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BIOFILMS IN POROUS MEDIA

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5.1 INTRODUCTION

The high specific surface area of porous media suggests that most bacteria in such systems are attached to the solid surfaces. Under low organic and nutrient concentrations, attached bacteria can outnumber suspended bacteria by several orders of magnitude and account for a major portion of all microbial activity (van Loosdrecht et al., 1990). An understanding of biofilm accumulation in porous media provides significant opportunities for improving the performance of environmental and industrial processes. Subsurface biofilms offer the potential for biotransformation of organic contaminants, thereby providing an in situ method for treating contaminated groundwater supplies (National Research Council, 1993). The rate of biotransformation is strongly influenced by porous media mass transport characteristics including media permeability and pore velocity distribution, as well as by biofilm surface roughness and other variables that affect the delivery rate of substrate and nutrients to growing cells. Industrial applications include microbially enhanced leaching of metals from ores and recovery of metals from solutions, deliberate plugging of high-permeability zones to enhance oil recovery operations, and bioreactors for water and waste treatment (Cunningham et al., 1991).

Biofilms II: Process Analysis and Applications, Edited by James D. Bryers.
ISBN 0-471-29656-2

Accumulation of microorganisms on surfaces as biofilms often enhances the potential for biotransformation of organic contaminants. Biofilm growth permits greater accumulation of cells per unit volume in comparison to suspended cultures, particularly for slow-growing microorganisms, and spatial positioning may promote interaction and gene exchange between different microbial species (Ehlers and Bouwer, 1999). Attached microorganisms have an advantage over suspended cells as they are continually exposed to the source of fresh substrate and nutrients contained in either (1) the aqueous phase that flows past them or (2) sorbed to the porous material itself.

This chapter addresses some important issues governing the influence of porous media hydrodynamic properties and bacterial cell surface characteristics on the accumulation rate and spatial distribution of biofilms in porous media. These issues include relevant processes, bacteria/solid surface interactions, transport of bacteria, and biofilm accumulation. The relevance of biofilms to subsurface remediation of organic contaminants is also presented.

5.2 EXISTENCE OF BIOFILMS IN POROUS MEDIA AND CONTROLLING PROCESSES

Biofilm morphology in porous media systems can be highly variable, ranging from patchy, discontinuous colonies to thick, continuous films. For example, packed bed bioreactors for treating high-strength wastewaters or volatile organic emissions may develop biofilms several millimeters thick (Grady et al., 1999). At the other extreme, subsurface biofilms capable of biologically converting trace organic compounds generally consist of isolated microcolonies of native soil bacteria in the range of 10^5 to 10^7 cfu/g of soil (Table 5.1). The microbial distribution in the subsurface and its ramifications for modeling remains a controversial issue (Rittmann, 1993). Several studies have stressed the importance of biofilm kinetics to describe microbial growth in the subsurface (Cunningham et al., 1991; Taylor and Jaffe, 1990a; Taylor and Jaffe, 1990b). Others have questioned the existence of a "biofilm" (Baveye et al., 1992; Vandevivere and Baveye, 1992a; Vandevivere and Baveye,

TABLE 5.1. Reported Bacterial Numbers in Shallow and Deep Aquifer Materials

Location	Depth	Bacterial Numbers per Gram	Reference
Lula, OK	0–8 m	10^7–10^9	Beloin et al., 1988
Coal tar site (unidentified)	1–10 m	10^6–10^8	Madsen et al., 1991
Coal tar site, Baltimore, MD	2.7–28.5 m	10^4–10^6	Durant et al., 1995
Vejen, Denmark	4–31 m	10^7–10^9	Albrechtsen and Winding, 1992
Bordon, Ontario	<50 m	10^3–10^6	Barbaro et al., 1994
Nemaha, KS	26–86 m	10^6–10^8	Sinclair et al., 1990
Idaho	70 m (unsaturated zone)	0–10^6	Colwell, 1989
Coastal plain Sediments, MD	182 m	10^3–10^6	Chapelle et al., 1987
Depart. of Energy, Savannah River, SC	50–260 m	10^4–10^7	Hicks and Fredrickson, 1989

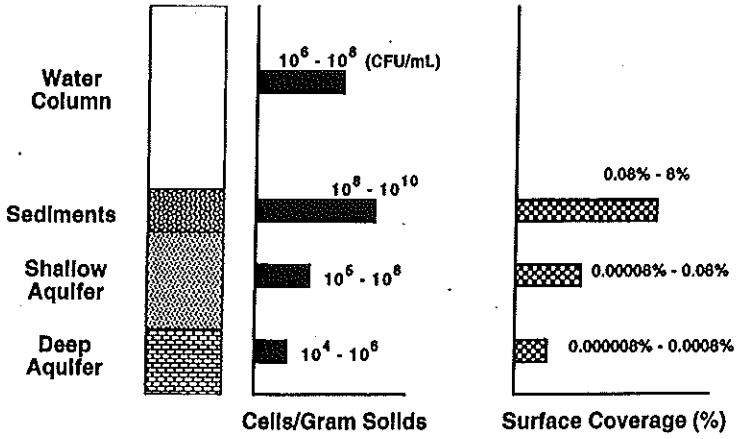

Figure 5.1 Distribution of microorganisms in water and in the subsurface environment.

1992b) because observations with soil and other natural materials have only revealed isolated bacterial aggregates.

Because soils, sediments, and aquifer materials have very high specific surface areas (range of 10 to 1000 m^2/kg, (Bear, 1964)), it is likely bacteria only colonize a small fraction of the soil surface area in the absence of significant pollution (i.e., low substrate concentration and limited nutrient availability). Assuming a dimension of 1 μm for spherical microorganisms, a single cell would occupy a projected area of about 0.8×10^{-2} m^2. For a porous media with a specific surface area of 0.1 m^2/g (100 m^2/kg) and microorganisms uniformly distributed over the solid surface, the cells at 10^{10} cells/gram of solids would occupy about 0.8×10^{-2} m^2 or 8% of the total surface area. Sediments adjacent to the water column have the highest numbers of microorganisms (10^8–10^{10} cells/gram), which correspond to 0.08 to 8% surface coverage (Fig. 5.1). The aquifer solids in shallow and deep aquifers (up to 100 feet deep) usually have low microbial numbers (10^4–10^8 cells/gram) (Table 5.1) corresponding to 0.000008% to 0.08% coverage of the surface area.

Irrespective of the amount of the attached bacteria, their net accumulation on a surface initially free of bacteria is controlled by four processes: particle transport, particle–surface interactions, microbial responses, and particle detachment. These fundamental biofilm processes have been described previously (Chapter 3) in the context of their individual contributions to net accumulation of biofilm on a substratum. The basic concept behind biofilm accumulation is that some cells will deposit on the media, utilize substrate, and grow. Biofilm is removed by biomass decay or detachment; the latter causes cells and biofilm debris to move back into suspension. The relationship between these processes and biofilm accumulation in porous media is illustrated in Figure 5.2 using in situ bioremediation as an example. A typical groundwater plume is shown in Figure 5.2*a*. As chemicals in the source region move with the groundwater, concentrations of the chemicals undergo physicochemical and biological changes. An engineered bioremediation system usually includes extraction and injection wells and equipment for addition and mixing of nutrients.

The accumulation and activity of biofilms varies from point to point along individual pore channels (Fig. 5.2*b*), and thus are considered to be microscale phenomena. The first category of microscale phenomena, transport processes, include the movement of suspended cells with the flow (termed *advective* or *convective* transport) and dispersive

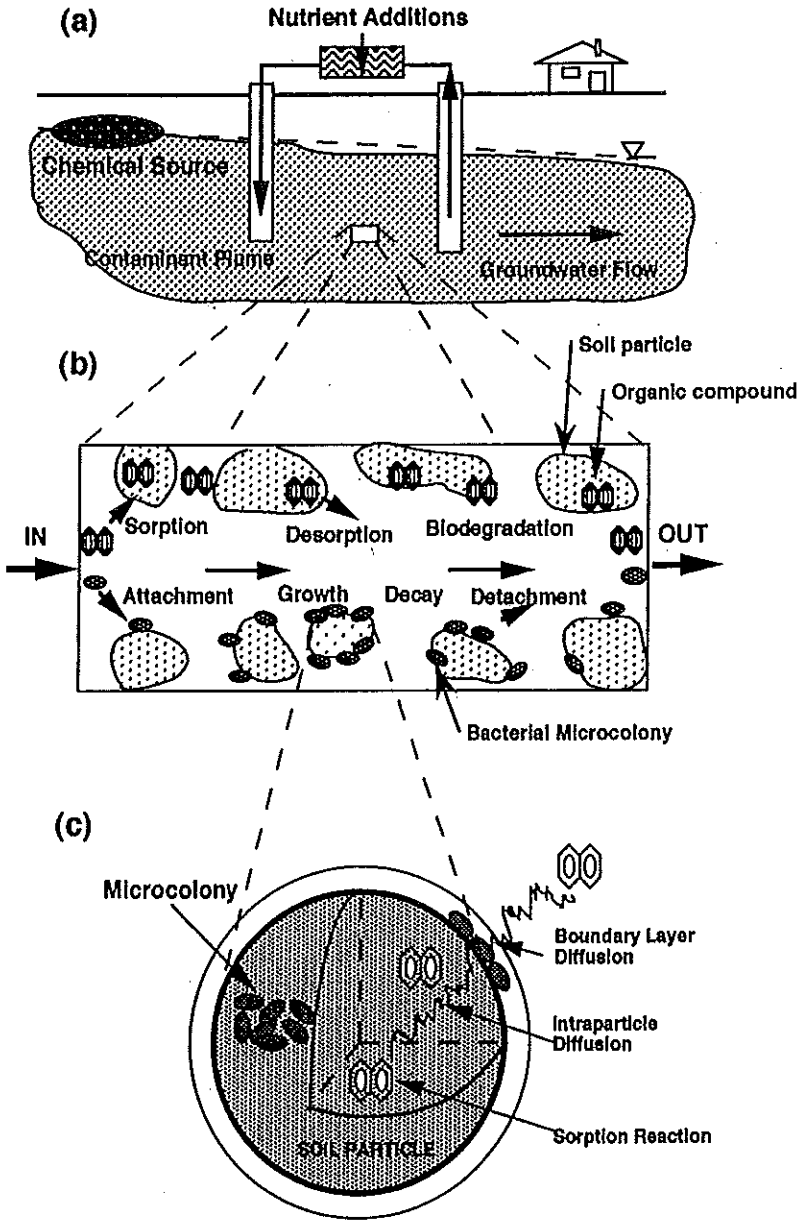

Figure 5.2 Processes controlling biofilm accumulation and contaminant behavior during in situ bioremediation. (*a*) Schematic of in situ bioremediation, (*b*) major microscale mechanisms influencing the movement and fate of bacteria and contaminants, and (*c*) idealized (model) soil particle.

processes of mixing and particle diffusion. Sedimentation of cells and the self-generated motion of motile bacteria also fall into the process of transport. These processes control the rate at which cells collide with surfaces. The phenomena that come into play during such collisions and that cause cells to be either immobilized on the surface or returned to the bulk flow constitute the second category. These particle–surface interactions depend on the

chemistry of the surfaces involved and that of the aqueous phase, and influence the extent to which cells penetrate or are retained by a porous medium. Together, these processes of transport and adhesion constitute deposition. The third category, microbial responses, is reserved for phenomena that are due to the nature of bacteria as viable biological entities. Alteration of size, shape, and surface composition during growth might influence cell transport and adhesion while growth, death, and dormancy control the eventual development of the biofilm. The final category is detachment, where biomass from the biofilm is transferred to the bulk liquid. Detachment rates are generally proportional to the amount of attached biomass and the hydrodynamic shear stress (Rittmann, 1982), although other factors can be involved (Peyton and Characklis, 1993; Speitel and DiGiano, 1987).

An idealized model of a soil particle is shown in Figure 5.2*c*. The solid matrix is porous and sorption sites for organic contaminants are homogeneously distributed. Diffusion is the only major mass transfer mechanism within the solid, and it is assumed to occur only in the radial direction. The typical size of bacteria, 1 μm, is larger than the majority of pore diameters in soil particles. Consequently, the pore structure within the particle is assumed to be too small for penetration of bacteria. Thus bacteria tend to be located on the outer soil particle surfaces as shown in Figure 5.2*c*. If sufficient substrate and nutrients are available, microbial cell accumulation will develop into either patchy discontinuous colonies or colonies that have merged to form a continuous film.

5.2.1 Interactions Between Bacteria and Solid Surfaces

As just noted, the initial step in biofilm formation and growth is the deposition of planktonic cells to the substratum. Deposition is commonly viewed as sequential steps of transport and adhesion. Transport is hydrodynamically determined, whereas the adhesion step is controlled by cell–substratum interactions. This section summarizes several advances recently made in quantifying factors influencing bacterial adhesion.

Experiments with model systems and under well-defined conditions were performed to assess cell–substratum interactions in bacterial adhesion (Rijnaarts et al., 1993; Rijnaarts et al., 1995). Negatively charged Teflon and glass were used as the model substrata. Eight coryneform bacteria and four pseudomonads were selected. At pH = 7, these organisms had different negative cell surface charges (Rijnaarts et al., 1993). The hydrophobicity of solids and bacteria were determined by measuring the contact angle of drops of water on flat pieces of solid and on air-dried bacterial lawns. Glass with a contact angle (θ_w) of 12° ± 2° is hydrophilic and Teflon with θ_w of 105° ± 1° is extremely hydrophobic. Hydrophobicities of the dried bacteria varied between strongly hydrophilic ($\theta_w < 20°$) and extremely hydrophobic ($\theta_w > 100°$). Two types of experimental setups were used: (1) static batch systems containing bacterial suspension and flat pieces of Teflon or glass and (2) dynamic model porous media that consisted of water-saturated columns packed with Teflon or glass beads to which bacterial suspensions were applied. Transport of cells from bulk liquid to substratum is much more efficient in dynamic columns that in static systems: Transport is controlled by convection and diffusion under dynamic conditions but by diffusion only in static systems (Rijnaarts et al., 1993).

At an ionic strength of 0.1 M, adhesion in the batch system was irreversible and the activation energies for adhesion varied between 0 and 5 kT (k (JK^{-1}) is the Boltzmann constant; 1 kT = 4×10^{-21} J at 20°C) (Rijnaarts et al., 1995). A Gibbs energy barrier, located between cell and substratum and several hundreds of kT high, is created by the DLVO (Derjaguin, Landau, Verway and Overbeek) interactions (Rijnaarts et al., 1993; Rijnaarts

et al., 1995). This barrier cannot be surmounted by whole cells. However, bacterial cell surface macromolecules can penetrate this energy barrier and hence reach the substratum at high ionic strength. Therefore, the interactions between the cell surface marcomolecules can penetrate this energy barrier and hence reach the substratum at high ionic strength. Therefore, the interactions between the cell surface macromolecules and the substratum, which are generally called *steric interactions*, determine adhesion at high ionic strength. The following two types of steric interactions generally occur: (1) bridging, that promotes adhesion and causes a lowering of the activation energy of adhesion, and (2) steric hindrance that inhibits adhesion and therefore increases the activation energy of adhesion.

The various mechanisms of adhesion as deduced for the different combinations of types of cell–surface coatings and solid substrata are summarized in Figure 5.3 (the capital letters given below refer to the various panels shown in this figure). Long-range electrostatic interactions as described by the DLVO theory (*A* and *B*) create strong repulsive barriers to adhesion that cannot be passed by whole cells. In addition, deep secondary minima exist for glass but not for Teflon. The secondary minima on glass result from strong Van der Waals attraction and are sufficiently deep for irreversible adhesion. Lipid or protein (nonpolysaccharide) cell surface macromolecules cause strong attractive bridging on the hydrophobic surface (Teflon) (*C*). On glass (*D*), these same cell surface macromolecules slightly inhibit adhesion and permit adhesion in the secondary DLVO minimum as demonstrated for two hydrophobic coryneforms (Rijnaarts et al., 1995). Bacteria with an amphiphilic cell surface may adhere by bridging on a hydrophobic surface (*E*) whereas strong steric hindrance prevents secondary minimum adhesion on glass (*F*). The adhesion of bacterial cells coated with anionic polysaccharides is strongly inhibited on both Teflon and glass (*G* and *H*).

At an ionic strength (*I*) of 0.1 M in a batch system, adhesion was irreversible. The bacteria/surface interactions could be quantified in terms of Gibbs activation energies for adhesion and detachment as determined from experimental adhesion and detachment rates. The adhesion efficiency α, which is the probability of a cell attaching upon reaching a sub-

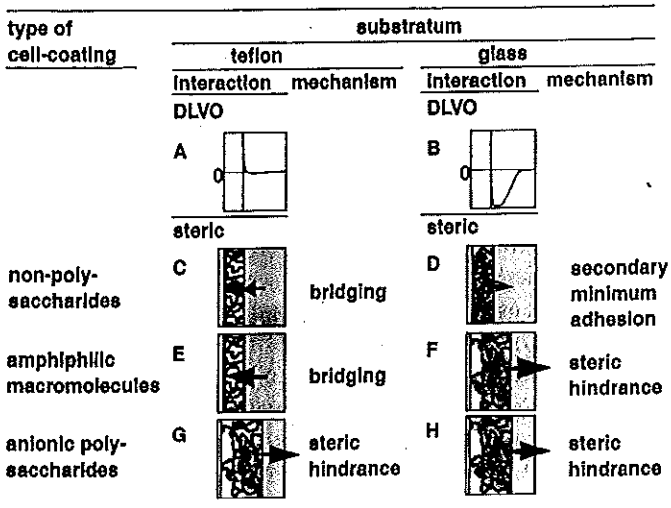

Figure 5.3 Summary of the adhesion mechanisms that occur among different bacterium/substratum combinations. The arrows indicate attraction due to bridging (arrow points to the surface, i.e., to the left) or repulsion due to steric hindrance (arrow points away from the surface, i.e., to the right).

TABLE 5.2. The Level of the Activation Energy for Adhesion and the Adhesion Efficiency α for Different Adhesion Mechanisms

Adhesion Mechanism	Activation Energy for Adhesion (kT)	Adhesion Efficiency, α
Bridging	0	1
Secondary minimum adhesion	1	0.3
Steric hindrance	2.5–5	0.01–0.05

stratum, is related to the activation energy for adhesion where the activation energy is proportional to $-ln\ \alpha$. Values of the activation energy for adhesion and the adhesion efficiency for the different adhesion mechanisms shown in Figure 5.3 are summarized in Table 5.2. The activation energy for detachment exceeded 5 kT for irreversible adhering bacteria. The greatest resilience against detachment was observed for hydrophobic bacteria on hydrophobic substrata. For hydrophobic/hydrophilic and hydrophilic/hydrophilic bacterium/substratum combinations, binding mechanisms not related to hydrophobicity inhibited cell detachment.

The effect of DLVO and steric interactions on adhesion as a function of the ionic strength is illustrated in Figure 5.4 (Rijnaarts et al, 1999). Steric interactions dominate at high ionic strength (0.1 M). Adhesion attains a maximum and is even 100% efficient ($\alpha = 1$) in the case of bridging. Long-range electrostatic repulsion, as described by the DLVO model, starts to exert its influence when the ionic strength is reduced below a critical value I_s (Fig. 5.4) and dominates adhesion at an ionic strength of 0.0001 M. The value of I_s is determined by the distance over which the cell–surface macromolecules penetrate the electrostatic barrier. For the bacterial strains tested, the extension of cell surface macromolecules varied between 5 nm and 80 nm, which corresponds to I_s values varying between 0.1 and 0.001 M. A cell–substratum separation of 165 nm was bridged by a flagellated *Pseudomonas putida* strain. The practical consequence of these findings is that studying and controlling bacterial adhesion in porous media should include the assessment of both DLVO and steric interactions since the ionic strengths of groundwater and wastewater generally fall between 0.001 M and 0.1 M.

5.2.2 Bacterial Transport in Porous Media

The movement of bacteria through porous media is a critical factor in the fate and remediation of many polluted surface and subsurface formations. When in situ bioremediation is chosen as the remedial action, successful cleanup depends on the transport and redistribution of the endogenous strains through the entire contaminant plume. When exogenous strains with specialized metabolic features are used, their transport from the injection source to and within the contamination plume is a major factor determining the efficacy of the operation (Thomas and Ward, 1989). Alternatively, if in situ biobarriers are desired, sufficient adhesion of introduced strains in the desired locale is required (Hanna and Taylor, 1996). Bacterial transport is also important when the organisms are pathogenic or contain a modified genetic element (Trevors et al., 1990).

Bacterial transport is typically governed by aqueous phase convective movement coupled with retardation by adhesion onto surfaces and straining or trapping in interstitial pores. Adhesion is commonly thought of as the main retarding factor, while straining is

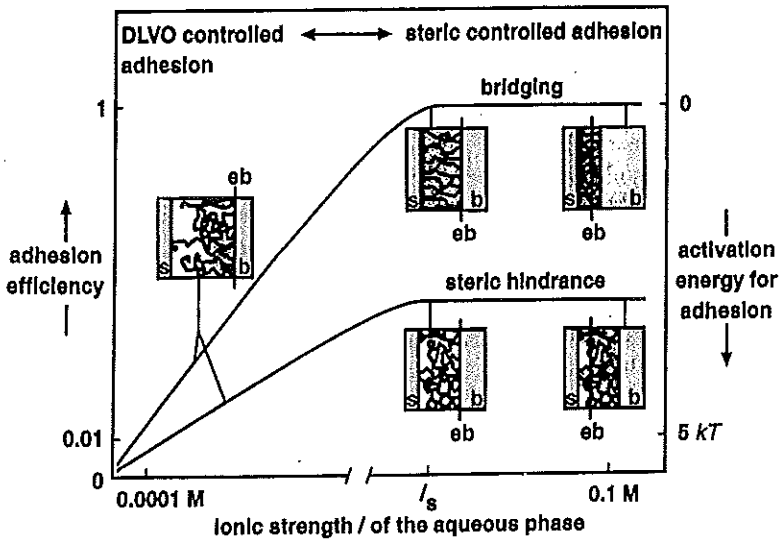

Figure 5.4 Illustration of the effect of the ionic strength on bacterial adhesion. eb = electrostatic barrier; b = bacterium; S = substratum.

important only when the diameter of the particle exceeds 5% of the mean interstitial pore size (Jenkins and Lion, 1993; McDowell-Boyer et al., 1986). Bacterial transport through porous media is influenced by several parameters including properties of the bacterial cells, solution chemistry, porous media characteristics, and interstitial fluid velocity.

Cell properties that may influence bacterial transport, retardation, and adhesion to surfaces include the presence of molecules such as proteins or polysaccharides on the cell surface (Caccavo et al., 1997; Fletcher, 1976; Fletcher and Floodgate, 1973; Rijnaarts et al., 1996b; Williams and Fletcher, 1996), the presence of pili (Busscher et al., 1990a; Busscher et al., 1990b; Busscher et al, 1992; Mueller, 1996) as well as motility and chemotaxis (Camper et al., 1993; Jenneman et al., 1985; McCaulou et al., 1995; Reynolds et al., 1989; Sharma and McInerney, 1994). Cell size and cell shape likewise have been correlated with transport through porous media (Bitton et al., 1974; Fontes et al., 1991; Gannon et al., 1991b; Weiss et al., 1995) as well as cell surface charge (Gilbert et al., 1991; Schäfer et al., 1998b; Sharma et al., 1985; van Loosdrecht et al., 1987a) and cell hydrophobicity (Caccavo et al., 1997; Fletcher and Marshall, 1982; Scholl and Harvey, 1992; van Loosdrecht et al., 1987b). In some cases the buoyant density of the bacterial cells influenced the sedimentation rates of bacteria in porous media environments (Harvey et al., 1997; Wan et al., 1995), but has also been considered negligible (Corpcioglu and Haridas, 1984). Many cell properties are influenced by the physiological state of the bacteria and can therefore be significantly different for the same bacterium, depending on environmental conditions (Grasso et al., 1996, van Loosdrecht et al., 1987a). The growth state of the bacteria and the presence of nutrients have, for instance, been shown to influence attachment (Grasso et al., 1996; Mueller, 1996; van Loosdrecht et al., 1987a). Starvation is another important physiological state of bacteria. Short-term starvation of bacteria can result in an increased tendency to attach to surfaces (Dawson et al., 1981; Kjelleberg, 1984). Long-term starvation (weeks to months) in contrast may enhance bacterial transport through porous media (Cusack et al., 1992; Gerlach et al., 1998; Lappin-Scott and Coster-

ton, 1992; Lappin-Scott et al., 1988a; Lappin-Scott et al., 1988b; MacLeod et al., 1988; Sharp et al., 1999).

Solute characteristics including ionic strength, pH, temperature, concentrations of dissolved organic matter, surfactants, and nutrients have also been shown to influence bacterial transport and adhesion to surfaces. Increased ionic strength has widely been correlated with increased attachment (Fontes et al., 1991; Gannon et al., 1991a; Jewett et al., 1995; Mills et al., 1994; Scholl et al., 1990). This effect is usually attributed to the compression of the electrostatic double layer in the presence of high ion concentrations. Bitton et al. (1974), Scholl and Harvey (1992), Kinoshita et al. (1993), and Jewett et al. (1995) investigated the effect of changes in pH values on bacterial transport and attachment. However, no uniform results were found. Increased attachment with decreasing pH was found by Scholl and Harvey (1992); Kinoshita et al. (1993) observed decreased attachment with decreasing pH. However, Jewett et al. (1995) stated that changes in pH from 5.5 to 7 did not affect bacterial transport through porous media columns. Temperature has also been shown to influence bacterial attachment (McCaulou et al., 1995; Sarkar et al., 1994). Johnson and Logan (1996) investigated the influence of dissolved and sediment organic matter (DOM, SOM) on bacterial transport through porous media columns and observed an increase in travel distance in the presence of SOM. The addition of surfactants or dispersants can result in decreased attachment and therefore facilitate the transport of bacteria through porous media, however the activity or viability of the bacteria may also be altered (Goldberg et al., 1990; Gross and Logan, 1995; Jackson et al., 1994; Paul and Jeffrey, 1984; Sarkar et al., 1994).

Porous media properties that have been reported to influence bacterial transport and adhesion include pore water velocity (Gannon et al., 1991a), hydraulic conductivity (Vandevivere and Baveye, 1992b), pore size (Sharma and McInerney, 1994), the presence of Fe minerals (Johnson and Logan, 1996; Mills et al., 1994; Scholl et al., 1990), the organic matter content (Johnson and Logan, 1996), and grain and pore size distribution (Fontes et al., 1991; Sharma and McInerney, 1994; Smith et al., 1985; Wood and Ehrlich, 1978). The surface charge (Johnson and Logan, 1996; McCaulou et al., 1994; Scholl et al., 1990) and surface hydrophobicity (Absolom et al., 1983; Fletcher and Loeb, 1979; McCaulou et al., 1994; Schäfer et al., 1998a) of the porous media can also influence bacterial attachment to surfaces. Geesey and Costerton (1979), Mueller et al. (1992), and Scheuermann et al. (1998) report that the roughness of surfaces can also influence attachment. The surface roughness in dynamic systems may even become more important than the chemical nature of the surface. Trevors et al. (1990) reports that the presence of plants and plant roots can have an effect on bacterial transport through soil.

Transport of bacteria through porous media may be influenced by some combination of the foregoing parameters. Camper et al. (1993) state that bacterial adsorption rate coefficients determined under the conditions of interest are a much better predictor of microbial transport phenomena than individual characteristics of cells or the porous medium. To predict bacterial transport through porous media, one should therefore perform experiments with the aquifer material of interest and as close as possible to the expected conditions in the field. Harvey (1997) gives a good overview on how to design and standardize bacterial transport experiments.

Basic bacterial transport models have been commonly based on the convection–dispersion equation written for bacteria (Harvey and Garabedian, 1991; Jewett et al., 1995; Taylor and Jaffe, 1990c). Empirically determined collision efficiency factors have typically been used to account for the extent of bacterial attachment to surfaces. Macroscopic models of

bacteria transport have, in general, ignored detailed treatment of interfacial forces between the solid surface and the bacterium. Column breakthrough experiments with well-characterized Teflon and glass collectors and bacterial strains were recently carried out to provide a better basis for bacterial transport modeling by identifying and quantifying the principal mechanisms of bacterial retention in coarse grain media (Rijnaarts et al., 1996a, 1996b).

Bacterial deposition on spherical glass and Teflon collectors was studied in vertical downflow columns (Rijnaarts et al., 1996a, 1996b). Deposition was analyzed in terms of the clean bed collision efficiency α_o (the probability of a cell attaching upon reaching a cell-free substratum), and a blocking factor B (the ratio of the area blocked by an attached cell to the geometric area of a cell). At an average interstitial fluid velocity of 200 $\mu m\ s^{-1}$ and 0.1 M ionic strength, α_o was close to unity (0.83 $\pm$ 0.01) for the two tested bacterial strains. The cell–solid interactions were nearly completely favorable for deposition and were similar to the results of the static batch systems shown in Figure 5.3 and Table 5.2. Values of B determined from model fits to column breakthrough data increased (enhanced blocking) as cell–cell repulsion increased due to decreasing (more negative) charge of the cell and increasing hydrophilicity of the cell–surface macromolecules.

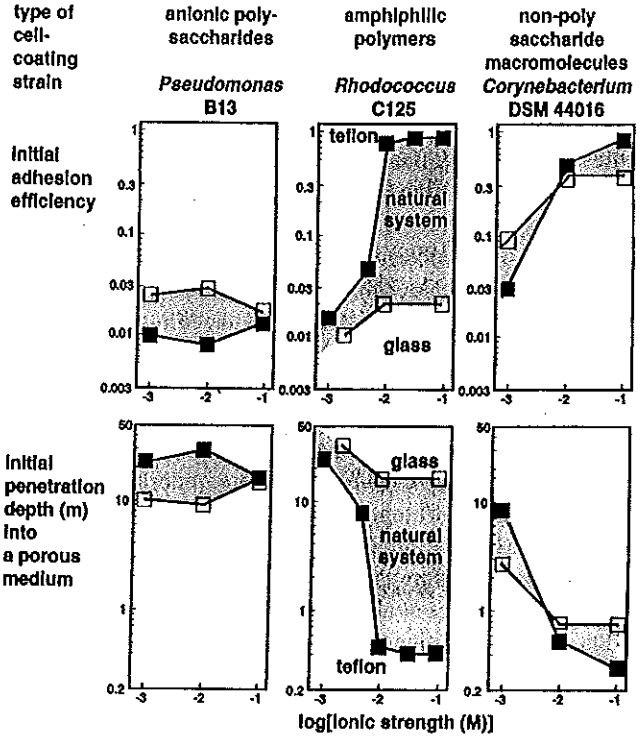

Figure 5.5 Effect of the ionic strength on adhesion and transport of bacteria in porous media. Results of the following organisms are shown: *Pseudomonas* strain B13, *Rhodococcus* strain C125, and *Corynebacterium* strain DSM 44016. Adhesion and transport for natural porous media (shaded area) probably fall between the values for glass (open symbols) and for Teflon (closed symbols). The penetration depth into the porous medium is the porous medium length required to reduce the influent cell concentration by a factor of 100 at an interstitial fluid velocity of 30 cm h^{-1}.

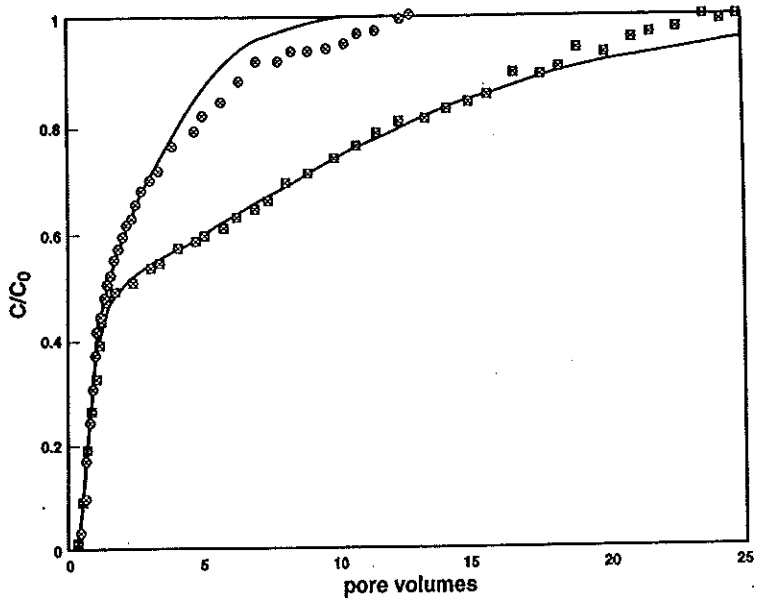

Figure 5.6 Measured data (points) and simulations obtained by incorporating α_o and B into groundwater flow models (lines) for *Bacillus sp.* breakthrough in sand columns at initial concentrations of 3×10^8 cells/cm^3 (squares) and 1.2×10^9 cells/cm^3 (circles).

Initial clean-bed collision efficiency (α_o) decreased with decreasing I, for I values smaller than the critical level I_s in a similar way as found for the static batch systems (Fig. 5.4). The level of B increased about one order of magnitude upon changing I from 0.1 to 0.001 M. The effect of cell–solid interaction and ionic strength (as inferred from the initial adhesion efficiency on bacterial transport in porous media is illustrated in Figure 5.5. Depths of bacterial penetration into a porous medium may be as high as 50 m at low ionic strength based on the laboratory conditions (collector radii of 190 μm ± 50 μm for Teflon and 225 μm = 25 μm for glass, porosity of 0.34 ± 0.02, and interstitial fluid velocity of 30 cm/hr). At high ionic strength, this penetration depth varies between about 0.25 m and 20 m depending on the type of cell–surface constituents, such as nonpolysaccharide, amphiphilic, or anionic polysaccharide macromolecules. The practical consequence of the research summarized in Figure 5.4 is that maximal control of bacterial mobility in porous media can be reached in systems for which B and α_o are high at I around 0.1 M. The high B value obtained from model fits minimizes the occurrence of pore clogging whereas the dependencies of α_o and B on I allow manipulation of deposition by varying the ionic strength. The concepts of initial adhesion (α_o) and blocking factor (B) were recently incorporated into a microbial transport model based on the widely used groundwater flow models termed MT3D and MODFLOW (Te Stroet et al., 1999). This model was able to simulate the microbial breakthrough data for sandy soil columns reported by Lindqvist and Enfield (1992) (Fig. 5.6). Consequently, this model appears to be superior to other models for describing microbial transport in natural heterogeneous subsurface systems.

In field-scale applications, however, manipulating the solute chemistry (e.g., lowering the ionic strength, changing the pH, changing the temperature of the groundwater), or

manipulating aquifer characteristics to facilitate bacterial transport may pose difficult problems both from a technical as well as economical standpoint. However, manipulating the physiological properties of the bacterial inoculum to facilitate bacterial transport through porous media may be more promising. Starvation of bacteria, for example, has been proposed as a means of facilitating transport of an inoculum injected into the subsurface (Cusack et al., 1992; Lappin-Scott et al., 1988a; Lappin-Scott et al., 1988b; MacLeod et al. 1988). Starvation of bacteria results in (radical) size reduction (Bryers and Sanin, 1994; Sanin, 1996) and a rapid decrease in metabolic activity until the bacteria approach complete dormancy (Kjelleberg, 1993).

The significance of starvation on bacterial transport is illustrated in a recent study by Gerlach et al. (1998) in which starved and active cells of *Shewanella alga* BrY were injected into porous media columns (length: 10 ft = 3.048 m; diameter: 1 in. = 2.54 cm) filled with 40 mesh quartz sand. *S. alga* BrY is a facultative anaerobic bacterium, which has been extensively studied and characterized (Caccavo et al., 1996a; Caccavo et al., 1992; Caccavo et al., 1996b; Caccavo et al., 1997). Caccavo et al. (1996a) showed that starvation of BrY results in a gradual decrease in the mean cell volume from 0.48 μm^3 to 0.2 μm^3 and a dramatic decrease in endogenous metabolic activity. The column transport experiments were carried out in an up-flow mode at flow rates of approximately 10 mL/min, resulting in interstitial fluid velocities of approximately 11.5 cm/min (nutrients were removed from the influent, and influent cell concentration was continuously monitored to ensure no-growth conditions for the duration of the experiment). The studies compared the porous media transport of starved BrY cells to their active counterpart as shown in Figures 5.7 and 5.8. The cell breakthrough data shown in Figure 5.7 show that the passage through 3.048 m of quartz sand leads to almost a 5-log removal of active cells. Starved cells, however, break through the column with about a 1.5-log removal. The influent concentrations of starved and active cells were approximately equal at 5×10^7 cfu/ml. The quantification of cells (as described in Gerlach et al. (1998)) sorbed to the quartz sand along the flow-path shown in Figure 5.8 supports the breakthrough data. Active cells tended to adhere more to the porous medium than the starved cells (Fig. 5.8).

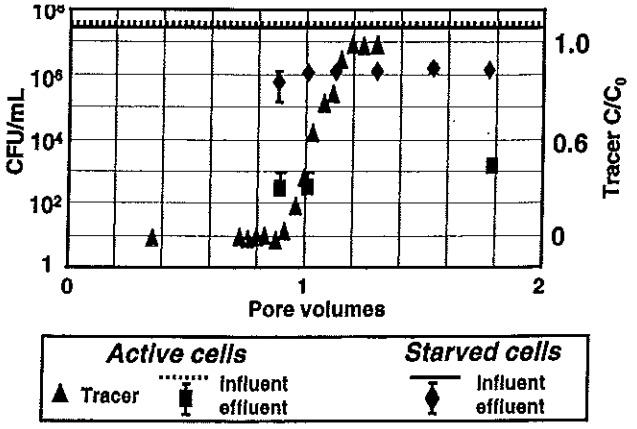

Figure 5.7 Breakthrough of starved and active *Shewanella alga* BrY cells through 10 ft columns filled with 40 mesh quartz sand. Starved cells break through in concentrations and numbers that are orders of magnitude higher than active cells.

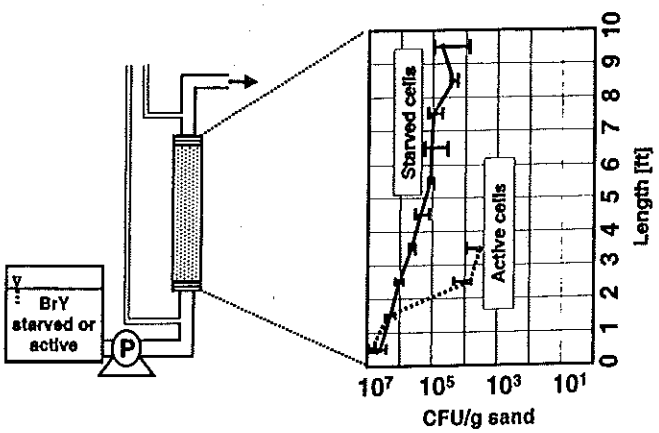

Figure 5.8 Transport of starved and active cells of *Shewanella alga* BrY (a dissimilatory metal reducing bacterium) through 10 ft columns filled with 40 mesh quartz sand. Approximately the same number and concentration of starved and active bacteria were injected. Starved cells are less retarded and more evenly distributed throughout the column.

High concentrations of adsorbed (adherent) active cells within the first few feet of the point of injection were observed. Starved cells, in contrast, were more evenly distributed throughout the system. The detection limit for culturable cells was 10^3 CFU/g sand for sorbed cells and 10^2 CFU/mL for planktonic cells. Results clearly indicate that starvation of *Shewanella alga* BrY provides a means for enhancing bacterial transport in quartz sand porous media.

5.3 BIOFILM ACCUMULATION

The microscale phenomena that govern biofilm accumulation and activity (i.e., chemical and hydrodynamic gradients) are difficult to measure in a porous media environment in comparison to other common reactor systems such as flasks, tanks, reservoirs, and pipelines. Biofilm growth, for example, is complicated by the nature of fluid and nutrient transport which, in porous media, occurs along tortuous flow paths of variable geometry. Similarly, the wide distribution of pore velocities introduces considerable variation in the processes of transport, adhesion, and detachment on a microscale. Consequently, most investigations to date have focused on quantifying the effects of biofilm development rather than on the behavior of individual biofilm processes.

In porous media flow systems, biofilm accumulation generally decreases with distance along the flow path, especially if substrate concentration is depleted through the medium. Laboratory (Taylor and Jaffe, 1990c) and field (van Beek, 1984) observations illustrate the plug flow nature of biofilm accumulation in porous media. In a plug flow system, as discussed in Chapter 2, the concentration of substrate and oxygen (or other electron acceptors) decreases in the downstream flow direction. Thus, cell growth also diminishes at some downstream point where substrate and/or oxygen become limiting to the growth process. The potential for both deposition and detachment are likewise highest in the upstream location experiencing maximum cell growth rates.

5.3.1 Hydrodynamics of Porous Media

The hydrodynamic principles presented here provide the necessary background for subsequent discussion of biofilm processes in porous media. For a more comprehensive treatment of flow through porous media, the reader should consult a standard groundwater text such as Bear (1979), H. Bouwer (1978), Freeze and Cherry (1979), or Todd (1980). These references also provide an overview of mass and momentum transport in porous media. In porous media, mass transport determines the capacity of the flow regime to transport dissolved or suspended substances (i.e., tracers, chemicals, nutrients, and microbial cells) while momentum transport determines the resistance to flow through the porous matrix. Mass transport processes influence the net accumulation of biofilm and the rates of degradation of organic contaminants, and they control the migration of contaminated groundwater. Momentum transport processes are likewise of importance to the analysis of biofouling in packed bed filters and porous media.

5.3.1.1 Darcy's Law. Consider flow through a cylinder of cross-sectional area A (L^2) filled with a granular porous medium. Analysis of this experimental system provides the basis for illustrating fundamental porous medium flow principles. If water is introduced into the cylinder until all the pores are filled, the inflow rate Q is equal to the outflow rate. If an arbitrary datum is chosen, the elevations of the column inlet and outlet water levels relative to the datum are h_1 and h_2. Then,

$$Q = \frac{-KA\,\Delta h}{\Delta L} \tag{5.1}$$

where ΔL = column length or distance traveled by the water (L), $\Delta h = h_1 - h_2$ (L), K = proportionality coefficient (Lt^{-1}).

Expressed in differential terms,

$$Q = -KA\frac{dh}{dL} \tag{5.2}$$

or

$$v = \frac{Q}{A} = -K\frac{dh}{dL} \tag{5.3}$$

where Q = volumetric flow rate (L^3t^{-1}), v = specific discharge (Lt^{-1}), K = hydraulic conductivity (Lt^{-1}), dh/dL = hydraulic gradient (−).

The preferred units for K and v are m day^{-1}, which result in values ranging from essentially zero for highly impermeable material (sandstone, clay, rock) to as much as 1000 m day^{-1} for very permeable media (gravel, fractured limestone). The negative sign indicates that the flow of water is in the direction of decreasing head. Equation 5.3 is known as Darcy's law and states that the specific discharge v equals the product of hydraulic conductivity and hydraulic gradient. Darcy's law is experimentally based, and its range of validity is determined by the magnitude of the porous medium Reynolds number

$$Re = \frac{vd\,\rho}{\mu} \tag{5.4}$$

where d = grain diameter (L), μ = fluid viscosity ($ML^{-1}t^{-1}$), ρ = fluid mass density (ML^{-3}).

d is the mean grain diameter, although alternative quantities such as d_{10} (the diameter such that 10% by weight of the grains are smaller) are used in the literature. According to Bear (1979), despite the various definitions for d, Darcy's law can be considered valid as long as Re does not exceed 1.

Darcy's law is of fundamental importance in the analysis of groundwater flow; it is also of importance in many other applications of porous medium flow. As indicated by Freeze and Cherry (1979), Darcy's law describes the flow of soil moisture and is used by soil physicists, agricultural engineers, and soil mechanics specialists. It describes the flow of oil and gas in deep geological formations and is used by petroleum reservoir analysts. It is relevant to the design of filters by chemical engineers. It has also been used by bioscientists to describe the flow of bodily fluids across porous membranes in the body.

5.3.1.2 Permeability. The hydraulic conductivity K is a function of porous medium properties and hydraulic properties; it provides a measure of the ability of a porous medium to conduct water. However, it is often useful to express the permeability of a porous medium as a property of the medium independent of the density and viscosity of the fluid. Thus, the intrinsic permeability is defined as

$$k = \frac{K\eta}{\rho g} \tag{5.5}$$

where k = permeability (L^2), g = acceleration due to gravity (Lt^{-2}).

Expressing g in cm s^{-2}, ρ in g cm^{-3}, and K in cm s^{-1}, then k is expressed in cm^2. The customary unit of k is the darcy, which is 0.987×10^{-8} cm^2. Intrinsic permeability is mainly applied in the petroleum and natural gas industries, which deal with underground flow of fluids of varying viscosity and density.

Because water movement occurs through the pores and cracks of the aquifer material only, the actual pore velocity of the water is greater than the specific discharge. If the effects of tortuosity are neglected, the relation between the actual pore velocity v_p and the specific discharge v is

$$v_p = \frac{A}{A_{\text{eff}}} v \tag{5.6}$$

where v_p = pore velocity (Lt^{-1}), A = total area normal to the flow direction (L^2), A_{ef} = effective area available for flow (L^2)

or

$$v_p = \frac{v}{\varepsilon} \tag{5.7}$$

where $\varepsilon = A_{\text{eff}}/A$ = porosity (−).

Equation 5.7 is theoretically correct only for the condition where the effective pore area is the same as the total pore area A_p. Also, if tortuosity is considered, the true microscopic velocities will be larger than v_p, since the fluid must travel along irregular flow paths that are longer than the linearly averaged path represented in v_p. However, according to H. Bouwer (1978), Eq. 5.7 reasonably estimates the actual velocity in most types of soil or aquifer materials.

5.3.2 Influence of Initial Bacteria Deposition

Laboratory-scale column (50 mm i.d. × 300 mm packed with 3 mm borosilicate glass beads) experiments were carried out to demonstrate the effect of the level of initial bacterial deposition on the rate of biofilm accumulation and organic substrate removal (Martin, 1990). Since the extent of substrate removal depends on the amount of active biomass, the measurement of substrate concentration along the column and over time provided an indirect indication of the spatial and temporal development of the biofilm. Each experiment comprised three phases designated "deposition," "flush," and "growth." In the deposition phase, the column was seeded with *P. aeruginosa* at a volumetric flow rate of 10 mL/min. The basic approach was to apply bacterial suspensions to the column and monitor the concentrations of cells in the effluent following the same procedure used for column deposition experiments described by Martin et al. (1992). Two trials were completed satisfactorily for which the concentration of cells applied to the column during the deposition phase differed by about 3 orders of magnitude. The average inlet cell concentration during the 60-min deposition phase for the low inoculum experiment was 70 cfu/mL and for the high inoculum experiment was 65,000 cfu/mL. During the deposition phase, the average effluent cell concentrations were 85% and 90% of the feed concentrations for the low and high inoculum experiments, respectively, indicating minimal changes in bulk liquid cell concentrations along the length of the columns. After 60 min, the column was flushed with sterile media containing 0.2 mg/L acetate at the same flow rate for a period of 120 min. The growth phase began at a total elapsed time of 180 min when sterile media containing 3 mg/L acetate was fed to the column. The concentration of acetate and cells in the bulk fluid were measured as a function of time and distance along the column.

The two different levels of cell concentrations applied to the column resulted in a pronounced difference in the time required to reach a steady state column effluent acetate concentration (Fig. 5.9). Data for the high inoculum experiment are represented by the squares and show between 5 and 6 days were required to reach 90% removal of the inlet acetate. For

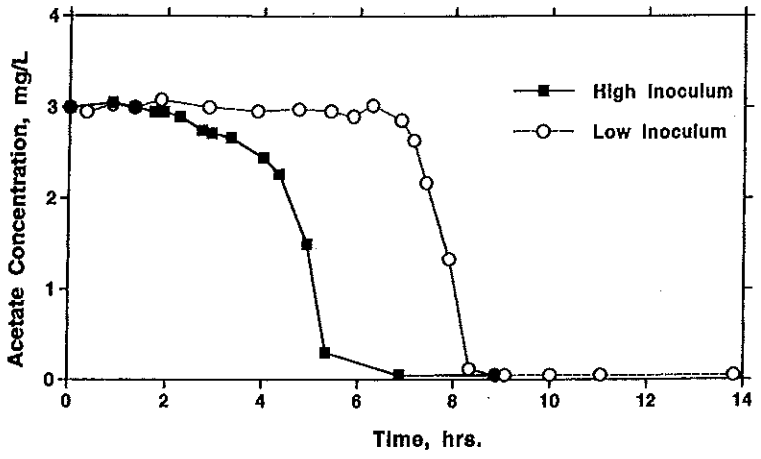

Figure 5.9 Variation in the effluent acetate concentration over time for both low and high inoculum biofilm growth experiments. The influent acetate concentration was 3.0 mg/L.

the low inoculum trial, indicated by the open circles, this time was just over 8 days. The steady state effluent acetate concentration was about 0.1 mg/L, representing about 97% removal. This level was reached within 7 and 9 days for the high and low inoculum trials, respectively. The implications of these laboratory findings to field-scale accumulation of biofilms for bioremediation of contaminants depend on the substrate removal kinetics. For rapidly biodegradable substrates, such as acetate, differences of a few days in the time scale to achieve steady state substrate biodegradation do not necessitate using a high inoculum over a low inoculum in the field. However, for slow contaminant biodegradation reactions, the time scale indicated in Figure 5.9 is markedly extended so a high inoculum greatly shortens the time needed to accumulate sufficient biofilm for successful bioremediation. Additional ramifications of a slow-growing species for application to biofilms utilizing toxic compounds are discussed in Chapter 7.

5.3.3 Influence on Porous Media Hydrodynamics and Permeability

Cunningham et al. (1991) carried out visual observations of biomass accumulation patterns occurring in specially designed packed-bed reactors under high substrate loading conditions (feed contained 25 mg/L glucose). Average biofilm thickness, as determined from 10 separate microscopic measurements of biofilm thickness on the exposed edges of media particles, was the variable used to estimate net accumulation. The experimental system consisted of parallel, horizontally mounted rectangular porous media reactors. Nutrients were supplied to the reactors under gravity flow from a constant head mixing chamber. A constant piezometric head drop of 0.5 cm/cm was maintained across the system throughout each experiment by locating the downstream end of each reactor effluent tube at an elevation ranging between 2.7 cm and 16 cm below the level in the constant head mixing chamber. Flow rate through each reactor varied in proportion to reactor permeability, which decreased substantially as biofilm accumulated during the experiments.

The progression of biofilm thickness for porous media of various sizes and composition followed a sigmoidal curve, reaching a maximum thickness after about 5 days (Fig. 5.10). The ultimate biofilm thickness varied directly with media pore space size and was not influenced significantly by media composition. The following interactions gave rise to the sigmoidal shape. As biofilm thickness increased, the diffusional path length within the biofilm increased, thereby decreasing nutrient concentrations in the base film. Since the piezometric head gradient remained constant, increased thickness also resulted in decreased interstitial pore velocity (decreased pore velocities were observed using nigrosine dye before and after biofilm development). Decreased pore velocities reduced both advective and dispersive transport, thereby lowering nutrient concentrations at the film–water interface, which subsequently reduced growth rate. Decreased pore velocities also reduced shear stress within the biofilm matrix. After biofilm thickness reached quasi-steady state (plateau between 5 and 10 days in Fig. 5.10), the volume of effective pore space (permeability) appeared to stabilize and remain constant.

Wanner et al. (1995) investigated microscale biofilm accumulation in a packed bed biofilm reactor inoculated with a pure culture of *P. aeruginosa* and operated under high substrate loading (feed concentration of 7 to 16 g C/m^3) and constant flow rate conditions. The 3.1 cm diameter cylindrical reactor was 5 cm in length and packed with 1 mm glass beads. Daily observations of biofilm thickness, influent and effluent glucose substrate concentration, and effluent dissolved and total organic carbon were made during the 13-day

experiment. A published biofilm process simulation program (AQUASIM) was used to analyze the experimental data (Reichert, 1994). Biofilm accumulation appeared to reach a quasi-steady state condition (based on thickness measurements) after 10 days. Analysis of the experimental data using AQUASIM identified three distinct phases of biofilm accumulation: (1) an initial phase in which substrate removal was determined by biofilm thickness and mass transfer resistance between biofilm and bulk fluid, (2) a growth phase in which thickness, mass transfer resistance, and area of the biofilm–bulk fluid interface were important, and (3) a mature biofilm phase in which the area of the interface was the impor-

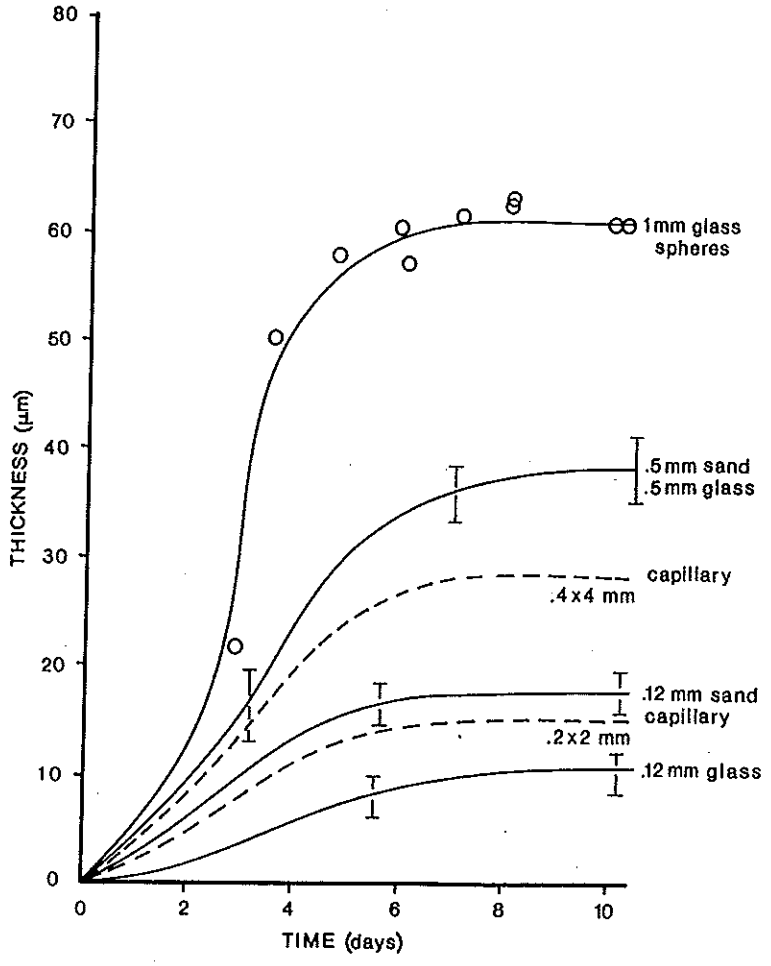

Figure 5.10 Progression of biofilm thickness (*P. aeruginosa*) for media of different diameter and composition. All reactors were run in parallel under a piezometric gradient of 0.5. Data indicate a quasi-steady state thickness is reached after about 5 days of reactor operation. Maximum biofilm thickness values of 63 μm, 40 μm, and 9–14 μm were observed for media particle diameters of 1 mm, 0.54 mm, and 0.12 mm. Clean surface permeability values of 2.1×10^{-5}, 2.17×10^{-6}, and 9.7×10^{-7} cm^2 correspond to these particle diameters, suggesting a direct relationship between media permeability and maximum biofilm thickness.

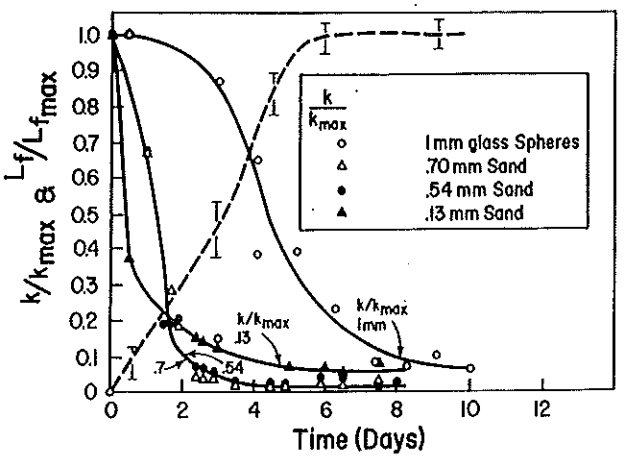

Figure 5.11 Porous media permeability decrease corresponding to increased biofilm thickness. L_f/L_{fmax} is a single composite dimensionless curve representing all thickness curves from Figure 5.10. K_{max} values are 2.1×10^{-5} cm^2 (1 mm glass spheres), 3.19×10^{-6} cm^2 (0.70 mm sand), 2.17×10^{-6} cm^2 (0.54 mm sand), and 9.7×10^{-7} cm^2 (0.12 mm sand). After 5 days of reactor operation, the media/biofilm permeability stabilized and remained essentially constant in the range of 3 to 7×10^{-8} cm^2.

tant parameter. In phase 3, the surface area of the biofilm in contact with the fluid had decreased significantly compared to phases 1 and 2. Substrate flux into the biofilm was utilized entirely within the outer layers of biofilm and thus the total thickness did not correlate with the substrate utilization rate. AQUASIM simulations also showed that biofilm detachment correlated directly with shear stress at the biofilm surface.

Investigations documenting permeability reduction due to biofilm accumulation in porous media have been widely reported (see review by Jaffe and Taylor, 1998). There appear to be two distinct mechanisms by which porous media biofilms reduce permeability. In case one, continuous films are formed, resulting in pore space reduction, causing both porosity and permeability to decrease, as demonstrated by Cunningham et al. (1991). An example of the permeability decrease, corresponding to increasing biofilm thickness for a laboratory porous media reactor system, is shown in Figure 5.11. Here it is seen that biofilm accumulation results in a 95–99% reduction in the original clean surface permeability. Medium porosity decreased between 50% and 96%. A minimum permeability (3 to 7×10^{-8} cm^2) persisted after biofilm thickness had reached a maximum value. These observations suggest that the biofilm accumulation process stabilizes and maintains a minimum permeability within the porous media system. No measurements have been made of "biofilm matrix permeability" yet.

In the second case, permeability is reduced by patchy biofilm aggregates accumulating mainly in pore throats. Under this condition, relatively small amounts of biomass (relative to free pore volume) can cause large reductions in permeability. According to Rittmann (1993), substrate flux to the biofilm is mainly responsible for determining whether case 1 or case 2 occurs. Rittmann developed an index variable, the normalized surface loading, which can be used to predict the occurrence of patchy versus continuous biofilm accumulation in porous media.

5.4 REMEDIATING SUBSURFACE CONTAMINATION

Contamination of groundwater and soils with hazardous organic compounds is widespread (National Research Council, 1994). Exploiting the metabolic capabilities of indigenous or introduced microorganisms in regions of subsurface contamination offers the prospect of converting dissolved and sorbed contaminants to harmless products. This represents a major new challenge for the application of biofilms in porous media. This section addresses some important issues concerning the design and implementation of in situ bioremediation.

5.4.1 Microbial Metabolism of Organic Contaminants

The metabolic capabilities of subsurface microorganisms are quite diverse. For growth of microorganisms, the presence of electron donors and acceptors, a carbon source, and essential nutrients is required. Besides natural compounds, many contaminants can provide these growth requirements. Most organic contaminants can typically be categorized as either aliphatic or aromatic compounds that contain different functional groups, such as -OH, -Cl, $-NH_2$, $-NO_2$, and $-SO_3$. As electron donors, these chemicals are oxidized during microbial metabolism to yield energy for growth and, in the best case, the organic carbon is converted entirely to CO_2. Some of the breakdown intermediates may also be assimilated as a carbon source for microbial growth. Functional groups (e.g., $-NH_2$, $-NO_2$, and $-SO_3$) may either be used as nutrients or cleaved from the carbon skeleton when the compound is reduced or oxidized. Oxidation can take place aerobically (in the presence of oxygen) or anaerobically (in the absence of oxygen). Oxygen serves two distinct functions. It can act as terminal acceptor of electrons that are released during the oxidation of electron donors, or it can react directly with the organic molecule. As an electron acceptor, oxygen can be replaced by other oxidized inorganic compounds such as nitrate, metal ions (e.g., Fe(III) or Mn(IV)), sulfate, or carbon dioxide, although the energy gains to the microorganism are then smaller. These alternate electron acceptors are reactants in anaerobic microbial processes, although they cannot substitute for the function of oxygen as a direct reactant (Schink, 1988).

Classes of organic contaminants known to be biotransformed by subsurface microorganisms are listed in Table 5.3 along with an indication of the required electron acceptor. In some instances, the compounds are the primary energy and carbon supply for the microorganisms (upper portion of Table 5.3). For other compounds, the biotransformation occurs as cosubstrate utilization where enzymes involved in the metabolism of one substrate are also able to degrade the contaminant (lower port of Table 5.3). Petroleum hydrocarbons and halogenated compounds are among the most prevalent organic contaminants at waste sites (Plumb, 1991). Petroleum hydrocarbons are easily degraded by certain aquifer microorganisms under aerobic conditions, but the anaerobic biodegradation of these compounds is much more variable and depends highly upon the compound, the terminal electron acceptor, and the specific site (Nales et al., 1998; Wilson and Bouwer, 1997). The aerobic co-oxidation of chlorinated solvents by methanotrophic bacteria while using methane as a cosubstrate (Table 5.3) is under intensive investigation for in situ bioremediation applications. In the absence of molecular oxygen (anaerobic conditions), halogenated organic compounds may be reductively dehalogenated during their degradation or be used as terminal electron acceptor for growth of microorganisms (Holliger et al., 1992; Mohn and Tiedje, 1991). In reductive dehalogenation, the halogenated

compound becomes an electron acceptor; and in this process, a halogen is removed and is replaced with a hydrogen atom. Detailed information on biotransformation of halogenated compounds is presented elsewhere (Adriaens and Vogel, 1995; Norris et al., 1994; Vogel et al., 1987).

TABLE 5.3. Some Important Classes of Organic Contaminants Susceptible to In Situ Bioremediation

Chemical Class	Frequency of Occurrence	Favorable Electron Acceptor(s)	Notes
		Primary Metabolism	
Monoaromatic hydrocarbons	Very Common	Oxygen[a], anaerobic[b]	Difficult to degrade if >4–5 rings
Polyaromatic hydrocarbons	Common	Oxygen[a], Nitrate[b]	
Aliphatic hydrocarbons	Common	Oxygen[a]	
Phenols	Infrequent	Oxygen[a], anaerobic[b]	
Nitroaromatics	Common	Oxygen[b], anaerobic[b]	
Alcohols, ketones, esters	Common	Oxygen[a], anaerobic[a]	
Some chlorinated solvents (CH_2Cl_2, CH_3CH_2Cl, $CH_2{=}CHCl$)	Common	Oxygen[a]	Biodegradable under a narrow range of conditions
Less halogenated aromatics	Common	Oxygen[a]	
Less chlorinated polychlorinated biphenyls	Infrequent	Oxygen[a]	Biodegradable under a narrow range of conditions
		Cosubstrate Metabolism	
Highly halogenated aliphatics	Very Common	Oxygen[a], anaerobic[a]	Aerobic cometabolism by methanotrophs in special cases; anaerobic cometabolism by many bacteria
Less halogenated aliphatics	Very Common	Oxygen[a], anaerobic[a]	Aerobic cometabolism by methanotrophs; anaerobic cometabolism by many bacteria
Highly halogenated aromatics	Common	Aerobic[a], anaerobic[a]	

[a]Many observations, confirmed in laboratory and supported by field evidence.
[b]Demonstrated, variable effectiveness in the field.

5.4.2 Treatment Approaches

The most important principle of bioremediation is that microorganisms (mainly bacteria) can be used to destroy hazardous contaminants or transform them to less harmful forms. The microorganisms act against the contaminants only when they have access to a variety of materials—compounds to help them generate energy and nutrients to build more cells. In some cases the natural conditions at the contaminated site provide sufficient quantities of essential materials that bioremediation can occur without human intervention—a process called *natural* or *intrinsic bioremediation*. In most cases, bioremediation requires the application of engineered systems to supply microbe-stimulating materials—a process called *engineered bioremediation*. Engineered bioremediation relies on accelerating the desired biodegradation reactions by encouraging the growth of more organisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions. Consequently, bioremediation can be considered as a continuum, extending from completely natural at one end to fully engineered at the other. The common and distinct issues for these two broad types of in situ bioremediation are discussed in the following two sections. An expanded discussion of the design considerations for in situ bioremediation of organic contaminants is given by Bouwer et al. (1998).

5.4.2.1 Intrinsic Bioremediation. Intrinsic bioremediation (also termed natural attenuation) relies on the innate capabilities of naturally occurring microbial populations to convert environmental pollutants to harmless forms. Intrinsic bioremediation occurs at many sites, sometimes at a rate significant enough to prevent further movement of the pollutant with the flowing groundwater. There is increasing interest in relying on intrinsic bioremediation for control of all or some of the contamination at waste sites (National Research Council, 1993; Rifai, 1998). Intrinsic bioremediation is attractive economically because it is relatively passive, requiring only a demonstration (via extensive site characterization) that natural biological processes are destroying contaminants in situ. Examples of sites where intrinsic bioremediation has been shown to play a significant role in attenuating organic contaminants in groundwater have been presented by Madsen et al. (1991), Godsy et al. (1992), Klecka et al. (1990), Davies et al. (1994), Cox et al. (1995), Bosma et al. (1997), Ravi et al. (1998), Kennedy et al. (1998), Mondello et al. (1998), Rijnaarts et al. (1998a, 1998b), and Rijnaarts (1998b).

Before intrinsic bioremediation can be considered as a legitimate cleanup method for a given site, the capacity of the indigenous microorganisms to metabolize the contaminants must be documented through laboratory and field testing (Chapelle and Bradley, 1998). Furthermore, the effectiveness of intrinsic bioremediation must be proven with a site-monitoring program to confirm the progress of contaminant biodegradation. Chemical analyses of contaminants, terminal electron acceptors, and/or other reactants and products indicative of biodegradation processes should be performed. Consequently, employing intrinsic bioremediation is in contrast to no-action alternatives.

For intrinsic bioremediation to be effective as a stand alone approach to aquifer or vadose zone restoration, the naturally occurring hydrogeochemical conditions at the site must allow the rate of biodegradation to exceed the rate of contaminant migration. Site conditions that favor intrinsic bioremediation include presence of microorganisms adapted to contaminant degradation, consistent and known groundwater flow throughout the year to ensure that contamination is not spreading with the flowing groundwater, presence of carbonate minerals to buffer acidity produced during biodegradation, ade-

quate supply of electron acceptors (for oxidative degradation), electron donors (for primary substrate and for reductive dehalogenation), and nutrients to meet the microbial growth requirements, and an absence of compounds that might be toxic to microorganisms (e.g., Hg, cyanide). Intrinsic bioremediation of petroleum hydrocarbons and monochlorinated compounds is especially applicable to sites where contamination is in the vadose zone and oxygen is more readily available. In areas where aquifers generally have low redox conditions (like in the Netherlands (Rijnaarts, 1998b)), these compounds are often not readily degraded by intrinsic processes. Microbial populations in such systems have not yet become adapted to the anaerobic degradation of petroleum hydrocarbons (e.g., benzene) which typically pose the greatest health risks and are the chemicals of most concern at the sites (van Heiningen, 1999). In contrast, highly chlorinated compounds (e.g., PCE, TCE, and hexachlorocyclohexanes) are often found to be naturally degraded by reductive processes in porous media with low redox conditions (Gerritse et al., 1999; Langenhoff et al., 1999; Rijnaarts et al., 1998b). This intrinsic bioremediation potential can be realized only when the contaminant load does not exceed the natural electron donor capacity.

5.4.2.2 Engineering In Situ Bioremediation. Although the potential for intrinsic bioremediation is substantial at many sites, it can often be unacceptably slow as a sole remediation strategy due to poorly adapted microorganisms, the limited availability of electron acceptors/donors and nutrients, cold temperatures, high concentrations of contaminants (NAPL), and mass transfer limitations in the subsurface. When site conditions are not favorable for natural biotransformation, bioremediation requires construction of engineering systems to supply microbe-stimulating materials. Engineered bioremediation relies on accelerating the desired biodegradation reactions by encouraging the growth of more organisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions. In some cases, bioaugmentation with adapted microbial consortia may be considered. Examples include the initiation of the anaerobic degradation of PCE and its degradation products cis-1,2-DCE and vinyl chloride (Morgan, 1998) or the stimulation of the anaerobic conversion of benzene to innocuous products (van Heiningen et al., 1999, in press).

Frequently, the necessary stimuli for microbial growth in the subsurface are oxygen and other electron acceptors (such as nitrate or sulfate) and nutrients (such as nitrogen and phosphorus) or organic substrates as electron donors. Typical engineered bioremediation systems therefore perfuse electron acceptors/donors and nutrients through the contaminated region as shown in Figure 5.2. Engineering in situ bioremediation near the land surface can be achieved by using infiltration galleries that allow water amended with nutrients and electron acceptors/donors to percolate through the soil. When contamination is deeper, in situ bioremediation systems inject the amended water through wells. Some in situ bioremediation systems use extraction and injection wells in combination to control the flow of electron acceptors/donors and nutrients and to hydraulically isolate the contaminated area. Another approach to engineered in situ bioremediation is extraction of contaminated groundwater combined with above-ground bioreactor treatment and subsequent reinjection of nutrient-spiked effluent (Bouwer et al., 1998; National Research Council, 1993).

The application of engineered bioremediation for aerobic cometabolic biodegradation of trichloroethylene (TCE) was recently demonstrated in field scale at the Edwards Air Force Base in California using toluene as primary substrate (McCarty et al., 1998). Groundwater contaminated with 500 to 1200 μg/L trichloroethylene was circulated between two

contaminated aquifers through two treatment wells located 10 m apart. Groundwater circulation was achieved by pumping groundwater from the 8 m thick upper aquifer to the 5 m thick lower aquifer in one well, while the second well pumped groundwater from the lower to the upper aquifer using flowrates between 25 and 38 L/min. Toluene (7 to 13.4 mg/L), oxygen, and hydrogen peroxide were injected into the recirculating groundwater to stimulate biofilm formation (bioactive zones) in the exit flow regions surrounding each well. With each pass through the bioactive zone in the upper aquifer, 87 ± 8% of the TCE in the contaminated groundwater was biodegraded. In the lower aquifer bioactive zone, TCE removals were as high as 83 ± 16% with each passage of contaminated water. The groundwater recirculation on a regional scale (multiple passes through the two bioactive zones) achieved 97–98% total removal of TCE. The average downgradient residual toluene concentration was 1.1 ± 1.6 μg/L, indicating that 99.98% of the primary substrate was biodegraded in the engineered system.

5.4.3 Bioavailability

Biodegradation rates in the field can be significantly slower than in the laboratory because of lower field temperatures and reduced bioavailability (Sturman et al., 1995). Bioavailability is generally defined as the availability of a chemical to biological transformation, and it is determined by the extent to which a chemical is exposed to organisms (Hamelink et al., 1994). Hydrophobic organic contaminants tend to partition among solid, liquid, and gas phases in the subsurface. For example, hydrophobic organic compounds can be found as a nonaqueous phase liquid, dissolved in the aqueous phase, sorbed to soil and sediment materials, and/or associated with colloids and other large organic molecules (e.g., natural organic matter) in water. Bioavailability of hydrophobic organic contaminants in the subsurface is affected by sorption/desorption in two important ways (Zhang et al., 1998). First, sorption diminishes the organic concentration in bulk water such that only a small fraction of the compound may actually be in the water phase. Most evidence indicates that microorganisms are most efficient in utilizing substrates dissolved in the aqueous phase (van Loosdrecht et al., 1990). Sorption causes high organic concentrations in microporous regions and impermeable zones to which bacterial access is obstructed. Separation of organic compounds by sorption from the aqueous phase is likely to reduce the rate and extent of biotransformation in the subsurface (Mihelcic et al., 1993). Second, because desorption and immobile zone diffusion must occur before biodegradation can proceed, the overall rate of bioremediation can be limited or even controlled by these mass transfer processes, not by the activity of the degrading microorganisms (Chung et al., 1993). The practical impact of reduced bioavailability is to decrease the rate of removal of the contaminants, thereby increasing the time required to achieve cleanup and the amount of chemicals that must be added to sustain microbial activity.

The influence of soil on the rate and extent of benzene, toluene, and naphthalene biodegradation has been demonstrated through a series of batch experiments (Zhang and Bouwer, 1997). Nearly complete aerobic naphthalene biodegradation (1.28 mg/L) by indigenous soil bacteria occurred within 60 h in aqueous solution (soil-free) while it took 2 weeks to degrade the same amount in the presence of 0.47 kg soil/L. The batch data also showed that the slower the desorption, the lower the biodegradation rate. Large soil particles exhibited a slower approach to equilibrium than smaller particles, implying intraparticle diffusion limited biodegradation. The rate of biodegradation was also observed to decrease with increasing organic compound hydrophobicity, soil/water ratio, and soil organic carbon content.

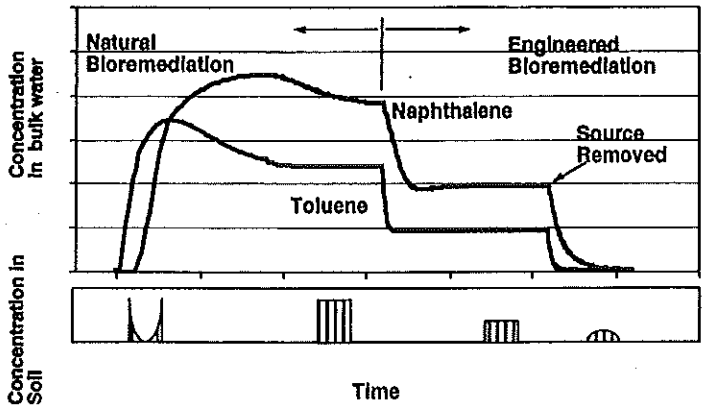

Figure 5.12 Simulations of sorption and biodegradation of toluene and naphthalene in the subsurface that illustrate the qualitative behavior of hydrophobic pollutants during bioremediation. The lower portion depicts the concentration profile along the centerline of the soil particle for different times.

A qualitative response of organic concentrations in aqueous and solid phases as a function of time during bioremediation in the subsurface is shown in Figure 5.12. Biotransformation is initially insignificant as the amount of biomass present is very small. Sorption removes a large portion of the organic compounds from the bulk water. As concentration gradients within soil aggregates diminish, organic concentrations in the bulk water increase quickly and finally level off and reach a steady state with biomass accumulated from utilization of the organic contaminant. As the contact time between the contaminant and the solid phase increases, the amount of the contaminant available for desorption or biodegradation tends to decrease. The mechanisms responsible for these contaminant aging effects are poorly understood (Luthy et al., 1997).

With engineered bioremediation, microbial activity is enhanced, which brings about a rapid decrease in the bulk water concentration (Fig. 5.12). This lowering of the bulk water concentration promotes desorption of the contaminants by increasing the local concentration gradient for diffusion (Rijnaarts et al., 1990). The desorbing contaminants can be biodegraded as they pass through the attached biomass, which keeps the bulk water concentrations low. Thus, the net rate of desorption is accelerated in the presence of biotransformation. However, if the rate of desorption is much slower than the biodegradation rate, stimulating microbial growth only has a minimal impact on accelerating the rate of soil decontamination. Furthermore, upgradient sources of contamination must be removed (e.g., removal and containment of separate phase organic liquids) in order to achieve effective in situ bioremediation of the contaminant plume.

5.4.4 Bioactivated Zones and Biobarriers

Often one of the first steps for remediation of groundwater pollution is containment of the dissolved contaminant plume. Bacteria can be injected into porous media to create either a bioactive zone to biodegrade contaminants in the flowing groundwater or a biobarrier by plugging of the formation (Cunningham et al., 1997). By reducing the overall permeability

and mass transport properties of the aquifer matrix, the creation of subsurface biofilm barriers (i.e., biobarriers) serves as an alternative technology for controlling the migration of contaminants from hazardous waste sites. Biobarriers may also be useful as a means of funneling contaminated groundwater through subsurface treatment systems (Fig. 5.13).

The first successful attempt at selectively plugging permeable strata with microbial biomass was conducted with ultramicrobacteria (UMB) (cell diameters from 0.3 to 0.5 μm) (MacLeod et al., 1998). The UMB are not motile, and therefore they followed the path of injected water in the subsurface environment. They penetrated wherever pore sizes were permissive, with a significant number retained in each pore structure by trapping and/or deposition. When a solution containing a suitable nutrient mixture for UMB resuscitation was subsequently injected into the porous medium, these nutrients initiated the relatively rapid process of resuscitation. The UMB returned to their active 1.0 to 1.4 μm form and began to reproduce. Immediately following their resuscitation, the active bacteria derived from these UMB began to grow, reproduce, adhere to surfaces, and secrete exopolysaccharides (EPS). The simultaneous nutrient-driven processes of reproduction and EPS secretion rapidly filled the available pore space (Lappin-Scott et al., 1988a). The processes of resuscitation and plugging could be controlled by selecting nutrients that were either fast acting or slow acting and exhibited different extents of assimilation by the resuscitating organisms and by the rate at which these nutrients were pumped into the porous medium. Rapid re-

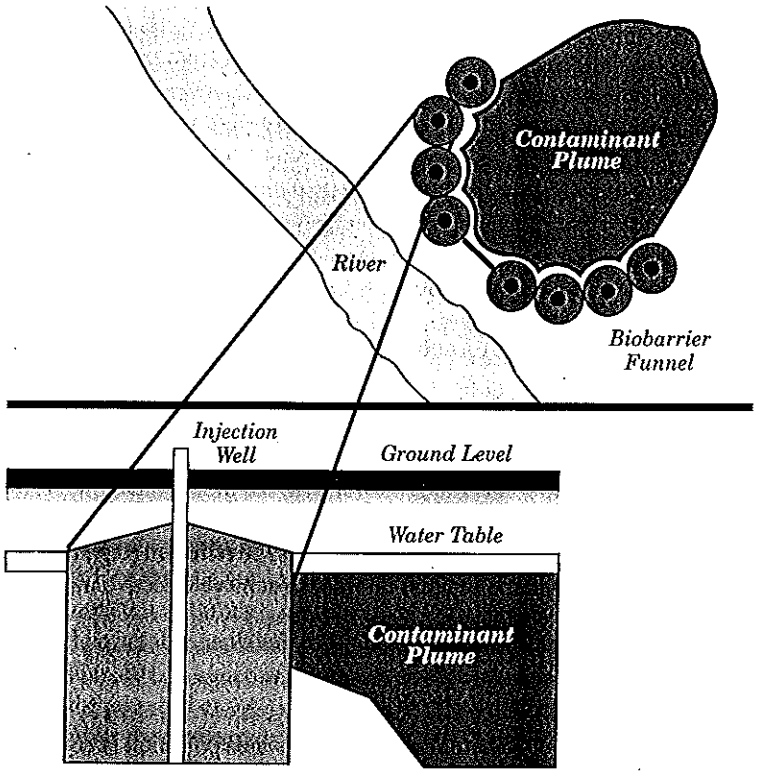

Figure 5.13 Schematic illustrating use of biofilm barriers to channel the flow of a dissolved contaminant plume through a zone of active treatment.

suscitation of UMB and slow rates of nutrient injection caused plugging very close to the injection point and limited the depth of the placed biobarrier.

Recent experiments with a series of packed-bed column and small tank reactors were conducted to evaluate biobarrier formation and persistence (Cunningham et al., 1997). An initial reduction in the hydraulic conductivity to less than 10% of the original value was observed in 91.4 cm long packed sand columns operated in a vertical mode. A reduction in hydraulic conductivity to less than 0.0001% of the initial hydraulic conductivity was obtained with simulated biobarrier formation in the small tanks (sand bed 91.4 cm wide, 30.4 cm deep, and 122 cm long). Biobarrier persistence in the columns and tanks was significantly different. Starvation conditions in the 91.4 cm long packed sand columns had a deleterious effect on biobarrier effectiveness. The sand column required continuous nutrient addition for barrier maintenance, while the tank barriers were able to persist for a considerable period without nutrient addition. The persistence of the biobarriers in the tanks compared to the column experiments may be due to the higher head pressure continually present in the column reactor design. The difference is approximately 122 cm of head above the top of the 91.4 cm column compared to approximately 3.6 cm of head across the 122 cm length of the tank. The higher head provides a higher flow rate and pore velocity through the columns, which may increase the detachment rate. The performance and formation of the biobarrier in the sand columns was not altered by the presence of 1 ppm strontium and 1 ppm cesium for periods up to 120 days. The column biobarrier challenged with 100 mg/L carbon tetrachloride for extended periods and with 300 mg/L carbon tetrachloride for short periods did not have any measurable effect on biobarrier stability as measured by changes in hydraulic conductivity. No detectable amounts of strontium or carbon tetrachloride were detected in the column effluent during these biobarrier challenge experiments.

Biobarriers are attractive for environmental purposes because of their potential to preferentially plug zones of high permeability. Also, they are conceptually not limited to near-surface application (i.e., 30 m or less) as is the case for sheet piling and grout curtains. Biobarriers are currently being considered for secondary oil recovery by water flooding (Cusack et al., 1992), protection of rivers and lakes from pollutant plumes in emerging groundwater (Cunningham, 1997), and for sealing of earthen dams, berms, and hazardous waste landfill liners (Lappin-Scott and Costerton, 1992).

Bioactivated zones constructed using strains that produce very little EPS would provide less resistance to groundwater flow. Bacterial cells within these "loose" biobarriers could be capable of pollutant degradation and/or metal reduction as groundwater passes through them. In some cases, a microbial population needs to be introduced to establish a certain biodegradation capability. In other cases, the indigenous microorganisms can be stimulated to degrade or immobilize contaminants. For petroleum hydrocarbons, chlorinated solvents, and chlorinated pesticides, feasibility and pilot test have been performed and implementation at full scale at several large contaminated sites is expected (Rijnaarts, 1998a). The hydraulic conductivity within bioactivated zones can be manipulated by seeding with bacterial strains with the desired clogging or nonclogging ability. This procedure was demonstrated by Koene and Rijnaarts (1996) in aerobically toluene degrading packed bed biofilm reactors under continuous flow conditions (Fig. 5.14). The reactors were packed with activated carbon (0.25–0.50 mm grain diameter), and parallel reactors were inoculated either with a pure culture of *Rhodococcus sp C. 125* (high blocking factor and unfavorable cell–cell interactions yielding a low α and low tendency to clog), an undefined mixed culture, or a pure culture of *Pseudomonas putida mt2* (low blocking factor and favorable cell–cell interactions yielding a high α and high tendency to clog). After 50 days of operation, hydraulic conductivity decreased from about 100 cm/day

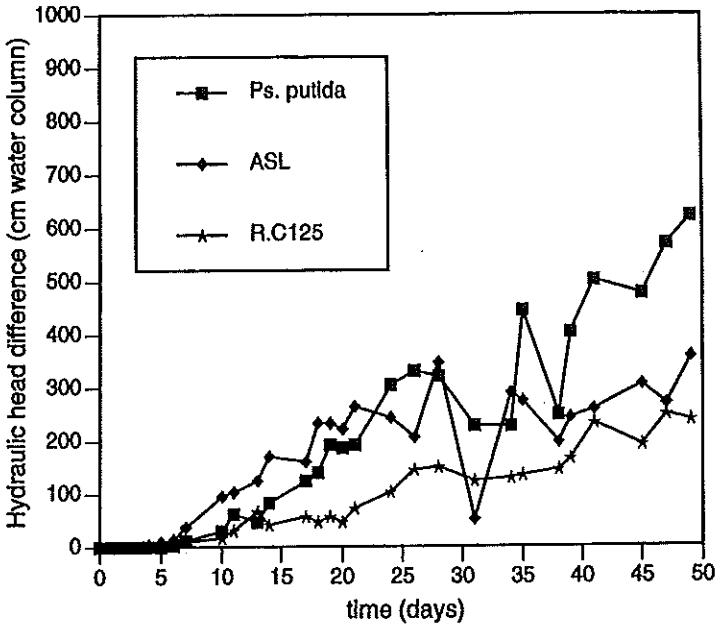

Figure 5.14 Pressure buildup in aerobic activated carbon-packed bed columns inoculated with a strongly blocking pure culture (*Ps. putida*), a weakly blocking pure culture (*R. C125*), and a mixed culture (termed ASL) and fed with toluene as the substrate.

to values of 24, 13, and 8 cm/day, for *R. C125*, the mixed culture, and *Ps. putida*, respectively. This finding shows that the nature of the cell–cell interactions of the dominant microbial population influences the hydraulic performance of a bioactivated zone or biobarrier.

5.5 CONCLUDING REMARKS

Biofilm accumulation in porous media is controlled by the processes of transport, adhesion, microbial growth, and detachment. Interactions between bacteria and solid surfaces strongly influence the behavior of bacteria in natural and engineering environments. Subsurface microorganisms are capable of transforming many organic contaminants. This has led to great interest in exploiting biological processes for in situ treatment including intrinsic bioremediation, engineered bioremediation, and biobarriers. More attention must be directed to the role of biofilms in contaminant transformations because biofilms predominate in many natural aquatic systems and have several advantages in engineered treatment systems. Future research should be especially directed toward developing methods to improve the bioavailability of hydrophobic organic contaminants.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the National Science Foundation (NSF) through Cooperative Agreement ECD-8907039 between NSF and Montana State University.

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