in sand columns. Findings such as this may facilitate delivery of microbial inocula to contaminated sites with no indigenous degraders.

15.3.3 Gene Transfer

Bacteria are intentionally introduced into soil systems in order to manipulate components and/or processes that occur within the soil profile. Typically, enhancement of microbial activities, e.g., organic degradation or metal resistance/immobilization, is the driving force behind their introduction. It may be possible to circumvent some of the factors that limit microbial transport and thus the success of bioremediation in soil via genetic exchange. Gene transfer between organisms may occur through conjugation, transduction or transformation. Transfer events such as these may make it possible to distribute genetic information more readily through the soil. Gene transfer events between an introduced organism and indigenous soil recipients has been shown to occur and in some cases this results in increased degradation of a contaminant (DiGiovanni *et al.*, 1996) (see Case Study 3.1).

Studies have addressed the transport potential of transconjugants, which arise when indigenous bacteria receive a plasmid from an introduced donor through conjugation. In a column study involving a donor inoculum at the column surface, Daane *et al.* (1996) found that transconjugants were limited to the top 5 cm of the column. However, when earthworms were also introduced into the column, not only did the depth of transport of donor and transconjugants increase, depending on the burrowing behavior of the earthworm species, but also the number of transconjugants found increased by approximately two orders of magnitude.

In a separate study, Lovins *et al.* (1993) examined the transport of a genetically engineered *Pseudomonas aeruginosa* strain that contained plasmid pR68.45 and the indigenous recipients of this plasmid in nonsterile, undisturbed soil columns. The surface of the column was inoculated and unsaturated flow conditions were maintained. Transconjugants survived longer in the columns and were found to have moved farther down the column than the donor. The greater survival rate of transconjugants would be expected because these organisms have previously adapted to the particular conditions of the soil. The increased transport could be the result of plasmid transfer to smaller, more mobile bacteria.

In addition, consecutive gene transfer events between indigenous microbes have been suggested as a mode of transfer (DiGiovanni *et al.*, 1996). This would be especially feasible when microbes are present in high densities, such as stationary microbes growing within a biofilm on soil surfaces or in the rhizosphere.

15.4 MICROBIAL TRANSPORT STUDIES

15.4.1 Column Studies

In situ transport experiments involving microorganisms are difficult to conduct. There are many obstacles associated with sampling and manipulating a complex environmental system that make the determination of critical transport factors difficult. As a result, most studies are performed in the laboratory in soil-packed columns where factors that affect transport can be varied individually (Figure 15.17). However, these usually represent homogeneous media of one soil type with no soil structure. Thus,

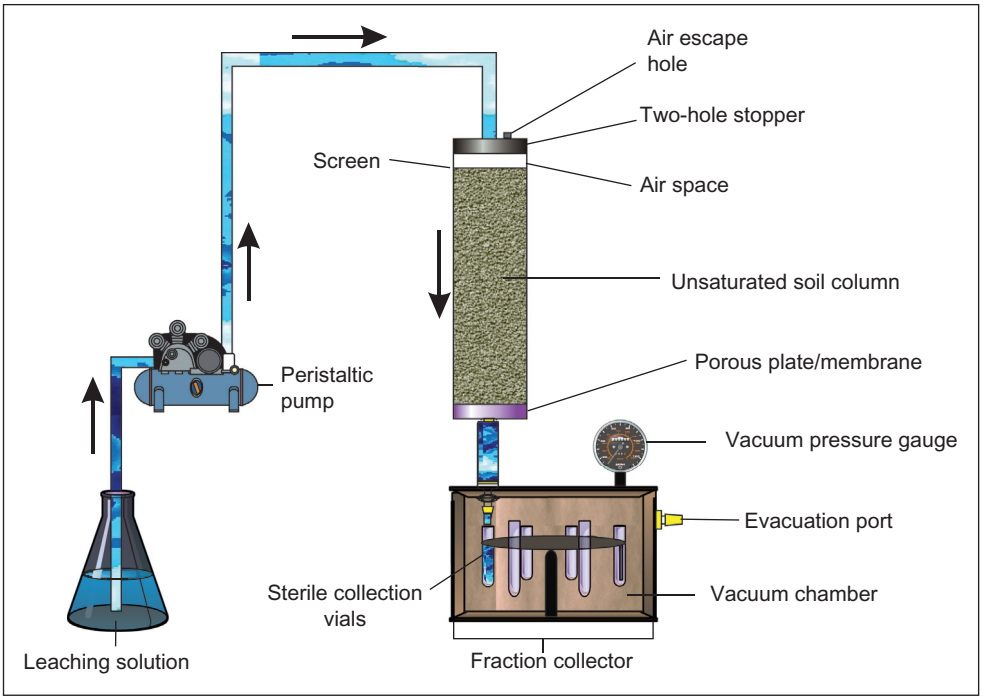

FIGURE 15.17 The setup used to run an unsaturated soil column. Modified with permission from Wierenga (1996).

while they are useful to study individual factors, like infiltration rates, they do not reflect the heterogeneous nature of subsurface environments. To overcome this limitation intact soil cores obtained from a site which retains site heterogeneity rather than using hand-packed more homogeneous columns can be used. For example, Smith *et al.* (1985) observed that under saturated flow conditions packed columns retained at least 93% of the bacterial cells applied, whereas intact cores retained only 21 to 78%, depending on the matrix texture and structure. Increased heterogeneity in soil structure preferential flow and flow velocity are probably key factors accounting for the increased transport within the intact soil cores.

Another limitation of soil cores is that the length of the columns does not reflect full-scale transport over long distances. However, generally, greater removal of microbes occurs in the first meter in most soils, and removal rate tends to decrease with travel distance. Microbial removal determined from laboratory column (usually one meter in length or less) studies can be one to three orders (10 to 1000) of magnitude greater than observed under field conditions (Pang, 2009). Thus, caution should be used in extrapolating results from column studies to distances greater than one meter in the field.

15.4.2 Field Studies

Field studies, in which multiple factors may change simultaneously, provide more relevant information than column studies. Field studies may involve both the use of tracers (e.g., bacteriophages) and/or the detection of naturally occurring enteric organisms near a source (i.e., septic tank or land treatment of wastewater). This requires the use of sampling wells near the point of injection or source. In an extensive review of field studies on microbial transport, Pang (2009) found that a log function best describes removal for most organisms, with removal rates decreasing with increasing distance from the source, contradicting the conventional transport models and filtration theory. This reflects the hypothesis that heterogeneity among microbial particles themselves (type, size, density, charge, strains, survival characteristics and aggregation with colloids) affects their transport. Approximate microbial removal rates in different subsurface media are shown in Table 15.2.

15.4.3 Tracers

Tracers, chemical or particulate in nature, are often used to estimate microbial transport potential. Tracers are chosen so that their transport will closely mimic that of the microbe of interest. Their use is especially informative with regard to abiotic processes that influence movement of bacteria and viruses through subsurface media. Tracers are advantageous, particularly for field studies, for a variety of reasons. They can be added to a system in high numbers, their transport can be monitored without introducing a risk of infection, and they are typically easy to detect. A number of different tracers have been used in microbial transport studies, including microspheres,

TABLE 15.2 Magnitude of Removal Rates for Different Subsurface Media

Category	Magnitude of Removal Rate Log/Meter	Conditions
Soil	1	Most soil types
	0.1	Clayey soil, clay loam and clayey silt loam
Vadose zone (unsaturated soil)	0.1	Clay and silt, sand, sand-gravels, coarse gravels
Sand aquifers (velocity < 2 meters/day)	1	Sand aquifers
Sand and gravel aquifers (velocity < 3 meters/day)	0.1	Less than 17 meters travel distance
	0.1 to 0.01	Less than 177 meters travel distance
	0.0001	210 to 2930 meters travel distance
Coarse gravel aquifers (velocity greater than 50 meters/day)	0.01	
Fractured rock aquifers	1 to 0.1	Clean fractured clay till
Karst limestone aquifers	0.1 to 0.01	Less than 85 meters travel distance
	0.0001	5000 meters travel distance

Modified from Pang (2009).

halides, proteins and dyes. The use of microbe-sized microspheres has the advantage over the use of dissolved tracers since the microspheres should follow the same flow paths as bacteria, even in highly heterogeneous subsurfaces. However, their surfaces may interact with subsurface particles very differently from bacteria. Studies indicate that the tracer choice is often critical in determining relevant estimates of transport but that all tracers have limitations in terms of mimicking microbial transport through the terrestrial profile. Coliphages and bacteria containing a marker (antibiotic resistance, stained) are often used (Harvey, 1997).

Powelson *et al.* (1993) compared the transport of a tracer with that of two phages. This group used potassium bromide, a conservative chemical tracer, and the bacteriophage MS-2 and PRD-1, which were selected because of their low adsorption to soils and their long survival time in the environment. Both the viruses and the conservative chemical tracer arrived at sampling depths in irregular patterns, indicating preferential flow. Virus breakthroughs were later than bromide except when viruses were added after pore clogging had reduced infiltration of the surface-applied sewage effluent. This study demonstrates the variability of relative transport rates that can exist between microbes and a chemical tracer.

Gitis *et al.* (2002) developed fluorescent dye-labeled bacteriophages to provide an additional tool to study virus transport. Advantages of these modified bacteriophages over conventional tracers include the ability to uncouple inactivation and transport phenomena, decreased costs associated with sample preservation, simple quantitation by optical methods and enumeration of individual virus concentrations instead of aggregates.

The ratio of the time it takes for the maximum concentration of a conservative tracer (a tracer that is nonsorbing) to be detected in the column effluent to the corresponding time for the coinjected microorganism defines the retardation factor for the microbe. This factor can be used for comparisons involving the same microbe and different soils or different microbes and the same soil. For example, Bai *et al.* (1997) found that in the absence of rhamnolipid (a surfactant) the retardation factors for three *Pseudomonas* strains through sandy soil ranged from 3.13 to 2.12. This is in comparison to a value of 1, which indicates no retardation. In addition, the retardation factor can be used to assess the occurrence of preferential flow. Preferred flow paths are suggested when the retardation factor is less than 1.0, indicating enhanced microbe transport relative to mean flow velocity (tracer transport). Gerba *et al.* (1991) suggested a worst-case value of 0.5 for the retardation factor when using models to predict microbial transport. One possible explanation for increased microbial transport is pore-size exclusion. Microbes may be excluded from smaller pores, where, on average, water travels more slowly (see Figure 15.14A). Thus, they are forced to travel in the larger pores, with velocities that are higher than that of the soil solution as a whole. Accordingly, transport of microbes can be faster than that of a conservative tracer through the same porous medium.

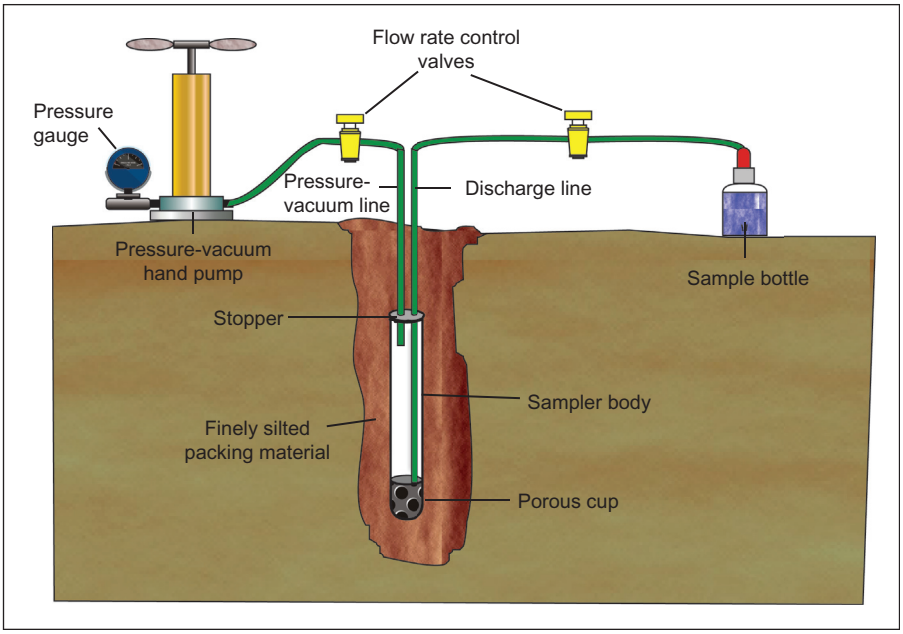

FIGURE 15.18 Example of an experimental setup that allows *in situ* sampling of soil pore water, which can subsequently be examined for microbial or chemical contaminants. Modified with permission from Soil Moisture Equipment Corp., Santa Barbara, CA.

15.5 MODELS FOR MICROBIAL TRANSPORT

Models have been used to predict not only the extent of movement of microbes but also the time required for the microorganisms to arrive at a specific location. Mathematical models designed to predict microbial transport through the subsurface should take into consideration a variety of factors that influence both survival and adsorption. In addition, they should account for changing conditions that the microbe encounters as it migrates through the subsurface profile. Laboratory and field studies should be conducted in an attempt to verify the accuracy of conceptual transport models. Such studies indicate that although models can be useful tools, they can also generate predictions that are off by orders of magnitude (Section 15.4.2). Accordingly, model predictions should be treated with caution.

The heterogeneity of microbes within a population can further complicate the application of models. Each bacterium or virus even of the same species may have slight differences in isoelectric points or factors in their biochemical composition which increase or decrease their transport. Typically, a microbial species that exhibits transport characteristics at one end of the spectrum or the other is chosen. The choice of an organism that does not travel extensively may be useful in assessing problems that may be encountered with bioaugmentation efforts. On the other hand, a microbe known to have high transport potential may be chosen when attempting to determine the minimum distance between a sewage release point and a well for drinking water. Another approach involves modeling the transport of each microbe individually and then combining the results to determine an overall concentration at a specified time and location. This method may be more accurate but is also much more involved.

A term is generally incorporated in equations to reflect the survival characteristics of the microorganism. This term represents the loss of the microbe, via death or inactivation, resulting from adverse chemical, physical or biological processes. Microbial growth counteracts a portion of this decay, and thus a net rate decay term (i.e., net decay rate = growth rate − death or inactivation rate) is often used (Figure 15.18). Microbial decay rates have been found to vary by several orders of magnitude (Table 15.3), making it necessary to evaluate survival characteristics for each particular microbe. Contaminant transport models, specifically advection−dispersion models and filtration models, are often modified for application to microbial transport. Factors that influence microbial transport can be incorporated into equations governing either of these models. However, the fundamental basis of each model differs, and thus under certain conditions use of a particular model may be preferable.

15.5.1 Advection−Dispersion Models

Unmodified advection−dispersion models assume that the contaminant is in solution and thus has the same average velocity as the matrix solution. Values for average velocity and dispersion of a contaminant are generally obtained from conservative tracer tests. These values may not be appropriate for microbial transport because microbes are not dissolved but are instead suspended particulates in the liquid medium. In order to obtain accurate adsorption data for input into the advection−dispersion

TABLE 15.3 Survival of Microorganisms in Groundwater vs. Temperature

Organism	Temperature (°C)	Mean Inactivation Rate (Log/Day)	Inactivation Rate Range (Log/Day)
Poliovirus	0–10	0.02	0.005–0.05
	11–15	0.08	0.03–0.2
	16–20	0.1	0.03–0.2
	26–30	0.08	0.006–1.4
Hepatitis A virus	0–10	0.02	0–0.08
	20–30	0.04	0.009–0.1
Echovirus	11–15	0.1	0.05–0.2
	16–20	0.1	0.05–0.2
	21–25	0.2	0.06–0.6
Coxsackievirus	8–20	0.06	0.002–0.2
	25–30	0.1	0.007–0.3
Rotavirus	3–15	0.4	one study
	23.2	0.03	one study
Adenovirus	4	0.0076	one study
	12–22	0.028	0.01–0.047
Coliforms	0–10	0.07	0.03–0.4
	15–20	0.4	0.02–1.5
	21–37	0.3	0.007–2.5
Cryptosporidium	22	0.039	0.025–0.072

Data from John and Rose (2005) and Regnery et al. (2013).

equation, it may be necessary to conduct site- and microbe-specific adsorption studies. Furthermore, both irreversible sorption and reversible adsorption should be considered. The advection–dispersion model often incorporates a decay term along with terms that account for transport with the bulk flow (advection), transport resulting from diffusion and mechanical mixing (dispersion), and adsorption. Advection–dispersion equations can be expanded in order to take into account hydrogeological heterogeneities in addition to the variety of factors that determine microbial survival. Figure 15.13 illustrates the influence of advection, dispersion, adsorption and decay on the transport of a contaminant. A relatively simple advection–dispersion equation is:

$$R_f \partial C/\partial t = -V\partial C/\partial x + D\partial^2 C/\partial x^2 \pm R_x$$

where:

C is the concentration of microbe (mass/volume)
x is the distance traveled through the porous medium (length)
V is the average linear velocity constant (length/time)
R_x is the microbial net decay term (mass/time-volume)
t is time, and
R_f is the retardation factor, accounting for reversible interaction with the porous medium.

Bai *et al.* (1997) used a one-dimensional advection–dispersion model to assess bacterial transport through a sandy soil in the presence of rhamnolipid (Figure 15.16). They found that three parameters were especially important: R, the retardation factor, which represents the effect of reversible adsorption on cell transport, and two irreversible sticking rate constants, one for instantaneous sorption and the other for rate-limited sorption. They found that all three constants decreased with increasing rhamnolipid concentration; however, the rate-limited sorption sites were affected the most.

15.5.2 Filtration Models

Filtration models, on the other hand, assume that the contaminant is particulate in nature and that its removal is

dependent on physical straining and sorption processes. These processes are often combined into a filtration coefficient for the system. Such models take into account mechanisms by which colloids (i.e., microbes) come in contact with particle surfaces and the relative size of the microbe compared with the pores in the medium. The premise of these models is that as the microbial suspension passes through the terrestrial profile, microbes will be removed. Yao *et al*. (1971) demonstrated that filtration models may be applicable in terms of predicting bacterial immobilization during transport through the subsurface. A general filtration equation is:

$$\partial C/\partial x = \lambda C$$

where:

C is the concentration of colloid (mass/volume)
x is the distance traveled through the porous medium (length), and
λ is the filter coefficient (1/length).

According to this equation, microbial removal would be exponential with depth, as is sometimes observed. As with the advection−dispersion equation, terms can be incorporated into the equation to account for net microbial decay.

QUESTIONS AND PROBLEMS

1. Compare and contrast the major factors influencing bacterial versus viral transport through the terrestrial profile. Which type of microbe would you expect to find deeper in the profile following surface application?
2. Choose either a bacterium or virus and design a column experiment to assess its transport potential. Your discussion should include items such as column design, type of matrix material, flow conditions, percolating solution, inoculation and sampling approaches. Support your choices.
3. What are UMB? How can they be used to facilitate bioremediation of contaminated sites?
4. As both soil particles and microbes generally have a net negative surface charge, why is adsorption to matrix material often a factor limiting microbial transport?
5. Why do microbes introduced to a site often die within a few days to weeks? What impact does this have on the transport potential of the introduced microbes?
6. Discuss the advantages and disadvantages of using a soil column to assess microbial transport potential as compared to the use of a column.
7. Why is microbial removal rate greater near the soil surface than at greater distances?
8. Determine the time in days for inactivation of 10^6 adenoviruses in ground water at 4°C.
9. You have packed a column with a sandy soil and are preparing to determine the transport of a particular bacterial isolate through the porous material. Indicate whether the following changes to your experimental system might be expected to increase or decrease the transport of the bacterium:
   a. an increase in cell size
   b. a decrease in ionic strength
   c. an increase in organic carbon content of the soil
   d. an increase in particle size of the sandy soil
   e. a bacterium produces copious amounts of exopolymeric material
   f. an anionic surfactant is added
   g. an anionic surfactant is added
   h. the cells are carefully washed with a phosphate buffer three times before the experiment
   i. a bacterium covered with fimbriae is used
   j. a bacterium with an extremely hydrophobic cell surface is used.

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