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Processes in microbial transport in the natural subsurface

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Abstract

This is a review of physical, chemical, and biological processes governing microbial transport in the saturated subsurface. We begin with the conceptual models of the biophase that underlie mathematical descriptions of these processes and the physical processes that provide the framework for recent focus on less understood processes. Novel conceptual models of the interactions between cell surface structures and other surfaces are introduced, that are more realistic than the oft-relied upon DLVO theory of colloid stability. Biological processes reviewed include active adhesion/detachment (cell partitioning between aqueous and solid phase initiated by cell metabolism) and chemotaxis (motility in response to chemical gradients). We also discuss mathematical issues involved in upscaling results from the cell scale to the Darcy and field scales. Finally, recent studies at the Oyster, Virginia field site are discussed in terms of relating laboratory results to field scale problems of bioremediation and pathogen transport in the natural subsurface.
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1. Introduction

Concern about pathogen contamination of groundwater and the use of bacterial agents in the cleanup of groundwater has highlighted the need for an improved understanding of the fate and transport of microbes in the subsurface. In particular, in situ bioremediation of contaminated groundwater may involve microbial transport promoted by intrinsic bioremediation (as part of natural attenuation; e.g., [21,173,178,188]), biostimulation (by the addition of substrates or electron donors; e.g., [113,184]), or bioaugmentation (by the introduction of microbial cells with specific function to the subsurface; e.g., [149,164]). Bioaugmentation in this context includes both injection of bacterial suspensions in the saturated zone near a contaminant plume and emplacement of solid media with attached bacteria as a 'biobarrier' through which mobile contaminant is expected to pass and be degraded [65,129]. In general the science underlying the processes involved in in-situ bioremediation is currently topical and well within the public view [13].

The design of remediation schemes involving subsurface biodegradation requires understanding the processes governing the fate and transport of the microbes under the particular physical, biological, and geochemical conditions involved. Most bioremediation techniques rely on the advective dispersive transport of chemical species to modify metabolism and on the transport of the microbial cells themselves. Cell transport occurs both by convection of aqueous-phase organisms and by generation of new aqueous-phase microbes through growth. Other important processes that can limit the effectiveness of such schemes include cell predation, cell decay, and cell attachment to solid surfaces [78,113].

In this article we focus on the physical, chemical, and biological processes involved in the transport of bacteria

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Nomenclature

C_{mm}	concentration of aqueous "mobile microbes" (per unit pore volume, ML^{-3})
C_{im}	concentration of attached "immobile microbes" (per unit pore volume, ML^{-3})
C_A	volumetric aqueous biomass concentration
$C_{A\gamma}$	concentration of microorganisms in the fluid phase
C_{As}	surface concentration of microorganisms
$\mathbf{D}$	dispersion tensor (L^2T^{-1})
$\mathbf{D}_O$	position dependent diffusion tensor
$\mathbf{D}_{eff}$	effective dispersion tensor
$\mathbf{D}_\gamma$	microbial diffusion coefficient
d_μ	random motility coefficient (L^2T^{-1})
d_s	diameter of microorganism (L)
d	diameter of particle (collector, L)
g	acceleration due to gravity (LT^{-2})
K_f, K_r	forward and reverse attachment/detachment rates (T^{-1})
k_f	forward (adhesion) kinetic constant
k_r	reverse (detachment) kinetic constant
kT	thermal energy of microbe
$\mathbf{V}$	pore water velocity (LT^{-1})
$\mathbf{v}_\gamma$	average particle velocity of microbe in the pore space
$\mathbf{V_s}$	sedimentation velocity (LT^{-1})
V_ω^{mm}	rate of displacement of aqueous biomass in the residence time dimension (TT^{-1})
V_ω^{im}	rate of displacement of attached biomass in the residence time dimension (TT^{-1})

Greek letters

α	collision (or sticking) efficiency parameter in filtration theory
ε_γ	porosity
η	collection (or collector) efficiency parameter in filtration theory
ρ	fluid density (ML^{-3})
ρ_s	biocolloid particle density (ML^{-3})
ω	generalized exposure-time (here residence time; T)

in the saturated subsurface. Since our main focus is on the basic science of bacterial transport processes, we do not treat in great detail either conventional models of bacterial attachment/detachment kinetics (recently reviewed in Clement et al. [24], and in Murphy and Ginn [82]), or bulk/column experiments elucidating apparent effects of aqueous or mineralogical physicochemical conditions (such as grain size distribution, presence of mineral oxides or organics, aqueous ionic strength, pH, and velocity; recently reviewed in Murphy and Ginn [82], and in Harvey and Harms [55]). Transport of other microorganisms (e.g., viruses) is often treated by considering similar approaches, but will not be considered in detail in this work (the interested reader is referred to Schijven and Hassanizadeh [110]).

We will first review the various mathematical representations of bacterial phases in the subsurface, and then review the subsurface physicochemical and biological microbial processes controlling behavior on short (e.g., bioremediation) time scales. Next we describe quantitative representations of these processes, in the context of both continuum and particle-based models and their mathematical linking at the microscale. Then we introduce the macroscopically relevant biomass-balance model, and summarize particular forms that arise with the incorporation of certain attachment–detachment processes. Finally we briefly review field bacterial transport experiments and discuss a number of issues that impact the application of current process descriptions and models at the field scale.

1.1. Conceptual and mathematical representation of subsurface biomass

The mathematical description of the kinetics of biomass transformation resulting from processes such as growth and cellular attachment to surfaces first requires a conceptual model of the biotic phases. In Fig. 1 we have illustrated (schematically) several scales of the hierarchy of length-scales that are important to microbial processes in porous media. In this picture we have represented the biomass as being contained within one of two phases: the aqueous phase (γ-phase) and the solid-associated biofilm phase (ω-phase).

Aqueous-phase biomass is commonly treated as a dilute suspension of 'free-living' (i.e., aqueous) cells, and is typically mathematically represented as a dilute reactive solute (e.g., [168,191]). The dilute assumption neglects interactions between aqueous cells, and this assumption will not be valid when there are significant cell interactions such as those encountered in cell clumping and quorum sensing [125]. Approaches that invoke the interactions among cells have not yet been developed; however, such effects have been rigorously incorporated in the description of the transport of simple colloids [89,90]. A similar analysis for representing the interactions among microorganisms represents a significant challenge, and is an open area for continuing research.

Unlike aqueous-phase biomass, biomass associated with the solid-phase is usually treated via one of three

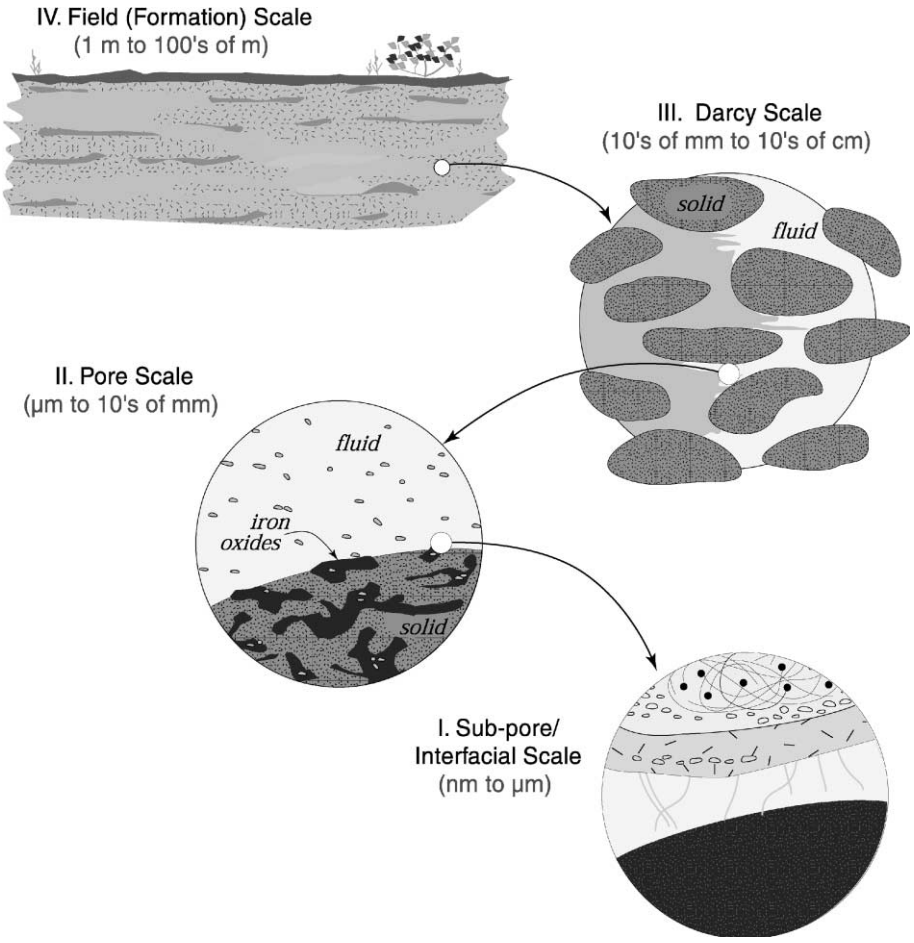

Fig. 1. A representation of the hierarchy of process length-scales important to microbial transport.

conceptual models, as discussed originally in [142,143, 157,186]. Biofilm models include those which consider the structure of the biofilm phase and those that do not. The primary differences between these two descriptions is that the structured models have the potential to represent the influence of biofilm structure on mass transfer (both diffusion and convection within the fluid and biofilm phases) and on momentum transfer (i.e., the change in permeability due to reduction in the pore volume), whereas unstructured models do not.

Typically, structured biomass models are represented as either (1) continuous biofilm on the solid surface [182, 183] or (2) discontinuous patchy film [123,175,187]. As discussed by Baveye and Valocchi [142], there has been perhaps too much emphasis on making the distinction between the two kinds of models rather than focusing on the fundamental transport and reaction processes that apply to the biofilm system (the early debate regarding the appropriateness of the various conceptual models is reflected in the exchanges of [141,142,157,186]). A mathematically consistent model should be capable of accounting for any reasonable biofilm geometry, and research has begun to examine the potential for formally upscaling biofilm processes in porous media [36,135,136]. Several researchers have used the biofilm concept in modeling microbially mediated electron-transfer reactions in laboratory experiments [145,170,182]. Dykaar and Kitanidis [36] used a biofilm model within a structured porous medium to study continuum-scale reactive transport by averaging the pore-scale equations defining diffusion-limited mass transfer and biotic reactions to determine an effective mathematical model at the bulk scale. They found that in some cases the pore fluid is not well mixed, and mass transport limitations associated with biofilm/pore geometry can control macroreaction rates; for diffusion-limited cases the rate of degradation was found in model simulations to be strongly correlated with porefluid velocity. MacDonald et al. [71] use a structured biofilm approach as a basis for quantifying the effect of momentum transfer in incurring biofilm shear in situ bioremediation schemes. MacDonald et al. [72] examine the role of growth in a structured subsurface biofilm in the clogging of porespaces during bioremediation. Refs. [34,35] are the first to combine pore-scale biodegradation and bacterial growth, using a structured approach, with quantitative treatment of the mechanics of

biofilm deformation. Biomass is modeled as a continuous uniform isotropic hyperelastic material, using both structure colony (aggregate) and biofilm conceptual models, whose expansion and deformation are governed by material mechanics stress–strain relations. Results in the context of a 2D lattice porous medium suggest that aggregates have a much greater potential impact on clogging than biofilms.

In the so-called unstructured biophase approach, no structural presumption is imposed on the biophase [22,23,162,180,191] and the biomass is treated as a suspended, but kinetically sorbing/desorbing species [169,191]. Therefore, in unstructured models the biomass is a fully penetrable volumeless component which assumes that a linear relation exists between mass of substrate consumed and mass of biomass produced and that no diffusion limitations affect the transfer of substrate mass from solution into the biomass. This approach has been taken in model construction [25,144,159,162], in column studies that focus on bacterial transport [69,120] and in intermediate-scale flow cell studies that focus on active degradation and growth and coupled transport [130,169]. For instance, Macquarrie et al. [162] used this approach in treating biomass involved in aerobic degradation as a volumeless species undergoing transport, with equilibrium partitioning of biomass between aqueous and attached phases. Wood et al. [130] used the unstructured approach in treating the biophase as irreversibly attached, in analysis of experiments in layered medium.

The degree to which the particular structural assumption impacts the resulting expressions for reaction transformation is incompletely understood. For example, the process of metabolic lag [131,153] may be *experimentally* indistinguishable from a substrate diffusion limitation through a biofilm. Both processes result in a delay in the onset of degradation, and the correct labeling of the process may or may not have an effect on the ultimate amount of contaminant degraded. Challenges in validating any conceptual model are associated with the difficulty in gathering data at the proper (pore) scale, with non-linearities in models associated with biofilm growth, and with the tremendous spatial and temporal variability of biofilm properties. Peyton and Characklis [171] report that published biofilm density values range from 10 to 130 kg/m^3 bulk volume. These values typically vary even more greatly under conditions of active biodegradation [82]. However, a quantitative foundation for understanding microbial processes in porous media may best be achieved through structured models explored at the pore-scale. Recently, several studies have used volume averaging to formally upscale the processes of mass transport and reactions in biofilms [36,132–135], and experimental work is beginning to be conducted to expose the structure of biofilms within experimental systems [34,123,136]. Continuing investigations such as these may help to refine our understanding of the role of biophase structure in the subsurface.

In Section 1.1, we have considered the different conceptual models that have been used to represent the distribution of biomass between the aqueous phase and the solid-associated biofilm phase. These conceptual models provide the framework within which the processes described in the remainder of this review can be modeled. The only conceptual model in common use for aqueous-phase biomass is that of a dilute suspension with no cell-to-cell interactions. Accounting for such interactions poses a challenge for future research. Three different conceptual models have been used for solid-phase biomass: continuous biofilm, discontinuous patchy biofilm, and unstructured biophase. The first two are physically structured models that consider the influence of biofilm geometry on mass transfer, while the unstructured model treats the biomass as a suspended but kinetically sorbing/desorbing species. It is unclear which of these models is most appropriate for representing biofilm reaction transformations, but ongoing theoretical work aimed at upscaling biofilm reactions via volume averaging and experimental work aimed at exposing biofilm structures may clear up these uncertainties.

2. Microbial transport processes

Microbial transport in the subsurface involves a host of complex and interacting processes. It is thus not surprising that the literature contains many inconsistencies regarding the effects of bacterial variables such as size, shape, hydrophobicity, and electrostatic charge [67]. As such, it is not currently possible to state definitive correlations between bacterial properties and transport. Because microbes are living organisms, their transport in the subsurface is more complex than is the case for abiotic colloids. Not only are they subject to the same physicochemical phenomena as are colloids, but there are also a number of strictly biological processes that affect their transport (e.g., temporal changes in surface properties due to changes in metabolic state; predation by other subsurface organisms). Because of the complexity of the combined physicochemical and biological processes, these processes are described in separate sections in the material following.

There is not necessarily a clear taxonomic distinction between processes that are physicochemical and those which are biological; in fact many processes important to microbial transport are in fact coupled physicochemical-biological phenomena (e.g., the effects of cell-surface macromolecules on bacterial partitioning and adhesion). In this section we will focus on the fundamental physics of the processes affecting microbial

transport, with the idea that these process may change over time due to associated changes in the biological state of the microorganisms (which is described in the subsequent sections).

2.1. Physical processes

Most reactive transport models incorporate a variety of physical processes, such as advection, dispersion, straining, and physical filtration. Unlike the biological processes, physical processes affecting microbial transport have been the focus of numerous experimental and numerical modeling studies. These important processes provide the framework of bacterial transport and reaction in porous media. Indeed, the impact of biological processes in a flowing groundwater system can only be evaluated within this physicochemical framework. Readers are referred to reviews by Harvey et al. [51] and McDowell-Boyer et al. [167] for more thorough discussions of these processes.

2.1.1. Transport

Microbes undergo convective transport as a particulate or a dissolved species moving with the pore-water whose velocity is governed by the hydraulic pressure gradient, porosity, and permeability distribution. The occurrence of nutrient and/or electron acceptor as a solute undergoing transport may be coupled to the transport process through the effects of these constituents on the fluid properties of density and viscosity. Convective transport in porous media is also associated with hydrodynamic dispersion, the mixing process arising from the tortuosity of the convective paths compounded by molecular-scale (diffusional) or particle-scale (Brownian) mixing. The resulting convective–dispersive flux $\mathbf{J}$ is given by the classical form $\mathbf{J} = C_{\mathrm{mm}}\mathbf{v} - \mathbf{D}_{\mathrm{eff}} \cdot \nabla C_{\mathrm{mm}}$ where $\mathbf{v}$ is the average pore-water velocity vector and $\mathbf{D}_{\mathrm{eff}}$ is the 2nd-order hydrodynamic dispersion/diffusion tensor [26]. See Nomenclature for all symbols.

2.1.2. Straining and filtration

Straining and physical filtration represent the removal of microbes from solution by physical (geometric and intermolecular/surface) forces. Straining is the trapping of microbes in pore throats that are too small to allow passage and is exclusively a result of pore geometry [25]. Estimates based on purely geometric relations between the effective diameter of biocolloids and the diameter and packing (coordination number) of grains suggest that mass removal by straining is not significant where the colloid diameter is less than 5% of the porous media grain diameter [25,51,155,167,176,177]. It should be noted that in natural heterogeneous porous media, a fraction of the pore diameters may be small enough to cause straining of colloidal particles even though the average grain diameter passes this rule of thumb for non-significance of straining.

Physical filtration is the removal of particle mass from solution via collision with and deposition on the porous media; here the term includes both attachment and sedimentation. Attachment of bacteria in the natural subsurface via filtration is often (but not always; e.g., [169]) treated using colloid filtration theory (CFT) [94], which posits the kinetic rate of attachment as

$$\text{kinetic attachment rate} = \left\lfloor \frac{3}{2}\frac{1-\varepsilon}{d}\alpha\eta\|\mathbf{V}\|C_{\mathrm{mm}} \right\rfloor \qquad (1)$$

where C_{mm} is the bulk aqueous concentration of mobile microbes, $\mathbf{V}$ is the parameter velocity ε is porosity, d is average diameter of the porous media grains, η is the collection efficiency (defined as the fraction of microbes approaching an idealized porous media grain that actually collide with the grain) and α is the collision (or 'sticking') efficiency (defined as the fraction of microbes colliding with the idealized porous media grain that actually attach to its surface). Generally, η is calculated a priori based on bacterial and porous media properties, and then α is calibrated with the use of data from column experiments. The literature contains a helpful clarification on the use of the analytical expressions for η [70], as well as a summary of CFT's major assumptions and the implications for modeling bacterial transport in natural porous media [83].

Sedimentation is filtration due to gravity [25,167] and depends on particle buoyancy [185]. Many natural bacteria and viruses are neutrally buoyant, in which case sedimentation is negligible. However, cultured microorganisms are typically larger and sometimes more dense than their native counterparts [54] and may involve sizeable buoyancy-driven filtration. The effect has been approximately quantified in Harvey et al. [54] by augmenting the advective pore-water velocity with an additional downward component whose magnitude is given by the classical Stokesian velocity of a dense sphere falling through a fluid; $\mathbf{V_s} = (r_s - r)gd_s/18\mu$, where $\mathbf{V_s}$ is the sedimentation velocity (acting vertically downward), ρ_s is the cell density, ρ is the solution density, g is the gravitational acceleration, μ is the dynamic viscosity, and d_s is the cell diameter (treated as a sphere). It should be pointed out that the sedimentation velocity sometimes appears as an *additional* velocity magnitude in the CFT kinetic rate of attachment (above); however, this velocity acts only downward. The proper magnitude for use in the CFT is that of the total sum of velocity components (i.e., one takes the magnitude of the sum of the velocities, not the sum of the magnitude of the velocities).

2.1.3. Size exclusion

Size exclusion results in bacterial and ion tracer breakthrough times that are different than those of a

non-reacting tracer. Size exclusion effects have been observed in laboratory columns [37,92,114,156,164] and in field experiments [50,172,189]. Exclusion is a phenomenon where transported particles move faster than the mean pore-water velocity, and involves an increase in the transport rate due to the size or charge of the material conveyed. Field experiments have reported cell transport velocities as much as 70% greater than the mean pore-water velocity, apparently due to exclusion [59]. Identification of exclusion from observations of tracer and particle breakthrough is not straightforward and has led to some confusion in the literature. Often, the time to arrival of the peak breakthrough concentration or center of mass of the breakthrough curve are used as indicators of exclusion. However, attenuation by kinetic attachment with minimal detachment can have the effect of shifting the peak (and center of mass) to earlier times even in the absence of enhanced velocity, rendering these indicators unreliable [28,138]. Time to first breakthrough is also not a reliable indicator because of differences in sensitivity of detection methods for microbes versus solutes. The most reliable indicators of exclusion are (1) a significant difference in fitted advective velocities in a model parameter estimation exercise, or (2) significantly higher normalized concentrations (C/C_0) of suspended microbes during the rising limb of the breakthrough curve. These issues are discussed further by Zhang et al. [138].

With regard to processes causing exclusion, one may distinguish anionic and size effects, and further divide size effects into classical chromatographic and "pore exclusion" processes. Anion-exclusion involves velocity enhancement by channeling of anionic molecular-scale solute particles in finer-grained porous media away from pore walls due to electrostatically repulsive forces that act on nanometer scales, and as such are not generally significant for bacterial transport (although it may amplify size-exclusion when bacteria are like-charged to fine-grained media). Pore-water velocity within a capillary or pore throat is generally parabolically distributed, with the maximum velocity occurring at the centerline and that at the pore walls equal to zero [26]. Conventional Taylor–Aris transport theory assumes that molecular-scale solutes eventually thoroughly sample the full distribution of velocities. Microbes and large colloids, by virtue of their size, preferentially experience the higher velocities near pore center-lines, yielding an average velocity that is higher than that of a dissolved tracer. Thus microbes can precede the tracers down gradient. The occurrence of exclusion typically requires the bacterial diameter be less than 1% of the media mean grain diameter, which is common for transport in sandy aquifers [26,148].

When the colloidal particle is of the same scale as a significant fraction of pore channels, not all pores are accessible. The presumed rerouting of particles to alternate pore throats (or alternate porous materials at the macroscopic scale) in this case occurs on a relatively larger detour scale than does chromatographic or ionic exclusion. Termed pore exclusion [127], this has been suggested as a velocity enhancement of the excluded material in natural aquifers (Gvirtzman and Gorelick [48], for anions in unsaturated flow; Rehmann et al. [97] for virus transport). Note that pore exclusion is also termed specifically "size exclusion" by some authors [98], as well as various other terms such as "volume exclusion" [4], "pore size exclusion" [116], or "size exclusion chromatography" [50]. The term "differential advection" has also been introduced [138,139] to generally describe the phenomenon of earlier breakthrough of colloids relative to a solute tracer without specific regard to the mechanism. The mechanics and modeling of pore exclusion has been recently debated [44,98]. In coarse-grained media size exclusion is a larger factor than pore exclusion [49], because the excluded particle is far smaller than almost all pores. However, exclusion is not consistently manifest in larger-scale field studies, cf. [53] even under consistent conditions, and so the final impact over long-term transport is unknown [49].

Modeling tools for exclusion in hydrogeology are generally introduced as modifications of classical transport or attachment/detachment coefficients. Engfield and Bengtsson [37] examined the effects of unaccounted exclusion on the effective value of partition coefficients for macromolecular transport, and Shonnard et al. [114] analyzed early breakthrough of microbes relative to phenol red in a capillary in the context of Taylor–Aris dispersion theory [181]. Shonnard et al. [114] assign the microbe a lower radial diffusivity than a molecular solute so that the microbial transport is dominated by convection, on the basis of which they use the high Peclet limit solution of the capillary convection–dispersion equation. This approach is critiqued in Ginn [45]. Another approach, used in anion exclusion, involves reducing the kinematic porosity by an "excluded" fraction that is unavailable to the anion. The remaining porosity is then divided into mobile and immobile fractions [48]. However, Mailloux et al. [76] found that porosity reduction according to an idealized media model was insufficient for treating the evident size-exclusion of a bacterium in intact sand cores.

Ginn [45] develops a mathematical approach to incorporating exclusion effects in a lagrangian context, relating the distribution of excluded particle travel-times to that of non-excluded, or "ideal" particles, such as reflected by a dye tracer test. The analysis involves a constitutive 'speedup' function that tells how travel-times of non-excluded particles map to those of excluded particles. An inverse operator that identifies the speedup function given experimentally observed cumulative arrival distributions (e.g., breakthrough curves) of excluded and unexcluded particles in the same flow field is derived,

and used in analysis of data on cryptosporidium breakthroughs in saturated columns, from Harter et al. [49]. Scheibe et al. [106] modeled exclusion phenomena observed in core experiments using a modified particle tracking approach. In this approach, the distribution of local dispersive displacements (corresponding to the value of dispersivity estimated from conservative tracer breakthrough observations) was truncated at the lower end to represent the exclusion of bacteria from regions of the pore space with very small local velocity (i.e., very near pore walls). This approach was demonstrated to be effective at simulating observed large exclusion effects (bacterial velocities nearly double that of conservative tracers) with minimal truncation (on the order of 5%) of the dispersive displacement distribution. This approach also leads to decreased apparent dispersion of the bacteria relative to conservative tracers, consistent with theoretical considerations and observations [116].

In Section 2.1, we have considered the physical processes governing microbial transport in subsurface environments. Advection and dispersion are described by a flux expression that depends on fluid and porous media properties, the coupled effects of nutrient and electron acceptor solutes on fluid density and viscosity, the tortuosity of convective paths, and Brownian diffusion. Straining describes the trapping of microbes in pore throats due to the relative size and geometry of the microbes and the pore throats, while filtration describes the removal of microbes from solution via collision with and attachment to the porous media. Sedimentation is filtration in which the collision step occurs due to the force of gravity. The attachment step of the filtration process is not well understood and is the subject of ongoing research. Exclusion, another topic of ongoing research, is preferential transport in which microbial size or charge causes the cell to experience the higher values of the pore-water velocity distribution. These physical processes are relatively well understood and provide the context within which we must strive to better understand the more elusive chemical and biological processes described in Sections 2.2 and 2.3.

2.2. Electrostatic and chemical processes

Although the physical processes described above are well understood by most hydrologists, the influence of electrostatic and chemical interactions between microorganisms and solid surfaces are not as familiar. These forces may act over characteristic lengths that are only fractions of nanometers to microns, but ultimately they determine how microorganisms adsorb and desorb from the solid surface, and thus can dramatically affect microbial transport at the largest scales. In this section, we examine the nature of some of these forces, and discuss the need to examine microbe–surface interaction forces from a fresh perspective.

2.2.1. Understanding the interaction potential: is the DLVO appropriate for microbes?

Colloid filtration theory, introduced above, assumes that attachment is a two-step process. First, the bacterium must be transported to the porous media grain (the "collector"). Second, the physicochemical interactions that occur upon contact of the two surfaces determine if the bacterium attaches to the surface of the collector. Traditionally, the transport mechanisms have been thought to be better understood than the attachment mechanisms. Consequently, the collection efficiency parameter, η, that represents the transport step is calculated a priori from characteristics of the bacterium, the porous media, and the flow field. The sticking efficiency (α, representing the attachment step in filtration theory) is then treated as a fitting parameter in conjunction with experimental data. Recent work suggests that this conceptual model is erroneous in that the transport and attachment steps may in fact be coupled [77].

Once a bacterium is transported to within a separation distance on the order of fractions of its own radius away from a collector, a complex set of interactions occurs that dictates the outcome of the attachment possibility. The Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloid stability has been widely employed as a model for describing the interaction forces between a microbe and a solid surface. The DLVO force is the sum of the London–van der Waals force and the electrostatic force (i.e., the "double layer" force which may be repulsive or attractive depending on the charges of the two interacting surfaces); the theory has been extended to cover a host of other possible interactions, including so-called Lewis acid–base forces, and steric interaction forces [3,16,100].

Although the DLVO theory is a useful paradigm for thinking about the interactions between microorganisms and solid surfaces, Ninham [84] has pointed out that it applies only for certain well defined conditions that are not in general met for microbial cells. The assumptions most flagrantly violated are that the colloidal surface is molecularly smooth, solid, and inert. Detailed critical assessment of assumption validity can be found in Ninham [84]. The unsuitability of the DLVO theory (or its extensions) is supported by direct measurements of the interaction forces between bacteria and solid surfaces obtained via atomic force microscopy. Such measurements often show substantial disagreement with the predictions of DLVO theory both in magnitude and decay distance [16,137]. Similar conclusions have been reached by the observation of *Escherichia coli* taxis along a glass surface; the bacteria–surface interactions could not be explained by DLVO theory [124]. Although DLVO theory has been extended to include acid–base (hydrophobic) interactions [122], hydrophobic interactions are often negligible under common conditions of subsurface bacterial transport [79,112].

Moreover, bacterial adhesion data often also deviates from the predictions of the extended DLVO model [63].

It has been suggested that the failures of DLVO and extended DLVO theory are most likely caused by the presence of polymers, other macromolecules, and structures such as pili and flagella on the bacterial surface [16,63,86]. Thus, an understanding of polymer-mediated interactions would seem to be a priority for the successful prediction of bacterial attachment rates in porous media. Bacterial polymer layers can cause a steric repulsive force [63,100,112,121] as well as an attractive bridging force [63,64,101,150]. The occurrence of attraction or repulsion depends on the coverage degree, polymer characteristics, and type of solvent [101]. Of critical importance to the interplay of attractive and repulsive tendencies is the relative affinity of the polymers for the solid surface and for water. Both types of steric interaction may operate for a given polymer and since the attraction and repulsion potentials will depend differently on the separation distance, the net result is likely to change signs as separation varies [101]. Heterogeneity in the physical and chemical properties of the polymers on a bacterium would yield a force potential that is distributed over the cell surface. In the case of chemical heterogeneity, the force distribution would be related to the distribution of polymer affinities. In the case of physical heterogeneity, the force probability could be related to the distribution of polymer lengths. Polymer bridging may be enhanced by polymer layers having a very small frequency of long polymer chains, which are able to extend far beyond the reach of the bulk of the polymer layer. The long chains would not encounter the same energy barrier as the rest of the polymer layer, and if only a few of them form hydrogen bonds, the binding force would be sufficient for irreversible attachment. This scenario has been proposed as an explanation for the observed adhesion of *Stenotrophomonas maltophilia* [64]. Similar arguments can be made for larger cell-surface structures such as flagella [86].

In the last decade, atomic force microscopy (AFM) has surfaced as a viable technology for making the interaction force measurements required for the development of theory for predicting polymer-mediated interactions. In addition to force measurements, AFM can be used to characterize cell surface properties (e.g., polymer conformations—[17]; for a review of AFM cell surface characterization in general, see [32]). The basic set-up involves a probe attached to a cantilever. The probe is moved towards the microbial sample until attachment occurs, and then it is retracted. The force is calculated from the deflection of the cantilever via Hooke's Law (or more complicated analyses that incorporate cantilever geometry). In general, data from the approach can provide information on the transport step, and data from the retraction can provide information on the attachment step [15].

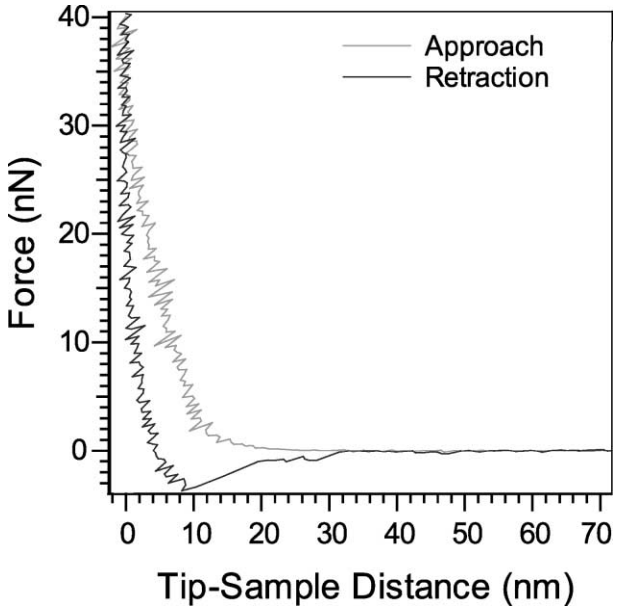

Fig. 2. A force curve obtained via AFM. The force curve was measured for a 5 μm silica sphere interacting with a single bacterial cell (*B. cepacia*) at an ionic strength typical of groundwater at the Oyster site (described in following sections).

An example of a typical microbe-mineral surface interaction force curve obtained via AFM is presented in Fig. 2. For this interaction force curve, *Burkhoderia cepacia* (strain 866A) were attached to a glass slide, and probed using a 5 μm silica sphere attached to a silica nitride cantilever. Two different force curves are observed; one for the approach of the silica sphere to the bacterial surface, and one representing the retraction of the sphere once contact had been made. Several interesting features have been commonly observed in such curves [38,56]. First, note that the approach curve represents almost exclusively repulsive forces. Second, note that the force curve is hysteretic; that is, the retraction curve is substantially different from the approach curve (in fact, the retraction curve shows the presence of an *attractive* force). Such curves have been observed for purified biopolymers [41], and it is likely that macromolecules on the cell surface are responsible for much of the observed behavior.

This interpretation is further supported by direct observation of macromolecules (and larger functional structures such as pili and flagella) on the surfaces of microorganisms. In Fig. 3, two electron micrographs are shown illustrating the potential extent of macromolecules and physiological structures on cell surfaces. Fig. 3(a) shows an SEM micrograph of *B. cepacia* (for which the force curve given in Fig. 2 was generated). It is clear in this picture that substantial amounts of cell surface macromolecules are present; from additional TEM mi-

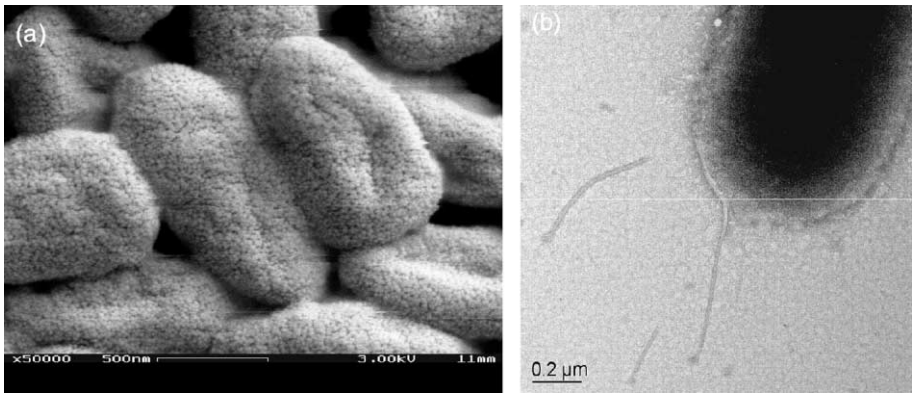

Fig. 3. (a) An SEM micrograph of *B. cepacia* showing copious extracellular macromolecules. (b) A TEM micrograph of *S. putrefaciens* showing the presence of pili.

crographs it was determined that this polymer layer is on the order of 30–50 nm thick. Fig. 3(b) shows a TEM micrograph of a whole-mounted cell counterstained with uranyl acetate. The bacterium is *Shewanella putrefaciens* (a metal reducing organism) and in this micrograph the presence of several long (~0.8–1 μm) pili are evident. It is intuitive that the presence of these surface features plays a substantial role in mediating the transport of these organisms. In the case of *B. cepacia*, the force curves in Fig. 2 provide compelling evidence of the influence of the surface macromolecules. We refer to these surface structures as extracellular polymeric substances (EPS). A general description as to how the particular features of the force curve illustrated in Fig. 2 are obtained is depicted schematically in Fig. 4.

For this hypothesis, we envision the cell surface exhibiting a repulsive force due to rearrangement of the macromolecules (Fig. 4, step A) as the tip approaches the surface; note that this is essentially a steric (entropic) interaction. Although attractive forces may exist between the tips of individual macromolecules and the probe tip surface, these attractive forces are overwhelmed by the net repulsive force cause by rearrangement of the macromolecular network structure. As the tip is withdrawn (Fig. 4, step B), the force gradually decreases (although not necessarily along the same trajectory as the approach curve). At some point, the net force will reach zero and then begin to become attractive. This attractive force is cause by the adhesion of the macromolecules to the probe tip (Fig. 4, step C). This description is supported by the shape of the retraction curve, which shows some jumps in the interaction force. This is typical of polymers as individual or clusters of macromolecules break away from the probe surface [11].

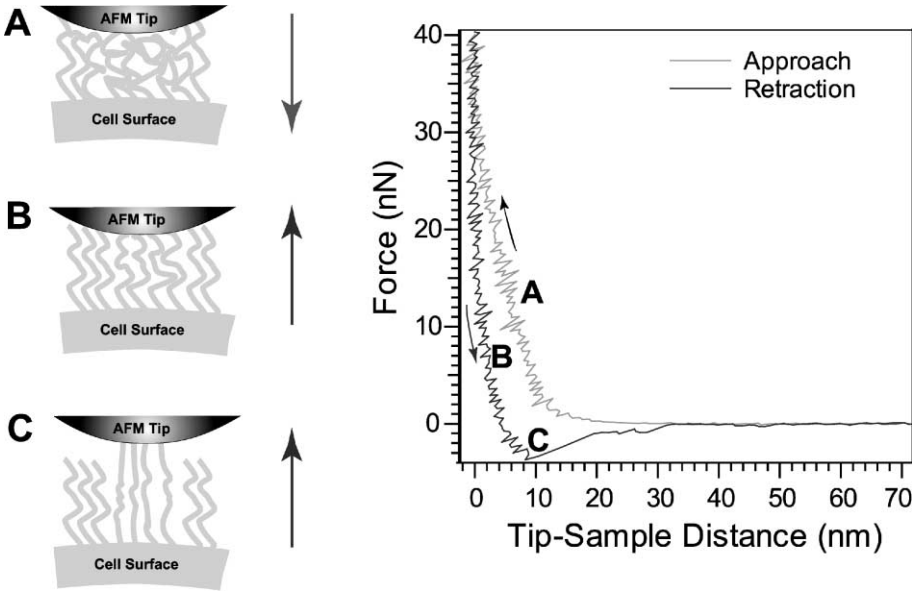

Fig. 4. A general description of behavior of cell-surface macromolecule behavior that might lead to the observed interaction force curve.

Much of the difficulty that has been encountered in using the potential function as a measure of the tendency for microorganisms to adsorb to surfaces may be due in part to surface macromolecules and the presence of hysteresis. In many instances, the length of the surface macromolecules (or surface structures) is greater than the characteristic distance of interaction forces predicted by conventional methods. In these instances, the microbial adsorption process is dominated by the interactions between the macromolecules and the solid surface; the bulk of the cell itself may not play a significant role in terms of the interaction forces. Similarly, conventional methods do not predict potential functions that are hysteretic, and this may be a crucial step in understanding microbial adsorption and adhesion. Hysteresis in microbial interactions with solid surfaces is most likely due to the formation of polymer bridges, and so the development of a hysteretic potential function will require an understanding of the biomechanics of polymer bridge formation (e.g., the effects of polymer conformation, elasticity, and affinity for the solid surface).

As a final example of the potential role of macromolecules and cell surface structures on the transport of microorganisms in porous media, we have plotted the breakthrough curves of two different transport experiments using the same bacterium with different surface properties. Fig. 5 depicts the results of transport experiments conducted in 10 cm columns packed with clean 40/60 silica sand (Accusand). The bacterium DA001 (*Comamonas* sp., cf. Section 3) was prepared for these transport experiments using three different protocols (after Cacavo [14]): (1) treatment with the enzyme β-glucuronidase (at a concentration of 100 Units/ml), (2) treatment with protease and chymotrypsin (at a concentration of 250 μg/ml for each enzyme), and (3) no treatment. As described by Cacavo, these enzymes can alter specific macromolecules on cell surfaces (polysaccharides, polypeptides, and proteins), and this allows some (semi-empirical) determination of what macromolecules most strongly affect adsorption and adhesion under the experimental conditions.

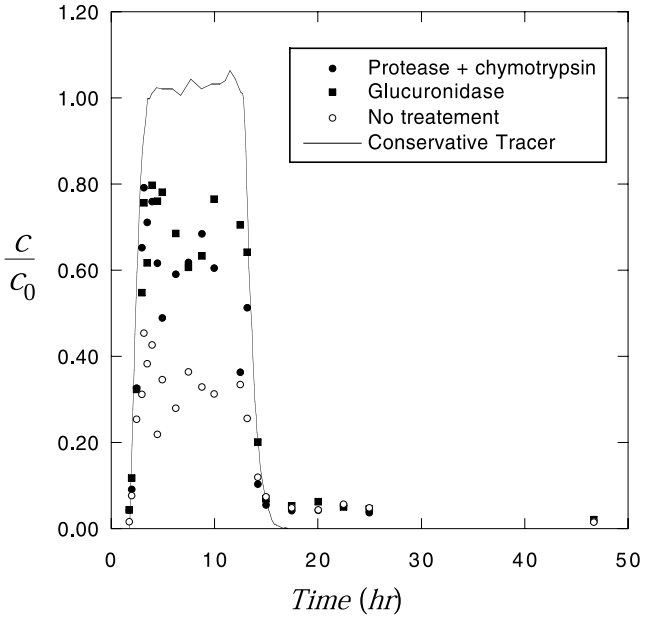

Fig. 5. The effects of surface macromolecules and structures on the transport of the bacterium DA001 (*Comamonas* sp.). Organisms were prepared using three different surface treatment protocols. The effects of the treatment protocols can be clearly seen in the breakthrough curve results.

The bacteria were injected into the columns at a concentration of 1×10^7 cells/ml (along with a bromide tracer) for 12 h at a flow rate of 100 cm d^{-1}; after 12 h, cell-free solute was injected at the same flow rate. Cell concentrations were measured in the effluent using AODC direct counts. The treatment with the enzyme β-glucuronidase substantially reduced the adsorption of organisms to the solid surface. The treatment with protease and chymotrypsin also reduced the adsorption of cells to the solid surface, although to a slightly lesser degree. These results suggest that the adsorption of these cells may be strongly mediated by cell surface macromolecules, and both polysaccharides and proteins may be involved in cell adhesion. Post modeling of these data using a linear adsorption–desorption model (cf. Eqs. (3)–(6)) showed that the effective forward kinetic rate parameter for β-glucuronidase treatment was less than one-half of that for the untreated cells, indicating the forward adsorption rate was lower for the β-glucuronidase treated organisms than for untreated cells. These experiments provide indirect, although compelling, evidence that extracellular polymers can be altered to affect microbial adsorption and adhesion.

2.2.2. Recent progress on modeling and understanding microbial adsorption and adhesion

Some initial progress in representing the role of EPS on adsorption and adhesion has been made. Ortiz and Hadziioannou [85] used an atomic force microscope (AFM) to directly measure the entropic elasticity of individual polymer chains. After a polymer was tethered to an AFM probe tip, retraction of the tip yielded single, continuous, attractive peaks. These peaks were fit to two different entropic-based statistical-mechanical models of polymer elasticity, the 'freely jointed chain' (FJC) model and the 'wormlike chain' (WLC) model. Camesano and Abu-Lai [18] performed similar experiments on individual surface polymers of *Pseudomonas putida* KT2442. Their retraction curve data was fit to the FJC model, and their results indicated that biopolymer heterogeneity on a single cell cannot be explained by differences in molecular weights alone but may be influenced by chemical differences as well. They hypothesized that the

presence of multiple polymers with different properties on a cell surface may be the chief cause for the difficulty in predicting bacterial adhesion. The magnitude and decay of repulsive forces encountered on the approach of the AFM tip have been shown to be much better described by an electrosteric repulsion model [140,147] than the predictions of classical DLVO [16]. The data from both approach and retraction curves have been correlated with type of microorganism, physiological state (e.g., dormancy vs. germination), and ionic strength [33]. In spite of these initial advances, Camesano and Logan [16] suggest that presently too few studies have been completed to state definitive relationships between bacterial polymer properties and the interaction forces that may either promote or inhibit attachment. However, further use of AFM for force and elasticity measurements coupled with detailed characterization of the polymers being studied may be able to produce such relationships.

A number of researchers have investigated the relationship between bacterial polymer properties and attachment likelihood via adhesion experiments on smooth surfaces. The isoelectric point (IEP) of a bacterium was found to be correlated with adhesion to Teflon and glass [102]. An IEP $\leqslant 2.8$ indicated the significant presence of cell surface polysaccharides containing negatively charged phosphate and/or carboxyl groups, which may inhibit adhesion. An IEP $\geqslant 3.2$ indicated the absence of polymers that inhibit adhesion. In a more detailed study of bacterial surface polysaccharides, lipopolysaccharides (LPS) were extracted from five Gram-negative bacterial strains and the adhesion of the isolated LPS to SiO_2, TiO_2, and Al_2O_3 was studied [64]. However, even with information on the chemical structure of the O-antigen monomers, the composition of repeating units, the presence of branched or straight O-antigens, or the length of the O-antigens, the adhesion of the isolated LPS to the surfaces could not be predicted. Two different studies have demonstrated a possible link between the loss/attenuation of O-antigens on bacterial LPS, decreased hydrophobicity, and decreased adhesion [30,75]. Other studies have shown an opposite linkage between O-antigen loss/attenuation and adhesion [118,126] and an opposite linkage between O-antigen loss/attenuation and hydrophobicity [57,73,118]. A general conclusion of Williams and Fletcher [126] was that multiple polymers are likely to be involved in determining the adhesiveness of a given bacterial species, which would be consistent with the variability and flexibility of attachment properties that have been observed with different substrata and environmental conditions.

In Section 2.2, we have considered the interactions that occur between bacteria and solid surfaces at close separation distances. The experimental results and theoretical observations of several researchers suggest that the DLVO theory of colloid stability does not adequately describe such interactions when the colloids in question are microbes. Elucidating the ways in which cell surface macromolecules influence the adhesion process is a topic of current research.

2.3. Biological processes

Additional biological processes affecting microbial transport are expressed through the growth/decay process and include active adhesion/detachment, survival, and chemotaxis. The biological nature of these processes presents a challenge for transport modeling in that one biological mechanism is often dependent on and/or influenced by another biological mechanism. Thus, it may be necessary to consider the interdependency of the various biological processes.

2.3.1. Active adhesion/detachment

Active adhesion/detachment is treated here as a biologically driven process. Several studies have reported that microorganisms exhibit active adhesion/detachment processes that may be a response to local nutrient availability [121,146,160], survival mechanisms [146,152,190], and/or growth ([60], 1986; [99,179]). No generally accepted quantitative treatment of dynamic biologically mediated adhesion/detachment processes exists. It is possible that the evidently dynamic attachment/detachment processes are in fact ramifications of EPS mechanics or other cell-driven factors under transient metabolic states. Smets et al. [117] reported experimental results indicating the adhesion of a pseudomonad to glass was significantly more favorable in the exponential growth phase than in the stationary or decay phase. They hypothesized that differences in the cell surface structure, the cell physicochemistry, or the hydrodynamic behavior of the cells were the most likely reasons for the enhanced adhesion of exponential phase cells. Thus the distinction between a microorganism's response to nutrient availability, survival stress, and growth are not necessarily separable nor independent processes.

2.3.2. Chemotaxis

Microorganisms that have the capability to move in response to a chemical gradient are termed chemotactic. Both random motility (taxis in the absence of a chemical gradient) and chemotaxis have been cited as potential means of transport for subsurface organisms [8,25,60,80,99]. Quantitatively, random motility is an effective diffusive flux for microorganisms that depends on the local spatial gradient in aqueous microorganism concentration, and chemotaxis is a flux of microorganisms associated with the gradient in nutrient supply. Chemotaxis requires energy and therefore is closely linked to growth processes in porous media. In oligo-

trophic environments nutrient gradients will be quite small and will likely be associated with either preferential flow paths (if the nutrients arise from recharge) or solid-phase chemical heterogeneity. Chemotaxis may be a very important transport mechanism in these low-nutrient environments. Mercer et al. [80] found that bacteria subjected to oligotrophic conditions displayed enhanced chemotactic response. A contaminant plume will result in large chemical gradients that may also contribute to microbial transport via chemotaxis. Like virtually all microbial characteristics, tactic capability varies widely among organisms. In addition, the chemotactic capability of a given organism can vary depending on the nutrient to which it is being attracted. However, only a small fraction of the many organism-nutrient systems found in natural subsurface conditions and bioremediation schemes have been studied (see Lewis and Ford [68] for a compilation of random motility and chemotactic sensitivity coefficients that have been reported for a few different organisms and nutrients). Therefore, these organism-specific and nutrient-specific transport characteristics have not been incorporated into predictive models of microbial transport applicable for field-scale hydrogeological applications.

Much work has been done on developing basic models of chemotactic transport of cell populations in response to gradients in aqueous-phase nutrients. These efforts and the resulting models are beyond the current scope. The interested reader is referred to Ford and Cummings [39], which delineates the relationships between the various models and derives a reduced form of the rigorous three-dimensional cell balance equations of Alt [2], as well as the review by Ford and Cummings [40], which includes a comparison of model predictions with experimental results. Applying our understanding of chemotaxis in a bulk aqueous medium to the modeling of chemotaxis within a porous medium is still a matter of considerable uncertainty. However, Barton and Ford [8,9] derived expressions that relate the standard random motility and chemotactic sensitivity coefficients for bulk water to effective values that reflect the impact of the porous medium on an individual cell's swimming behavior. The application of their model to experimental data of bacterial migration through sand cores was consistent with the observed reduction in random motility and provided an explanation (the chemoattractant gradient was too small) for the observed lack of a chemotactic response. The Barton and Ford model modifies the advection–dispersion transport operator (see Section 2.1.1) to represent random motility and chemotaxis as microbial transport fluxes driven by the random motility coefficient (d_μ) and the chemotactic velocity ($\mathbf{v}_\chi$), respectively. d_μ describes diffusion-like random motions, and $\mathbf{v}_\chi$ describes the cell's velocity in the direction of increasing substrate concentrations. This modeling approach is an adaptation for porous media of the classical chemotaxis models reviewed in Ford and Cummings [39,40].

In Section 2.3, we have considered biological processes such as active adhesion/detachment and chemotaxis that may be represented through the growth/decay process. There is no generally accepted quantitative treatment for biologically driven active adhesion/detachment. Moreover, there is uncertainty in distinguishing the occurrence of active adhesion/detachment. The process of chemotaxis also lacks a validated model for use in porous media environments. Nonetheless, both of these processes may have significant impacts on subsurface microbial transport and warrant further theoretical and experimental research.

2.4. Combining physical, chemical, electrostatic, and biological processes: the Smoluchowski equation

The transport of microorganisms within a fluid can be described by a convection–dispersion type equation when the suspension is dilute and the particles are far from phase interfaces. However, in porous media the size of the microorganisms may be comparable to length-scale associated with pores. Under these conditions the microbe–solid surface interactions become important for describing the microbial transport phenomena at the sub-pore scale, and in general these interactions must be accounted for.

For systems with physically realistic interaction force functions and that do not exhibit hysteresis, it is possible to develop a continuum transport equation that accounts for the particle–surface interactions. From a statistical-mechanical analysis [89,90,103], one can show that for incompressible flows the relevant transport equation takes the form:

Smoluchowski equation

$$\frac{\partial C_A}{\partial t} + \nabla \cdot (C_A \mathbf{v}_0) = \nabla \cdot (\mathbf{D}_0 \cdot \nabla C_A) + \nabla \cdot [(\mathbf{D}_0/kT \cdot \nabla \Phi_A) C_A] \quad (2)$$

The terms in this expression reflect change in volumetric biomass concentration, advective flux, dispersive/diffusive flux, and interactions force effects, respectively. In Eq. (2), C_A is the volumetric aqueous biomass concentration, $\mathbf{v}_0$ is the velocity field for the particles, $\mathbf{D}_0$ is the (position dependent) diffusion tensor for the particles, k is the Boltzmann constant, T is the temperature (in K), and Φ_A is the (position dependent) potential function for the microbe–solid surface interactions. In principle Φ_A can also be a function of time, and this time dependence (if known) would allow one to account for changes in the microbe–surface interactions as cell physiology changes in time.

At the sub-pore scale, Eq. (2) provides the complete description of the evolution of a suspension of particles.

There are three main difficulties in applying Eq. (2) to applications of microbial transport in porous media: (1) it is a micro-scale equation, and therefore applies at the pore scale rather than at the Darcy scale; (2) the length-scale associated with the potential function may be much smaller than the length scales associated with convection, and this makes the problem difficult to solve; and (3) the potential function Φ_A (a function of separation distance between the surface and the particle) is generally not known for microbes, and it may be hysteretic (for which case Eq. (2) does not apply). We will briefly address the first issue in the next paragraph, and the second issue in Section 3; the remaining issue is a topic for further investigation. We do note, however, that until an expression analogous to Eq. (2) is developed for hysteretic potential functions, it will not be possible to make further progress on the understanding of the Eulerian sub-pore-scale modeling of physical, electrical, and chemical processes when hysteresis in the force potential is significant.

3. Macroscopic representation of microbial transport

Ideally, the complex physicochemical and biological interactions that are manifest at the molecular-, cell- and pore-scale would be formally included in a description of cell transport at the Darcy (REV) scale and above. This connection can in principal be made using various upscaling methods that link sub-pore-scale (and below) processes to their effective representation at the Darcy scale (and above). However, these efforts are only just beginning, and because of the complexity of the processes there is still substantial research to be done for describing microbial transport in the subsurface. Additionally, this research is progressing on multiple fronts and at multiple scales. Below we will describe some of the work that is being conducted on upscaling and representing microbial transport in the subsurface, with a caveat that much of the material described below represents research in progress.

3.1. Scaling microbial transport from the cell scale to the Darcy scale

In any practical application, Eq. (2) would have to be solved by numerical methods. Some difficulties are encountered in this approach, primarily (1) the length scales associated with Φ_A, $\mathbf{D}_0$, and $\mathbf{v}_0$ are generally disparate, making the problem difficult to solve numerically; and (2) it is necessary that the solution be computed at the sub-pore scale, whereas in general we are interested in transport at the Darcy (or REV) scale. One solution to this difficulty is to link the sub-pore-scale processes to their effective Darcy scale representation via volume averaging [128,47] or another upscaling procedure [87].

The sub-pore-scale representation of microbial transport and adsorption is usually represented not by Eq. (2), but by the more familiar set of equations

$$\frac{\partial C_{A\gamma}}{\partial t} + \nabla \cdot (C_{A\gamma}\mathbf{v}_\gamma) = \nabla \cdot \left(\mathbf{D}_\gamma \cdot \nabla C_{A\gamma}\right) \text{ in the fluid phase} \quad (3)$$

$$-n_{\gamma\kappa} \cdot (\mathbf{D}_\gamma \cdot \nabla C_{A\gamma}) = k_f C_{A\gamma} - k_r C_{As} \text{ at the fluid–solid boundary} \quad (4)$$

$$\frac{\partial C_{As}}{\partial t} = k_f C_{A\gamma} - k_r C_{As} \text{ at the fluid–solid boundary} \quad (5)$$

Here, $C_{A\gamma}$ is the concentration of microorganisms in the fluid phase, C_{As} is the surface concentration of microorganisms, $\mathbf{v}_\gamma$ is the average particle velocity of the microorganisms in the pore space (note that this is in general different than the fluid velocity), $\mathbf{D}_\gamma$ is the microbial diffusion coefficient, $\mathbf{n}_{\gamma\kappa}$ is the unit normal vector pointing outward from the fluid phase toward the solid phase, k_f is the forward (adhesion) kinetic constant, and k_r is the reverse (detachment) kinetic coefficient.

The constitutive interfacial flux condition given by Eq. (5) represents a reasonable but empirical formulation (research efforts to relate Eq. (2) to the interfacial constitutive flux condition given by (5) are currently in progress [137]). The right-hand sides of Eqs. (3) and (4) represent attachment–detachment kinetics in the simplest form (first-order reversible kinetics independent of suspension velocity). Note that, although not explicitly identified, this sub-pore formulation implicitly accounts for other colloid processes such as gravity settling, straining, taxis, and interception through the velocity field and through the kinetic parameters k_f and k_r. More complex kinetic forms that have appeared in the literature will be introduced below. If one adopts Eqs. (3)–(5) as the sub-pore-scale starting point, it can be shown that the Darcy-scale representation takes the form [137]:

Darcy-scale transport equation

$$\frac{\partial \langle C_{A\gamma}\rangle^\gamma}{\partial t} + \langle \mathbf{v}_\gamma\rangle^\gamma \cdot \nabla(\langle C_{A\gamma}\rangle^\gamma) = \nabla \cdot (\mathbf{D}_{eff} \cdot \nabla \langle C_{A\gamma}\rangle^\gamma) - \frac{a_\gamma}{\varepsilon_\gamma}(k_f \langle C_{A\gamma}\rangle^\gamma - k_r \langle C_{A\gamma}\rangle_{\gamma\kappa}) \quad (6)$$

Here $\langle C_{A\gamma}\rangle^\gamma$ represents the volume averaged intrinsic (pore-water) concentration, $\langle C_{A\gamma}\rangle_{\gamma\kappa}$ is the surface-averaged surface concentration, $\langle \mathbf{v}_\gamma\rangle^\gamma$ represents the volume averaged intrinsic (pore-water) velocity, $\mathbf{D}_{eff}$ is the effective dispersion tensor, a_γ is the area per unit volume of the fluid–solid interface, ε_γ is the porosity, k_f is the adsorption kinetic parameter, and k_r is the desorption kinetic parameter (in the context of linear reversible kinetics for attachment/detachment).

Although Eq. (6) is of the form that is typically proposed on the basis of a strictly macroscopic analysis, the method of volume averaging lends the additional benefit of explicitly tying the sub-pore-scale parameters and problem geometry to the definitions of the effective parameters that appear; this connection is made through the development of a closure problem [128]. In this case, the only effective parameter that appears is the effective dispersivity, $\mathbf{D}_{\text{eff}}$, and Quintard and Whitaker [93] have provided an extensive analysis and associated closure problem for predicting $\mathbf{D}_{\text{eff}}$ for aerosol filtration. Although the closure problem required for predicting $\mathbf{D}_{\text{eff}}$ for microorganisms will be slightly different than the aerosol problem, the essential features and details of the analysis have been carried out in the work presented by Quintard and Whitaker [93].

Note that in the development of Eq. (6) we have used notation that is consistent with the volume averaging literature. For consistency in the notation used elsewhere in this paper, note that Eq. (6) can be put in the form

$$\frac{\partial C_{\text{mm}}}{\partial t} + \mathbf{V}\cdot(\nabla C_{\text{mm}}) = \nabla\cdot(\mathbf{D}_{\text{eff}}\cdot\nabla C_{\text{mm}}) - (K_{\text{f}}C_{\text{mm}} - K_{\text{r}}C_{\text{im}}) \tag{7}$$

where we have made the correspondences:

$$C_{\text{mm}} = \langle C_{\text{A}\gamma}\rangle^{\gamma}, \quad \mathbf{V} = \langle \mathbf{v}_{\gamma}\rangle^{\gamma}, \quad C_{\text{im}} = \frac{a_{\gamma}}{\varepsilon_{\gamma}}\langle C_{\text{A}\gamma}\rangle_{\gamma\kappa},$$
$$K_{\text{r}} = k_{\text{r}}, \quad K_{\text{f}} = k_{\text{f}}a_{V}/\varepsilon_{\gamma}$$

In Section 3.1, we have considered the upscaling of microbial transport processes from the sub-pore-scale (and below) to the Darcy-scale (and above). Volume averaging is an approach that can be used to upscale the disparate length scales associated with the advection, diffusion, and surface interaction terms of the Smoluchowski equation to the Darcy-scale relevant for transport problems. Volume averaging explicitly incorporates the sub-pore-scale parameters and problem geometry into the definitions of the Darcy-scale effective parameters.

3.2. Conventional models of bacterial attachment/detachment kinetics

Modeling of attachment/detachment kinetics is extensively reviewed in Clement et al. [24] and in Murphy and Ginn [82], as well as in Harvey and Harms [55]. Because we focus here on cyto-scale processes not well captured by conventional models, the reader is referred to those articles for details; but a brief summary of classical approaches serves as a useful reference point. The linear attachment/detachment kinetic model appearing (as the last two terms on the right-hand side) in Eq. (7) is perhaps the most basic approach, and reflects the essentially universal treatment of bacterial attachment/detachment as a kinetically controlled process. This model may be viewed as a modification of the colloid filtration theory (CFT) approach noted above, wherein a linear detachment process is included (no such detachment occurs in original CFT), and wherein dependence of the rate coefficient for attachment on pore-water velocity is ignored. Murphy et al. [169] extend the linear reversible model to include non-linear dependence of the rate coefficients on ionic strength of solution, manifest in intermediate-scale experiments. Tan et al. [120], Lindqvuist et al. [69], and Saiers and Hornberger [105] all introduce the classical site-saturation limiting factor on the attachment rate coefficient, in order to account for potential depletion of available surface sites as attached microbe densities increase, which may occur when aqueous microbes cannot attach to attached microbes. Ginn [43] modeled non-Markovian (i.e., residence-time dependent) attachment/detachment kinetics apparent in experiments of McCaulou et al. [165] using the exposure–time approach of Ginn [153], as described below.

3.3. Metabolic effects on microbial transport

Some macroscopic-scale evidence suggests that bacterial attachment/detachment kinetics are metabolically mediated, in ways distinct from EPS-associated mechanisms described above. In an experiment conducted in an intermediate-scale flow cell (100 cm × 20 cm × 10 cm dimensions), a substrate pulse resulted in an increase in aqueous-phase bacteria (Fig. 1, [169]), similar to observations in field bioremediation efforts (US DOE, 1993). Column experiments have suggested that the response observed in [169] may be cell-division mediated transport, a mechanism long recognized in the microbiology literature [60,99,161,179]. Cell-division mediated transport has also been referred to as mother–daughter or shedding cells and is when the "mother" cell, attached perpendicular to the mineral surface, grows, divides, and the "daughter" cell is released into the aqueous phase [163]. The mother cell remains attached.

Many investigators have noted that starvation or nutrient availability can stimulate a change in the partitioning of a microbial community between the solid and aqueous phases. A contaminant plume creates a dynamic nutrient environment, but it is not clear whether the corresponding response in partitioning of the microbial community will have any effect at all on the actual contaminant degradation. Therefore, Ginn et al. [154] investigated the relative importance of dynamic partitioning of the bacterial phase on contaminant de-

gradation by modeling the response of a consortium of anaerobic bacteria involved in the degradation of chlorinated hydrocarbons. This example concerns the stimulation of a natural subsurface microbial community that would be initially associated with the solid phase. When substrate is present, as in a contaminant plume, the propionate degrader displays dynamic partitioning (e.g., the forward attachment rate, K_f, changes with the level of metabolic activity). The simulation results showed enhanced degradation under dynamic conditions due to the aqueous partitioning of one member of the consortium that resulted in an increasing population moving with the plume, and hence increasing degradation. This simulation illustrates the importance of the potential partitioning of bacteria under dynamic growth conditions during in situ biodegradation.

3.3.1. Exposure time model for biofilm formation

One instance of dynamic partitioning occurs when the propensity for a microorganism to become irreversibly attached to a solid phase depends on the residence time of the microorganism near the mineral surface. Such a manifestation may arise as a result of hysteresis in EPS-associated near-field forces described above, and/or as a result of metabolic changes in overall surface-solid bonds (e.g., as in biofilm formation). The following is distilled from Ginn [43] and from Murphy and Ginn [82]. Residence time is defined here as the amount of time a microorganism is reversibly associated with a surface through a specific interaction, such as electrostatic, van der Waals, or hydrophobic interactions. Irreversible attachment is usually associated with active adhesion processes on the part of the microbe [151,174]. For instance a microbe may exhibit slow (relative to transport) cell-surface changes such as exopolysaccharide production [126,158] associated with biofilm formation that effectively increase the probability of irreversible attachment over a population of microbes. Conventional descriptions of partitioning kinetics at the bulk scale (e.g., Eq. (7)) are incapable of capturing this behavior because such models cannot track the distribution of biomass over the contiguous residence time. This limitation is noted in Johnson et al. [61] who provide a heuristic accounting of the effects of residence time on reversibility by zeroing the detachment rate for microbes whose residence time exceeds a particular threshold. A new theoretical approach allows the tracking of residence time effects on arbitrary reaction terms [153]. This approach supports both variable methods of accounting of residence time (e.g., cumulative vs. contiguous) as well as arbitrary specification of the effect of residence time on the overall partitioning kinetics.

In [153] a reformulation of the conventional fate and transport mass balance (our Eq. (7)) is developed that allows distributions of solutes such as biomass over space $\mathbf{x}$ and time t, and generalized exposure-time (here residence time) ω on surfaces, so that C_{mm} is $C_{mm}(\mathbf{x}, t, \omega)$. The result is a mass balance equation system just as Eq. (7), but with the addition of a *convection term* dictating the evolution of the biomass over space, time, and the residence time coordinate ω

$$\begin{aligned}&\frac{\partial C_{mm}}{\partial t} + \mathbf{V}\cdot(\nabla C_{mm}) + \frac{\partial (C_{mm} V_{\omega}^{mm})}{\partial \omega} \\ &\quad = \nabla\cdot(\mathbf{D}_{eff}\cdot\nabla C_{mm}) - (K_f(\omega)C_{mm} - K_r(\omega)C_{im}) \\ &\frac{\partial C_{im}}{\partial t} + \frac{\partial (C_{im} V_{\omega}^{im})}{\partial \omega} = (K_f(\omega)C_{mm} - K_r(\omega)C_{im})\end{aligned} \tag{8}$$

where now V_{ω}^{mm} is the rate of displacement of aqueous biomass in the residence time dimension (just as V_x^{mm} is the rate of displacement in the x-dimension) and V_{ω}^{im} is the rate of displacement of attached biomass in the residence time dimension. Also, note that the rate of detachment K_r is now expressed as a function of residence time ω, that is, $K_r = K_r(\omega)$. If indicated, one may also specify a dependence of attachment rate, K_f, on residence time, ω. We assume that in a time unit specified by the expressions K_f and K_r, the number of partitioning events is exactly equal to the number of partitioning cells. Thus during one unit time, exactly K_f of the local aqueous cells attach and exactly K_r of the local attached cells detach, and no other partitioning events (such as an attached cell detaching and then reattaching) occur in that same unit time. This assumption sets the characteristic timescale of the partitioning event, and in doing so, links the units of the first order rate coefficients (K_f, K_r) with the residence time on the surface. Details of this "no-recrossing" assumption appear in Ginn [42].

We can now specify a detachment coefficient that reflects non-Markovian detachment, as it depends on the residence time on surfaces, as $K_r(\omega)$, where K_r (e.g., detachment rate) decreases with increasing residence time, ω. Following the suggestion of Johnson et al. [61], Ginn [43] put $K_r(\omega)$ as a positive constant to some critical residence time, $\omega = \omega^*$, beyond which the rate of detachment $[K_r(\omega)]$ is zero. That is,

$$K_r(\omega) = \begin{cases} K_r, & 0 \leqslant \omega \leqslant \omega^* \\ 0, & \text{otherwise} \end{cases} \tag{9}$$

There are three ways to keep track of the residence time on surfaces as described in Ginn [43]. For illustration we consider only the *contiguous* case where kinetics of detachment depend on recent residence time on surfaces. In the contiguous memory model, the physiologic state of the microorganism depends on some finite memory of historical attachment, and so time spent in the aqueous phase after any given attachment event results in a kinetically controlled return to a pre-attached state (e.g., slow loss of memory of attachment). So time spent in the

aqueous phase between attachment events results in reversal of the physiological changes in the cell surface.

One way to achieve this reversal to unattached state is through the specification of the "aging" velocities appearing in Eq. (8) as $V_{\omega}^{\mathrm{im}} = 1$ to track accumulation of time on surface, and $V_{\omega}^{\mathrm{mm}} = -\omega$, to allow kinetic forgetfulness of time on surface, in which case our model takes the form

$$\begin{aligned} &\frac{\partial C_{\mathrm{mm}}}{\partial t} + \mathbf{V}\cdot(\nabla C_{\mathrm{mm}}) - \frac{\partial(C_{\mathrm{mm}}\omega)}{\partial \omega} \\ &\quad = \nabla\cdot(\mathbf{D}_{\mathrm{eff}}\cdot\nabla C_{\mathrm{mm}}) - (K_{\mathrm{f}}C_{\mathrm{mm}} - K_{\mathrm{r}}(\omega)C_{\mathrm{im}}) \\ &\frac{\partial C_{\mathrm{im}}}{\partial t} + \frac{\partial C_{\mathrm{im}}}{\partial \omega} = (K_{\mathrm{f}}C_{\mathrm{mm}} - K_{\mathrm{r}}(\omega)C_{\mathrm{im}}) \end{aligned} \tag{10}$$

Simulations of data reported in [166] using a variation on (10) are described in detail in Ginn [43].

Determination of the appropriate form for the velocity of reduction in residence time (when it matters) requires controlled experiments. It may also be useful to treat this velocity as a random variable, reflecting variability of bacterial adhesion process rates among different individual cells.

In Section 3.3, we have considered metabolic effects on microbial transport. Cell metabolism may influence attachment/detachment kinetics via cell division of attached microbes (and the subsequent release of "daughter" cells into the aqueous phase) and/or dynamic partitioning in which the aqueous-phase concentration of microbes increases in response to the elevated nutrient concentrations associated with a contaminated plume. Dynamic partitioning may also operate in the opposite direction when residence-time (i.e, amount of time a microbe is reversibly held in close proximity to a surface by adhesion forces) dependent cell-surface changes increase the probability of the cell becoming irreversibly attached. The exposure-time approach has been proposed as a way to account for this effect [42].

4. Case study: microbial transport at the oyster field site

Microbial transport processes as described above are conceptualized and defined at the pore to local continuum scale. Most of the experimental work on which these definitions are based have been performed in the laboratory on small, essentially one-dimensional sediment cores. Translation of these representations to field-scale transport of bacteria in natural aquifers involves additional factors for consideration, such as the effects of three-dimensional flow fields, multiple scales of heterogeneity in aquifer and groundwater properties, interactions with native microbial communities, and temporal variations due to natural transient fluctuations. These factors confound the straightforward extension of laboratory results to field-scale predictions. In this concluding section, we review field-scale studies of bacterial transport and discuss the impacts of these factors. Particular focus is given to a recent series of field-scale bacterial transport experiments conducted at a research site near Oyster, Virginia as a case study.

4.1. Previous field-scale bacterial transport experiments

Microorganisms were first used as environmental tracers to delineate subsurface flow pathways, particularly in fractured or karst environments. A number of field studies have also been performed to determine the potential for transport of pathogenic organisms from sources such as septic systems to drinking water supply wells. More recently, local-scale controlled injection and recovery experiments have been conducted at a number of sites to directly study the mechanisms of bacterial transport at the field scale in porous aquifers. Harvey [54] and Harvey and Harms [55] review the historical use of microbial agents as tracers, and describe several methods used for field-scale bacterial transport injection and recovery tests.

Detailed, local field-scale bacterial transport experiments were pioneered at the US Geological Survey's Cape Cod Toxic-Substances Hydrology Research Site in Falmouth, MA over the past two decades. Several forced- and natural-gradient tests were conducted in glacial outwash sediments using bacteria, viruses, protozoa, inorganic colloids, and solute tracers. Harvey et al. [50] provide an initial overview of transport results using bacteria-sized fluorescent microspheres, native bacteria, and a conservative tracer. A forced-gradient experiment was conducted using a divergent flow design, with multi-level samplers (MLS) 1.7 and 3.2 m from the injection well. A natural-gradient experiment was conducted in a contaminated zone of the aquifer using microspheres only; samples were collected in a row of MLS 6.9 m down-gradient of the injection well. Transported bacteria and microspheres of varying diameter were observed at both sample collection points in the forced-gradient test, although relative breakthrough was less than 1% at the closest sampling point. Breakthrough of bacteria prior to a conservative bromide tracer was observed, particularly at the distant MLS. Peak abundance of microspheres increased with increasing diameter, qualitatively consistent with filtration theory. Behavior of bacteria and similar-sized microspheres was observed to be significantly different. Harvey and Garabedian [51] applied filtration theory to quantitatively model transport of bacteria in a subsequent natural-gradient experiment at the same site, and estimated a collision efficiency of 5×10^{-3} to 1×10^{-2} at a travel distance of 6.9 m. Harvey et al. [52] demonstrated the importance of physical heterogeneity in controlling field-scale bacterial transport. Observations of micro-

sphere, bromide, and bacterial transport 6 m down-gradient of the injection point varied significantly in three sampling ports separated by a total vertical distance of 0.7 m. Bales et al. [5] observed bacterial breakthrough at a distance of 11 m, but their experiment focused on effects of pH on phage (virus) attenuation and remobilization. Harvey et al. [53] studied the transport of groundwater protozoa at the Cape Cod site, and found that the protozoa were attenuated more rapidly than bacteria although the protozoa were of the optimal size for transport based on experiments with microspheres of various diameters. Pieper et al. [91] performed injections of viruses (bacteriophage) at the site, and found that attenuation was significantly lower in zones of the aquifer containing high concentration of sewage-derived organic matter. They attributed this observation to the blocking of attachment sites in the organic-rich zone. Ryan et al. [104] further evaluated the effects of geochemical conditions on virus transport at the site, specifically mobilization by a surfactant, high pH, and a reductant.

DeFlaun et al. [29] report preliminary results of forced-gradient injection and recovery experiments conducted in a sandy aquifer (shoreface sediments) near Oyster, Virginia. Although bacterial breakthrough was observed at the furthest sampling point (4 m from the injection), the large majority of bacteria (greater than 99%) were retained between the injection well and the first sampler only 50 cm away. A dual sub-population model was proposed as a possible explanation for the observed behavior.

Schijven et al. [109] injected viruses and bacteria into a deep sandy aquifer in the Netherlands and observed breakthrough at four monitoring wells ranging from 8 to 38 m distant from the injection. Although one bacterium (*E. coli*) was observed only at the closest well, the second (spores of *Clostridium bifermentans*) was observed at all four wells. A systematic decrease in attachment rate coefficients with distance was inferred using one-dimensional models of the experiment.

Bacterial transport experiments have recently been conducted in an alluvial gravel aquifer near Christchurch, New Zealand. Sinton et al. [115] introduced bacteria, bacteriophage, and rhodamine WT dye into a well and observed transport to wells as far as 400 m down-gradient. Groundwater flow rates in this aquifer are rapid, as high as 86 m/day. Transport from the ground surface through the vadose zone to the groundwater table was also observed in an irrigation experiment. They represented bacterial attenuation by an apparent decay constant (incorporating attachment losses as well as true bacterial decay), and estimated values on the order of 1.0 day^{-1} for coliform bacteria in groundwater. Pang et al. [88] describe results of a second experiment in which Rhodamine WT, Cl, and a bacterium were injected below the water table. They observed faster velocities of the bacteria relative to inert tracers, and estimated total removal rates of 2.4–9.4 day^{-1}. Sinton et al. [116] further examined the apparent velocities of microbial (bacteria and virus) and inert tracers, and found that apparent velocity increased, and apparent dispersion decreased, with increasing particle size.

Several field investigations of virus transport in groundwater have been conducted at a number of sites (e.g., [6,27,28,109,127] and others previously mentioned), but are not reviewed here. A detailed review of viral transport models, processes, and parameters from field studies is provided by Schijven and Hassanizadeh [110].

4.2. South Oyster research site

Three forced-gradient bacterial injection and recovery experiments were conducted in 1999–2001 at two locations near the town of Oyster, Virginia, referred to generally as the South Oyster site. These locations are near that of the preliminary experiments reported by DeFlaun et al. [29]. The motivation for these experiments was to understand the transport and attachment behavior of aerobic and facultative iron-reducing bacteria under oxic and suboxic groundwater conditions. Therefore, two separate experimental cells were developed, one in an oxic zone of the aquifer (referred to as the Narrow Channel or NC flow cell), and the second in a suboxic zone (the South Oyster Focus Area or SOFA flow cell). Each flow cell comprises a number of hydraulic control and monitoring wells around the perimeter, and a tracer injection well and gallery of multi-level samplers (MLS) in the center. Up to 24 MLS are installed in each cell, each with 12 ports vertically, for a total of 288 sampling points in three dimensions ranging from 0.5 to 7 m in distance from the injection well. Both flow cells are located within shallow sandy surficial aquifer sediments deposited by nearshore marine processes [119]. The NC site is relatively homogeneous and contains fine to medium sand with some gravel; NC groundwater is aerobic (dissolved oxygen concentrations of 4–7 ppm). The SOFA site is also predominantly sandy, but also contains some silty and muddy beds and a layer of peat. SOFA groundwater is suboxic (dissolved oxygen generally <1.0 ppm). An overview of the site characteristics, experiments conducted, and results is given by Balkwill et al. [7]. Fig. 6 shows the location of the South Oyster site; Fig. 7 shows a map of the site and configuration of wells in the experimental flow cells.

An aerobic bacterial strain (*Comamonas* sp. *DA001*) was cultured from site groundwater and selected for adhesion deficiency [29]. It was injected into the NC flow cell in 1999 along with a dilute bromide solute tracer. Scheibe et al. [107] describe the experimental design and

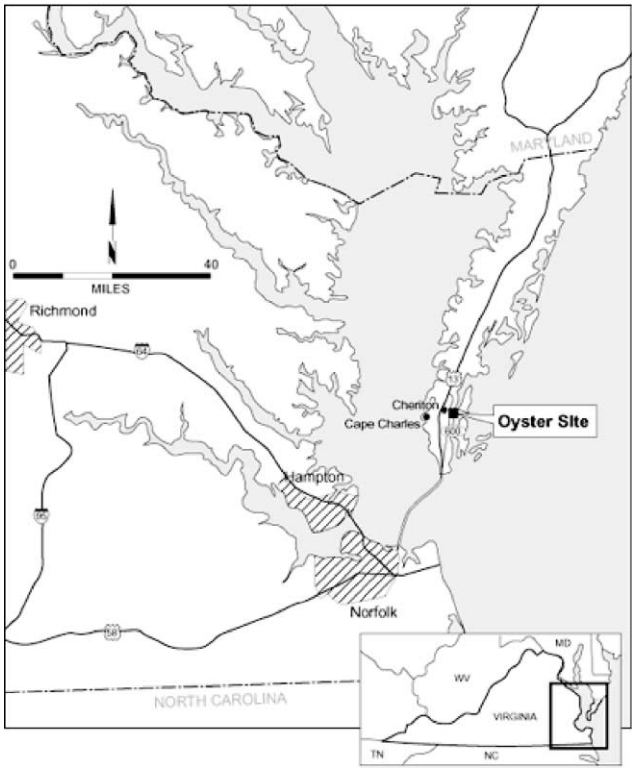

Fig. 6. Location of the South Oyster research site, from Scheibe et al. [107]. Copyright National Ground Water Association, 2001, used by permission.

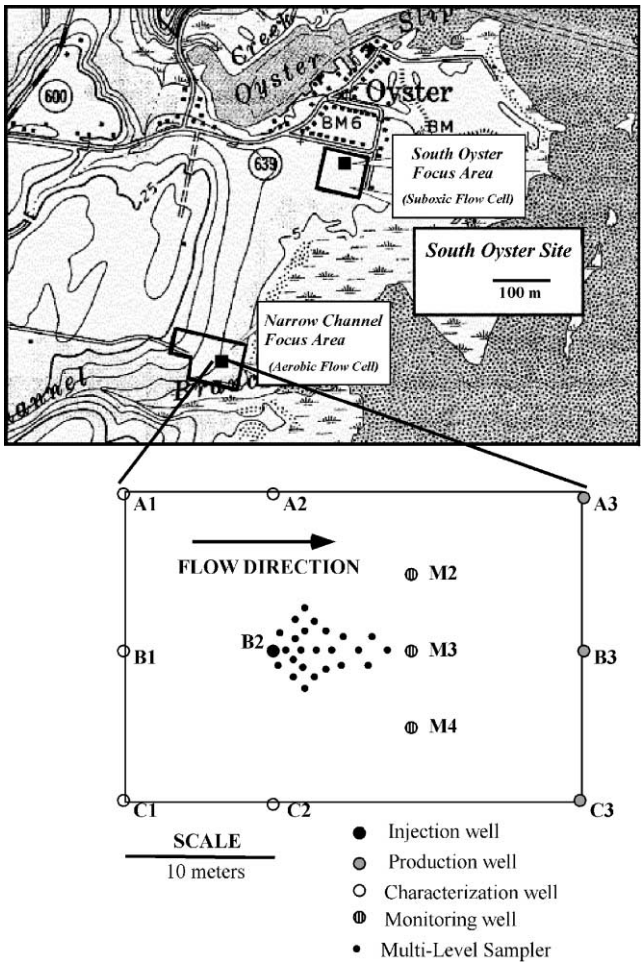

Fig. 7. South Oyster research site map. The well configuration of the Narrow Channel Focus Area flow cell is shown; the South Oyster Focus Area flow cell is configured similarly. Site map is provided courtesy of Golder Associates, Inc., based on the USGS Topographic Map, 7.5 Min Quadrangle Map Series, Cheriton Quadrangle, Northampton County, Virginia.

give a brief summary of experimental results. Bacterial breakthrough was observed at the most distant MLS at significant levels, and in general exceeded the extent of transport predicted based on extrapolation of transport experiments conducted on 50-cm intact sediment cores from the site. Even at this relatively homogeneous site, the effects of aquifer heterogeneity on transport were apparent. The bacterial transport patterns were similar to those of the bromide tracer; both correlated well with permeability patterns inferred from detailed geophysical observations [59].

In 2000, DA001 was co-injected into the SOFA flow cell with an iron-reducing bacterium (*Acidovorax* sp. OY107), also cultured from site groundwater and selected for relative adhesion deficiency. The initial injection was performed with a limited number of MLS in place, allowing the collection of intact sediment cores immediately following the injection during emplacement of the remaining MLS. Subsequently, in 2001, the two bacteria were again co-injected and monitored more extensively. In this manner, the spatial distribution of both aqueous and attached bacteria can be assessed. Because the results from the SOFA injection experiments are currently being analyzed and have not yet been published, the discussion here focuses on the results of the 1999 injection experiment at Narrow Channel.

4.3. Field-scale issues and discussion

4.3.1. Impact of physical heterogeneity

Physical heterogeneity (spatial variations in grain size, permeability, and porosity of the porous medium) clearly plays a significant role in controlling patterns of field-scale transport. Harvey et al. [52] reported dramatically different character of bacterial breakthrough at three adjacent elevations in a single multi-level sampler, separated by only tens of centimeters. At the South Oyster site, significant effort was invested in characterization of the spatial structure of physical aquifer properties using geological, hydrologic, and geophysical techniques [19,58,59]. At the NC flow cell, it was demonstrated that transport patterns of both bacteria and bromide were closely related to patterns of permeability inferred from cross-well radar tomography and that

apparent dispersivities were consistent with correlation lengths inferred from tomographic observations [59].

4.4. Apparent decrease in alpha/k_f with distance

The apparent rate of attachment (as expressed in terms of the fitted collision efficiency parameter or attachment rate coefficient) has been observed to decrease with distance traveled in field studies using bacteria [29,51,109], protozoa [53], and viruses [109,127]. This appears to be the key factor limiting the direct application of laboratory core experiments to predict the extent of field-scale transport. The phenomenon has occasionally been interpreted as reflecting in situ growth [51] or heterogeneity in aquifer properties [109,110]. Most often, however, it is interpreted as reflecting variability in the cell surface properties within a monoclonal bacterial population [1,12,29,110], which would cause the more "sticky" bacteria to attach rapidly at short travel distances while a smaller sub-population with less propensity for attachment transports much farther. Baygents et al. [10] and Glynn et al. [46] observed a bimodal distribution of surface charge density in selected bacterial strains, providing experimental support for this hypothesis. Redman et al. [95,96] proposed a multiscale (fractal) distribution of filtration length scales arising from microscale heterogeneity in surface properties of microbes and collectors, and provided experimental support for the model based on column experiments of virus transport. Mailloux et al. [74] estimated a field-scale distribution of apparent collector efficiencies from observations at various distances in the NC flow cell at the South Oyster site. The estimated values clearly decreased with distance, and the distribution inferred from small-scale core experiments was shown to be a subset of the field-scale distribution with the highest values.

If cell-to-cell surface interactions are more favorable than cell–grain surface interactions, initial attachment of cells near the injection point could increase subsequent attachment of more cells, thereby causing an apparent "ripening" effect that would lead to increased attachment rates nearer the point of injection. The effect of correlations between physical and chemical heterogeneity could also lead to enhanced transport at the field scale, particularly if more permeable zones had lower attachment rates such that preferential transport pathways could develop [108]. However, because attachment parameters are not yet predictable from basic property measurements, but rather are used as fitting parameters, it is also likely that the apparent systematic trend reflects some fundamental shortcoming with the assumed attachment model (generally treated as first-order kinetic). At any rate, it is clear that field-scale transport of microbes will usually be underestimated if predictions are based on attenuation rates estimated from laboratory column experiments [110].

4.4.1. Exclusion

At the South Oyster field site, exclusion phenomena were observed at the field scale only at a very limited number of locations, and at those locations it was minimal. However, exclusion factors (ratios of microbial velocity to solute velocity) as large as 1.6 were observed in laboratory core experiments using intact cores from the South Oyster site, and appear to be greatest in cores containing large amounts of finer-grained cross-bedded zones [31]. Why exclusion occurs more readily at laboratory scales than field scales in granular media remains a research question. A possible hypothesis is that restriction of flow in laboratory columns to 1D requires microbes to pass through zones with small grain size and low permeability in which exclusion processes are most significant, whereas in the field 3D flow paths allow microbes (and solute) to bypass low permeability zones.

4.4.2. Predation

Studies at the Cape Cod and South Oyster sites have both demonstrated that predation by native protozoan populations on injected bacteria can have a significant impact on transport distances and concentrations. At South Oyster, a dramatic increase in protozoan populations was observed in response to the injection of bacteria [20]. Although the total effect is difficult to quantify because of limited observations of attached protozoan populations, it was shown that an apparent increase in attachment rate coincided with the timing of the protozoan bloom at a time when a decrease in attachment rate would have been expected based on other factors [139].

4.4.3. Extended tailing/detachment

Typically, rates of detachment of attached cells are low and do not contribute significantly to observed bacterial concentrations during the main breakthrough pulse. However, slow release of attached bacteria at low concentrations over long periods of time may contribute significantly to transport over longer distances. At the South Oyster site (NC flow cell), inferred detachment rate coefficients were similar in magnitude to attachment rate coefficients [139]. This contrasts to laboratory experimental results reported in the literature, in which estimated detachment rate coefficients are one to three orders of magnitude smaller than attachment rate coefficients [139, Table 2]. Nevertheless, the inferred detachment rate coefficients were similar to those reported in the literature, so the investigators attribute the observed ratio to unusually low attachment rates of the organism, which had been selected for adhesion deficiency. Numerical simulations using the derived rate coefficients demonstrated that bacterial detachment

could have a significant impact on long-term distribution of attached bacteria in the subsurface.

Model fits to experimental data also support the hypothesis that there exist both reversible and irreversible attachment events (corresponding to heterogeneity in grain or bacterial cell surface properties). Zhang et al. [138] estimated that 70% of the attachment events were irreversible under forced gradient conditions, increasing to nearly full irreversibility under low-velocity natural gradient conditions.

4.4.4. Effects of iron oxide coatings/organic masking

Many sandy aquifers contain variable amounts of iron and other metal oxide minerals, commonly in the form of amorphous coatings on quartz grain surfaces. Under pH conditions commonly observed, quartz surfaces are negatively charged while iron oxides are positively charged. This leads to an enhancement of bacterial attachment to surfaces with iron oxide coatings that has been demonstrated in laboratory experiments [66,81,111]. In field settings, however, the effects can be modified by variable flow paths caused by physical heterogeneity or by other factors such as blocking of iron oxide sites by attached organic carbon [62]. Identification of the relative importance of physical and chemical heterogeneity is confounded by cross-correlation between the two; iron-rich minerals and coatings tend to be concentrated in finer-grained zones which also have lower permeability and higher collector efficiency. Dong et al. [31] performed a laboratory study using cores from the South Oyster site, and concluded that variations in bacterial attachment could be explained by differences in collector efficiency (a physically based control) and that physical heterogeneity was dominant over chemical heterogeneity despite the existence of discrete bands of metal-rich grains. At the field scale, physical heterogeneity also appeared to be the dominant control on transport at the NC flow cell [59]. At the more heterogeneous SOFA flow cell, however, preliminary comparisons do not establish a clear link between physical properties and bacterial transport observations (S. Hubbard, LBNL, personal communication), suggesting that geochemical conditions play a greater role at the suboxic flow cell.

4.4.5. Predictability/characterization issues

Field-scale bacterial transport has been observed in detail at a small number of highly instrumented sites. The results of these experiments clearly demonstrate that the processes that are active at field spatial and temporal scales differ from those operating at laboratory scales. A more fundamental understanding of the mechanisms of bacterial attachment to, and detachment from, aquifer grain surfaces is required before reliable quantitative predictions of field-scale transport can be made. Key areas for further study include

- Quantification of interactions between extracellular polysaccharides and grain surfaces of varying compositions.
- Discrimination of pore-scale processes leading to increased velocity of biocolloids relative to solute tracers (i.e., exclusion from intragranular porosity, redirection of colloids into preferential flow paths, or classical hydrodynamic chromatography).
- Mechanistic explanation of apparent decreases in bacterial attachment (attachment rate coefficients, collision efficiencies) with transport distance.
- Detachment mechanisms (i.e., growth-induced detachment, hydrodynamic shear, dislodging by suspended colloids, and/or active biological detachment).
- Relationship between microbial motility (chemotaxis) and attachment.

5. Summary and conclusions

This paper has reviewed the large volume of work that has already been done and the progress that has been made towards understanding and predicting microbial transport in natural porous media at a variety of characteristic length scales. This review has also revealed the many gaps that still exist in our understanding of the many interrelated processes affecting transport and how these processes fit together. Some of the findings to date and needs for future research are the following:

1. Aqueous-phase biomass is generally treated as a dilute suspension of cells with no cell-to-cell interactions, an assumption that may sometimes be violated. Attached phase biomass has been treated by different models, and it is unclear whether the use of any particular model is appropriate to represent the structure of attached biomass.
2. The use of volume averaging to upscale mass transport and reaction processes for both biofilm formation and for microbial transport may be useful in refining our understanding the macroscopic scale manifestations of microbial transport and the role of biophase structure in the subsurface.
3. The physicochemical processes affecting transport have been studied extensively and are reasonably well

understood. However, the colloid filtration theory approach that has commonly been used to model these processes neglects detachment. Attempts to remedy this deficiency have not yielded a definitive approach. Thus, the study of detachment processes merits further attention.
4. The effects of physicochemical aquifer heterogeneity should be studied further.

The biological processes affecting transport are less well understood. Needs for future work include the following:

1. Development of a modeling approach that accounts for the time a microorganism has been exposed to nutrients.
2. Modeling and experimental studies on the phase dependence of microbial reactions in porous media.
3. Studies on the possibly coupled nature of the effects of nutrient availability, survival stress, and growth on active adhesion/detachment.
4. Continued cytoscale, pore-scale, and Darcy (REV)-scale studies on chemotaxis for relevant bacteria-nutrient systems.
5. Studies on the interactions between bacterial surface polymers and soil surfaces; cell surface characterization for relevant bacterial species.
6. Increased research on the partitioning of bacteria under dynamic growth conditions and the transient movement of bacteria under changing chemical conditions.
7. Studies on the effects of heterogeneity between microbial populations.

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