Modeling Microbial Transport in Porous Media: Traditional Approaches and Recent Developments

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Abstract

A substantial research effort has been aimed at elucidating the role of various physical, chemical and biological factors on microbial transport and removal in natural subsurface environments. The major motivation of such studies is an enhanced mechanistic understanding of these processes for development of improved mathematical models of microbial transport and fate. In this review, traditional modeling approaches used to predict the migration and removal of microorganisms (e.g., viruses, bacteria, and protozoa) in saturated porous media are systematically evaluated. A number of these methods have inherent weaknesses or inconsistencies which are often overlooked or misunderstood in actual application. Some limitations of modeling methods reviewed here include the inappropriate use of the equilibrium adsorption approach, the observed breakdown of classical filtration theory, the inability of existing theories to predict microbial attachment rates, and omission of physical straining and microbe detachment. These and other issues are considered with an emphasis on current research developments. Finally, recently proposed improvements to the most commonly used filtration model are discussed, with particular consideration of straining and microbe motility.

1. Introduction

There is a considerable ongoing effort aimed at understanding the transport and deposition behavior of microorganisms in saturated porous media. A mechanistic understanding of these processes is of significant interest in various environmental applications such as *in–situ* bioremediation [125], riverbank filtration [136], and protection of drinking water supplies [45].

Certain indigenous microbes present in the subsurface can enhance the mobility of radionuclides, metals and other toxic contaminants. The natural degradative capabilities of native strains may be exploited in the application of biostimulation strategies targeting restoration of contaminated aquifers. Successful use of such remediation technologies and risk assessment of microbe–facilitated pollutant transport requires a means to predict the transport potential of microorganisms. An improved understanding of the factors controlling the fate and transport of microbes in porous media is also important in the design and function of engineered *in–situ* bioremediation schemes involving introduction of large volumes or high concentrations of bacteria (e.g., in the use of a biobarrier or in bioaugmentation) [125]. In such cases, the common objective is to achieve a target microbe concentration at a specific location.

Widespread microbial contamination of drinking water supplies has further prompted the need to better predict the transport behavior of microbes in the subsurface. Viruses, protozoa (e.g., *Cryptosporidium parvum*) and certain bacteria are pathogens of concern which may be introduced into the natural environment from land disposal of treated wastewater effluents or animal fecal deposits [81, 123, 143].

Microbial transport and deposition in porous media has traditionally been studied in experiments using bench–scale packed columns, where the suspended effluent microbe concentration is monitored as a function of time. One of the first studies of this type was reported

by Bales et al [4], where the transport of the bacteriophage f2 and MS–2 was investigated using columns packed with sandy soil and fractured rock. Since, a great deal of research has been conducted in the laboratory [57] and in the field [38, 58, 60, 70, 117, 145] in an attempt to elucidate the factors controlling the transport and fate of microorganisms in porous media.

Packed column experiments have been used to assess the influence of biological factors such as cell surface macromolecule length and composition [2, 105, 141, 144], cell motility [11, 24, 26, 83], cell size and shape [11, 46], organism type [35, 62, 64, 104, 118], and growth phase [122, 140] on bacterial transport and deposition kinetics in porous media. Other physical and chemical conditions which have been evaluated include grain size [46, 64, 78, 118] and shape [20], presence of surface coatings on collector media [11, 13, 47, 118], fluid velocity [11, 24, 49, 62, 104, 127], solution ionic strength and composition [13, 37, 46, 49, 64, 78, 79, 103, 105, 119], and cell concentration [24, 25, 127]. In studies of virus fate and transport, bacteriophages are often used as model particles [61, 114]. The removal of viruses in granular filtration has been examined using packed beds of quartz sand [28, 68, 91, 100-102], glass beads [5, 6], and natural sediments [4, 41, 72, 75, 115]. In general, viral attachment kinetics seem to be controlled by electrostatic interactions [91, 102, 114]. In comparison to bacteria and viruses, well–controlled laboratory studies on the removal mechanisms of protozoan (oo)cysts in flow through saturated porous media are relatively scarce. The limited body of literature on this subject is focused on the filtration behavior of *Cryptosporidium parvum* [1, 21, 33, 54, 65, 82, 133, 134] and *Giardia lamblia* [65] — two waterborne pathogens of considerable concern [81, 143]. Specifically, the role of solution ionic strength and composition [33, 65, 133, 134], grain size and shape [21, 54, 82, 134], fluid velocity [54], and collector surface heterogeneity [1] has been examined.

Although well–defined laboratory experiments provide an improved understanding of the fundamental mechanisms controlling microbial transport and deposition, the results of these studies are often not directly relevant to processes occurring in natural and engineered systems. The inherent physical, chemical and biological heterogeneity of such systems presents a highly complex environment to mimic [50]. Field–scale observations and experiments offer a practical compromise, however the results of these studies are often difficult to generalize. The role of physical heterogeneity in controlling bacterial transport through aquifer sediments has been demonstrated at two research locations — the US Geological Survey's Cape Cod Site, MA [59], and the Narrow Channel area of the South Oyster Site, VA [38, 66, 70]. Several field–scale studies have been used to assess the influence of chemical heterogeneities of grain (collector) surfaces, such as iron oxide [110, 117] and organic matter coatings [93, 110, 116], as well as the effect of aquifer media physical heterogeneity [86, 87, 94, 145] on virus transport and fate. In contrast, few investigations have been conducted to examine the removal of protozoan (oo)cysts at the field–scale [58, 60].

Typically, in such laboratory and field–scale studies, microbial transport and deposition kinetics are evaluated by measuring the suspended (fluid–phase) microbe concentration at a given travel distance. These findings may subsequently be utilized in the development of predictive models for microbial transport behavior in natural and engineered aquatic systems. However, recent theoretical [135] and experimental [10, 76-78, 130, 132] investigations suggest that monitoring fluid–phase particle concentration is inadequate in identifying the fundamental mechanisms controlling particle (or microbe) transport. Rather, it has been shown that the spatial distribution of retained microorganisms, in addition to fluid-phase concentrations, provides a more

accurate characterization of transport and deposition behavior in saturated porous media [76, 77, 130, 132, 135].

Mathematical models of microbial transport in saturated porous media generally involve a simplified form of the advection–dispersion equation, which can be derived from basic mass balance principles. In the most commonly used modeling approach, microbe removal is considered to be governed solely by attachment to sediment grain surfaces. Indeed, few modeling efforts take into account the influence of the numerous physical, chemical and biological factors which are known to affect microbe transport and fate in the subsurface [148]. The factors and mechanisms which influence microbial transport and removal in saturated porous media have been reviewed extensively [45, 55, 126, 148], however their incorporation into predictive models remains a challenge.

The purpose of this paper is to critically review traditional approaches used to model microbial transport and fate in saturated porous media. In the first section, the general governing equations typically considered in models of microbial transport and fate are presented. Next, the limitations associated to application of these mathematical models are reviewed; for instance, the inadequacy of the equilibrium adsorption approach, the difficulties in the prediction of microbial deposition rates and the relevance of straining for larger microorganisms. Finally, recently proposed initiatives which may improve the predictive capabilities of transport models are discussed.

2. Traditional Approaches to Predicting Microbial Transport and Fate

Several mechanisms act independently or simultaneously to control the movement and fate of microorganisms in natural and engineered aqueous systems. These processes can be classified

into three major groups: (i) transport, (ii) exchange between the liquid phase and the solid phase (due to attachment and detachment), and (iii) inactivation, grazing or death [128]. In modeling the transport and fate of microorganisms in porous media, the appropriate mathematical descriptions for these processes must be considered.

2.1 The general advection–dispersion equation

At the macroscopic level, the temporal and spatial variations of microbe concentration in a homogeneous, water–saturated porous medium are described by the advection–dispersion equation (shown here in a single spatial dimension for simplicity) [114, 148]:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \tag{1}$$

where C is the microbe concentration in the aqueous phase at a distance x and time t, D is the hydrodynamic dispersion coefficient, and v is the interstitial microbe velocity.

In eq 1, only the physical transport processes of advection and hydrodynamic dispersion are considered. As microorganisms are transported through porous media, they are removed from the pore fluid by physicochemical filtration (attachment to sediment grain surfaces). Physicochemical filtration has been modeled as either an irreversible (no detachment) or reversible process. In the case of reversible attachment, both equilibrium and kinetic mechanisms have been applied [148]. Regardless of the type of attachment mechanism used to describe removal of microbes from the pore fluid, the governing equation for microbial transport and fate in a one–dimensional, homogeneous, saturated porous medium becomes [114, 148]:

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{\varepsilon}\frac{\partial S}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \tag{2}$$

where S is the attached microbe concentration, ρ_b is the dry bulk density of the porous medium, and ε is the bed porosity.

2.2 Equilibrium "adsorption" of microbes

Both Langmuir and Freundlich isotherms have been used to describe the so–called equilibrium "adsorption" of microbes to solid surfaces [148]. For the special case of a linear adsorption isotherm, $S = K_{eq} C$. In this case, eq 2 can be rewritten as [114, 148]:

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \tag{3}$$

where $R = 1 + \rho_b K_{eq}/\varepsilon$ is commonly referred to as the retardation factor.

2.3 Kinetic attachment and detachment processes

In a suspension of microbes and sediment grains, an equilibrium adsorption state is not reached instantaneously [114]. Rather, a kinetic attachment mechanism which is composed of two processes controls the removal of microorganisms from the aqueous phase. In the first step, described as mass transport, microbes are transferred from the bulk fluid to the surface of the sediment grains. In the second step, microbes are attached to the surface as a result of physicochemical interactions [15, 114, 148]. The subsequent release (detachment) of microorganisms may also be controlled by a kinetic process. Under these conditions, the rate of change in retained microbe concentration can be described as follows:

$$\frac{\rho_b}{\varepsilon}\frac{\partial S}{\partial t} = k_{att} C - \frac{\rho_b}{\varepsilon} k_{det} S \tag{4}$$

where k_{att} and k_{det} are the microbial attachment and detachment rate coefficients, respectively. It should be noted that the processes described by eqs 3 and 4 neglect the effects of microbial growth

as well as microbe–microbe interactions which may occur under certain conditions (e.g., blocking, ripening and/or aggregation in the aqueous phase) [25, 44].

2.4 Irreversible attachment under "clean–bed" conditions

The most commonly used approach for evaluating microbial transport behavior in laboratory and field–scale studies is the classical colloid filtration theory (CFT) [56, 109, 114, 146]. In effect, this "clean–bed" filtration model is a special case of the general formulation described in section 2.3, whereby the attachment of microbes to sediment surfaces is considered irreversible, i.e., negligible release of microorganisms [135]. In the CFT, the mass transport process is reflected in the single–collector contact efficiency, η_0, and the surface attachment step is described by the attachment (collision) efficiency, α [44, 146]. This model is relevant to most applications of practical interest, where the system may be considered at steady–state and initially free of microorganisms, and the influence of hydrodynamic dispersion is negligible (i.e., the dispersion term is small compared to the advection term, or the *Peclet* number ($N_{\mathrm{Pe}} = vx/D$) is greater than about 5 [137]). Under these conditions, for a continuous particle injection at concentration C_0 (at $x = 0$) and time period t_0, the solutions to eqs 2 and 4 are [135]:

$$C(x) = C_0 \exp\left[-\frac{k_{att}}{v}x\right] \tag{5}$$

$$S(x) = \frac{t_0 \varepsilon k_{att} C_0}{\rho_b} \exp\left[-\frac{k_{att}}{v}x\right] \tag{6}$$

where the microbe attachment rate coefficient is related to η_0 and α via

$$k_{att} = \frac{3(1-\varepsilon)v}{2d_c}\eta_0\alpha \tag{7}$$

Here, d_c is the average grain size. In the case of soil grains that may not be spherical or uniform in shape, the average grain size can be characterized by the arithmetic mean diameter (d_a), the

geometric mean diameter (d_g), or the diameter for which 10% of all particles in the volume distribution are smaller (d_{10}) [78]. Since current theories are inadequate for predicting the attachment efficiency [44], it is common to use measurements of the normalized suspended microbe concentration (C/C_0) at a packed length x to determine α values for a given system. In this case, the theoretical value of the single–collector contact efficiency (η_0) may be determined numerically or using a closed–form equation [98, 131, 146].

2.5 Microbe inactivation or die–off

Inactivation or death is the second major mechanism which governs the removal of microbes during transport in porous media. This irreversible sink mechanism may affect both suspended or attached microorganisms and is commonly described by a first–order rate expression [32, 148]. In saturated natural environments, microbe inactivation may be affected by solution composition and pH, temperature, grazing, and attachment to sediment surfaces. However, the effect of these variables on the rate of inactivation is not clear [148]. It has been reported that temperature is the most important factor that influences virus inactivation, and some researchers have proposed formulations to describe the temperature dependence of the virus inactivation rate [114, 148]. These studies suggest that the virus inactivation rate is an exponential function of the porewater temperature [114, 148].

3. Limitations of Existing Modeling Approaches

Several different methods to model microbe transport and fate in saturated porous media have been employed. As described previously, these mathematical models generally differ in the description of fundamental microbe removal mechanisms. Thus, it follows that certain approaches

may have inherent weaknesses or inconsistencies which should be addressed. In this section, the limitations of existing modeling approaches and their implications are discussed.

3.1 The equilibrium adsorption mechanism may be inadequate

Attachment of microorganisms by a so–called equilibrium "adsorption" mechanism has been considered in both laboratory [5, 27, 28, 41, 54, 68, 90, 95, 99] and field–scale investigations [8, 56, 96, 121]. It should be noted though that equilibrium adsorption does not result in removal of microbes from the aqueous phase [114]. Rather, this process gives rise to retarded breakthrough of microorganisms in comparison to that of a conservative (inert) tracer, i.e., it is completely reversible. The retardation factor, *R*, is a direct measure of this delayed breakthrough, where an *R* value equal to one indicates no retardation. However, only a few laboratory studies of virus [5, 41, 90, 95] and bacteria [27] transport have reported retardation factors greater than one. In most controlled packed–column experiments where the transport of viruses [5, 6, 28, 68, 72], bacteria [35, 38, 40, 46, 62, 63, 103, 104, 119, 120, 141] or protozoa [54, 134] has been examined, the observed breakthrough has been comparable to that of a tracer. Similarly, little or no retardation has been reported in several tank and field–scale investigations of microbial transport [7, 8, 34, 56, 93, 110, 116, 118, 121].

Bales et al [5, 8] investigated the transport and removal of different bacteriophages in both laboratory and field–scale experiments. In these studies, the influence of equilibrium adsorption on the attachment of viruses to collector surfaces was found to be negligible. Instead, virus attachment was shown to be controlled by a kinetic process which is reflected in the slow–rising limbs and long tails of the measured breakthrough curves. A representative breakthrough curve measured with bacteriophage PRD–1 in a column packed with silica beads at pH 5.5 is presented in Figure 1a. This result clearly demonstrates the strong kinetic effect observed by Bales et al [5].

A typical breakthrough curve measured in a field study by Schijven et al [116] for bacteriophage MS–2 is shown in Figure 1b. Comparison of MS–2 transport behavior (open symbols) with that of a salt tracer (solid line) indicates that phage transport was not retarded by equilibrium adsorption. The slowly rising limb and significant tailing observed in the virus breakthrough curve reveal that virus transport was governed mainly by a kinetic process. Schijven and Hassanizadeh [114] described numerical results which show that predictions of microbial transport by an equilibrium approach and a kinetic approach may sometimes lead to similar conclusions. Their calculations illustrate how an investigation of tailing is required to distinguish between the two mechanisms. Reddy and Ford [99] further demonstrated how measurements of the microbe spatial distribution may be necessary to identify which of the two modeling approaches is more accurate.

[FIGURE 1]

As described above, equilibrium adsorption does not contribute to the removal of microbes from the pore space. Hence, in using this modeling approach to describe microbe attachment, actual removal of microbes may be accounted for only by including an inactivation (die–off) term and/or an additional sink term for attachment [114]. Some researchers have considered a microbial transport model which combines an equilibrium adsorption process with a first–order irreversible attachment mechanism (described in *section 2.4*) [27, 56]. In these studies, the microbial deposition rate coefficient (k_{att}) is defined within the context of colloid filtration theory. However, there is considerable evidence in the microbe transport literature that the contribution of equilibrium adsorption to microbe attachment is generally negligible and therefore this approach may be inappropriate for most situations. Obviously, significant errors can arise from application of an equilibrium sorption model to nonequilibrium conditions [5]. As a result, colloid filtration

theory (eq 5) has been used extensively to evaluate microbial transport and fate in saturated porous media [2, 6, 19, 24, 34, 37, 79, 91, 93, 100, 101, 103, 104, 134, 141].

3.2 Colloid filtration theory is not valid under unfavorable (repulsive) conditions

Traditionally, microbe attachment kinetics have been assessed using measurements of the fluid–phase microbe concentration. A theoretical evaluation of the factors controlling the transport and fate of microorganisms in laboratory–scale column experiments reveals that the spatial distribution of retained microbes is a more sensitive measure of filtration behavior than the profile of suspended microbes [135]. Furthermore, recent studies suggest that filtration of microorganisms may not be consistent with CFT [3, 10, 14, 24, 78, 100, 101, 133]. The most reliable method to assess the validity of CFT is to compare the measured spatial distribution of retained particles to that predicted based on the measured breakthrough curve.

Column experiments have been conducted under well–controlled physicochemical conditions using uniform spherical latex particles and glass bead collectors, where both the effluent fluid–phase concentration and the spatial distribution of retained particles have been examined [76, 77, 130, 132]. Results of these studies indicate that particle deposition behavior is in good agreement with CFT (eqs 5–7) under conditions which are *favorable* for deposition (i.e., in the absence of repulsive electrostatic interactions) (Figure 2a, b). However, CFT is generally observed to break down in the presence of repulsive Derjaguin–Landau–Verwey–Overbeek (DLVO) interactions [36, 139], namely, under conditions deemed *unfavorable* for particle deposition (Figure 3a, b).

[FIGURE 2]

[FIGURE 3]

Systematic experimental studies conducted with different–sized colloids over a broad range of physicochemical conditions recently revealed the controlling influence of secondary minimum deposition and surface charge heterogeneities on the observed deviation from colloid filtration theory [130, 132]. These findings further suggest that it is not necessarily the occurrence of an energy barrier in the particle–collector interaction energy profile that causes breakdown of CFT. Rather, the authors propose a dual deposition mode mechanism, whereby particles which overcome an energy barrier to reach the primary energy minimum deposit at a relatively "*slow*" rate in comparison to those particles which are retained in the secondary energy minimum or as a result of surface charge heterogeneities (where deposition is unhindered or "*fast*") [130, 132].

These results have important implications for predictions of microbial transport since the mechanisms identified are common to colloidal interactions in natural and engineered aquatic systems. In particular, since the depth of the secondary energy well is directly proportional to particle size, this mechanism can play an important role in the deposition behavior of larger microorganisms (e.g., bacteria and protozoa) [103, 133]. In a similar study using oocysts of *Cryptosporidium parvum* (*C. parvum*), Tufenkji and Elimelech [133] proposed that oocyst transport was controlled by "s*low*" deposition in the primary energy well, as well as the two aforementioned mechanisms of *"fast*" deposition — namely, secondary minimum deposition and retention due to charge heterogeneities. However, classic CFT (eqs 5–7) does not account for the combined effects of "*fast*" and "s*low*" oocyst deposition in the presence of repulsive electrostatic interactions. In general, predictions of oocyst removal assessed using CFT are greatly overestimated, thus revealing a significant risk of contamination when CFT is used to establish water treatment guidelines [133]. Other laboratory studies of microbial transport and deposition

involving viruses [100, 101] and bacteria [10, 129] also suggest that a model which takes into account a distribution in deposition rates may be better suited for prediction of microbe migration.

3.3 Determination of the attachment efficiency is complex

Classic theoretical approaches used to determine particle deposition rates in the presence of repulsive energy barriers include trajectory analysis for non–Brownian particles [98], and numerical solution of the convective–diffusion equation [44, 97]. In the latter case, an approximate analytical solution to the governing equation — the interaction–force boundary–layer (IFBL) approximation — is commonly utilized [44, 108, 124]. These approaches, which incorporate the DLVO theory, have traditionally failed to predict observed particle deposition rates under *unfavorable* conditions, even when experiments were conducted in well–controlled model systems [44, 109]. In general, particle deposition rates (or attachment efficiencies) calculated using these models are many orders of magnitude smaller than those measured experimentally.

An extended DLVO theory has been proposed by Van Oss et al [138] which includes short range Lewis acid–base interactions in microbial adhesion. A limited number of studies have been reported where experimentally–determined microbial deposition rates (using eqs 5 and 7) are compared with those calculated using the abovementioned theoretical approaches (which consider either classic or extended DLVO theory) [10, 39]. Most often though, these comparisons evoke the inadequacies of existing models used to calculate α; i.e., theoretical deposition rates are much less than measured values. In contrast, predictions of particle [48, 51, 53] and microbe [39] deposition rates which approach experimental results have been obtained by considering a model based on deposition in the secondary minimum. In this simple model, the kinetic theory of Maxwell is used to calculate the theoretical attachment efficiency for deposition in the secondary

energy well [51, 53]. Under conditions where repulsive electrostatic double–layer interactions predominate, microbe retention in a secondary energy well seems very likely. The considerable influence of this mechanism on bacterial transport behavior has been demonstrated using complementary experiments in a packed column setup and radial stagnation point flow (RSPF) system [103, 141]. Because of the design and hydrodynamics of the RSPF system, cells retained in a secondary minimum would be swept away by the radial flow component, allowing only the deposition of cells in a primary energy well [103]. Hence, by comparing the degree of bacterial deposition in the RSPF system to that measured in a traditional packed column experiment, the authors showed that secondary minimum deposition was a controlling factor [103, 141]. As mentioned previously, the influence of this mechanism on microbial transport has also been demonstrated in experiments using oocysts of *C. parvum* [133]. In effect, there is a growing body of evidence which suggests that the simple model for calculating α based on deposition in the secondary energy minimum may help improve predictions of microbial transport in porous media [48, 51-53, 132].

Inspection of eqs 5 and 7 reveals that assessment of the attachment efficiency, α, in packed–column experiments is dependent on the measured (or calculated) value of the single–collector contact efficiency, η_0. The classic filtration model of Yao et al [146] and the correlation equation of Rajagopolan and Tien [98] have been used extensively to predict values of η_0 for colloids and microorganisms in natural and engineered systems. Recently, a new correlation equation for calculating η_0 which overcomes the inherent limitations of these approaches was presented by Tufenkji and Elimelech [131]. Specifically, this new correlation improves upon earlier models by considering the influence of hydrodynamic (viscous) interactions and van der Waals interactions on the deposition of particles by Brownian diffusion. The resulting improvement in predictions of

η_0 has direct implications for studies of microbial transport, including viruses, bacteria, and protozoa. For instance, calculations based on the Tufenkji and Elimelech (TE) equation indicate that particles in the size range of ~ 2 μm (e.g., many bacteria) are nearly twice as mobile in porous media than previously believed [131]. Such improvements in calculations of η_0 translate into better predictions of microbial attachment efficiencies (α) when CFT is applied.

3.4 The influence of cell/cyst surface biomolecules is not well understood

It is well known that microbial cell/cyst surfaces are both chemically and structurally more complex and heterogeneous than most inorganic particles [15]. This inherent constitutional complexity of microbe surfaces, combined with variability across different strains and species, contribute to the difficulty in generalizing findings regarding the mechanisms of microbial adhesion. Surface biomolecules, which may include proteins, lipopolysaccharides (LPS), and extracellular polysaccharides, can occur in a broad range of sizes, compositions, and conformations. Recent studies have attempted to improve our understanding on the role of such macromolecules in microbial adhesion [2, 22, 23, 29, 30, 74, 105, 107, 141, 144]. In general, these findings show that the presence of surface biomolecules can either enhance [2, 22, 105-107, 141] or hinder [22, 74, 105-107, 144] microbe adhesion in aqueous media.

Burks et al [22] and Walker et al [141] used the same three strains of *Escherichia coli* (*E. coli*) K12 to investigate the role of LPS on microbial transport and attachment. The selected bacteria differed primarily in LPS length and composition. In these studies, bacterial adhesion could not be correlated with LPS length or microbe surface charge [22, 141]. Rather, it was proposed that a combination of DLVO–type interactions and surface macromolecule–associated interactions influence the degree of bacterial adhesion. Using atomic force microscopy (AFM) to

assess the influence of lipopolysaccharides on the attachment of *E. coli* K12 mutant JM109, Abu–Lail and Camesano [2] demonstrated qualitative agreement between measured adhesion forces and cell attachment to glass and quartz surfaces. However, in the same study, measurements of interaction forces upon approach of the AFM tip to the bacterium did not correlate with cell adhesion behavior to glass or quartz sand. Taken together, the results of these studies on bacterial adhesion draw attention to the difficulty in relating microbe and biomolecule characteristics to observed transport and adhesion behavior.

AFM measurements [29-31] and experiments conducted in a RSPF system [74] suggest that surface biomolecules can also affect protozoan adhesion in aqueous systems. Research shows that proteins of the *C. parvum* oocyst wall can give rise to a steric interaction (repulsion) thereby decreasing the degree of oocyst attachment to quartz surfaces. Considine et al [29-31] studied the force of interaction between *C. parvum* oocysts and silica surfaces over a broad range of physicochemical conditions relevant to granular filtration. The researchers proposed that the observed steric interaction between *C. parvum* and a siliceous material could be attributed to the presence of a "hairy" protein layer extending from the oocyst surface. Kuznar and Elimelech [74] provided further evidence in support of this theory by comparing the deposition behavior of viable oocysts with those of inactivated oocysts (both formalin and heat treated). Low deposition rates are measured with viable (untreated) oocysts even at high salt concentrations when electrostatic energy barriers are eliminated (Figure 4). This behavior is attributed to a steric repulsion between viable *C. parvum* oocysts and the quartz surface. Significant increases in oocyst deposition rates were observed after oocysts were inactivated with formalin or heat. Kuznar and Elimelech [74] linked the increased deposition rates to postulated changes in the structure of oocyst surface proteins caused by formalin and heat treatments. Additional experiments conducted with viable

C. parvum oocysts further suggest that specific surface interactions between oocyst wall proteins and silica surfaces could retard or even completely inhibit oocyst detachment (release) [29, 31, 73, 133].

[FIGURE 4]

We are only just beginning to have an understanding of the role of surface biomolecules in bacteria and protozoa transport and fate in saturated porous media. Upon approach of a microorganism to a surface, the presence of macromolecules such as LPS or proteins on cell/(oo)cyst surfaces can give rise to a broad range of interactions. This variability in the influence of biomolecules on microbial adhesion presents a major challenge to the incorporation of related mechanisms in microbe transport models.

3.5 Physical straining can be important for larger microorganisms

Predictions of particle straining potential based on system geometry suggest that physical straining could play a significant role when the ratio of the particle diameter to the median grain diameter (d_p/d_c) is greater than 0.05 [112, 113]. However, several experimental studies indicate that straining could be an important mechanism of particle removal during porous media transport when the ratio d_p/d_c is less than 0.05, and even when it is as low as 0.002 [16, 18, 134].

Bradford et al [16-18] examined the influence of colloid size and collector (grain) size on particle transport using columns packed with sand or glass beads. Two approaches were used to simulate particle transport in these systems: (i) a model which considers kinetic attachment and detachment (described by eqs 4 and 7) and (ii) a model which describes the irreversible removal of particles by a straining mechanism. Comparison with experimental results revealed that the straining model was generally in better agreement with measurements of effluent particle

concentration and spatial distributions of retained particles. These researchers also indicate that a model which considers removal of particles by both attachment and physical straining is more realistic, particularly for systems of intermediate particle and grain sizes.

Indeed, a systematic study of *C. parvum* transport behavior in columns packed with ultrapure quartz sand indicates that both straining and physicochemical filtration (attachment) affect the removal of oocysts from the pore fluid [134]. Experiments conducted in columns packed with glass beads of comparable size to the quartz sand revealed that grain shape contributes considerably to the straining potential of the porous medium. This effect was further examined by considering the transport of latex particles of increasing size in deionized (DI) water (Figure 5). In DI water, the electrostatic double–layer repulsion between particles and collectors is substantial so that physicochemical filtration (attachment) should be negligible. Under these conditions, any removal in the packed column can be attributed to the influence of a physical mechanism such as straining. As shown in Figure 5, the smallest latex particles (0.32 and 1.0 μm) exhibit no removal ($C/C_0 \approx 1$) after passage in the column packed with quartz grains. The degree of removal for the 1.9 μm particle is also minor. However, the 4.1 μm particle, which is comparable in size to the *C. parvum* oocysts, exhibits significant removal ($C/C_0 \approx 0.63$), indicating that straining can be an important capture mechanism in this type of porous medium. In addition, an observed increase in oocyst deposition rates with solution ionic strength confirmed that physicochemical filtration (attachment) also contributes to the removal of *C. parvum* in saturated porous media, possibly via deposition in a secondary energy minimum.

[FIGURE 5]

As indicated in Section 2 above, microbial transport in saturated porous media is generally described using classic colloid transport models where particles are assumed to be spherical in

shape. In reality, however, bacteria are known to exhibit a variety of shapes, including rods, ellipsoids, spirals, and coccoids [142]. Although it has been shown that cell shape can play a role in bacterial transport [46, 142], few studies have systematically investigated the influence of cell shape on physical straining. In an evaluation of the transport behavior of 14 different bacterial strains in porous media, Weiss et al [142] observed preferential removal of long, rod-shaped cells. Because of the small bacteria to grain diameter ratio ($d_p/d_c \approx 0.0014$), these researchers did not consider straining to be an important removal mechanism in their experiments.

Although straining has been shown to influence microbe removal rates under certain conditions, it is not considered in most existing modeling approaches. The numerical simulations of Bradford et al [16, 17] illustrate how classical colloid filtration theory — which does not consider removal by straining — is not always appropriate for describing particle (microbe) transport in porous media. Particularly, in settings such as bank filtration where straining is expected to be a dominant removal mechanism for relatively large pathogenic protozoa (e.g., *Cryptosporidium* or *Cyclospora*), CFT cannot be used to estimate (oo)cyst travel distances.

3.6 Microbial growth and inactivation are difficult to predict

In mathematical models of microbial transport and fate, the processes of microbial growth and inactivation (or death) are described as source and sink terms, respectively. These mechanisms may be relevant for both suspended (fluid–phase) and attached microorganisms, and are typically represented using first–order rate expressions [32, 114]. In general though, these mechanisms are not well understood within the context of microbial transport in porous media.

The Monod relationship can be used to describe bacterial growth in transport models, however its relevance to subsurface environments has not been demonstrated [32, 55]. A difficulty

associated with this approach is determining the values of the Monod parameters, namely, the maximum growth rate and the affinity constant. These microbe–specific "constants" are sensitive to changes in environmental conditions and substrate. Moreover, it is not easy to obtain accurate estimates of these parameters for *in–situ* conditions because of the difficulty in obtaining uncontaminated samples and accounting for temporal and spatial variations in microbial activity. The development and use of sophisticated imaging devices and flow chambers is likely to facilitate predictions of microbial growth rates in porous media [43, 147].

Survival of microorganisms in saturated porous media is affected by many factors, including the type of microbe, the presence of predators and parasites, pore water composition and temperature, and degree of attachment [55, 148]. Interaction amongst these factors is also probable and presents an additional challenge when predicting the role of inactivation (or death) in microbe transport. A substantial research effort has been directed at elucidating the influence of various environmental factors on the inactivation of viruses [61, 111, 114, 115, 148]. These studies demonstrate how difficult it is to draw any general conclusions on the role of certain factors in virus inactivation. For instance, the effect of attachment on the rate of virus inactivation is not clear — attachment to some geological media accelerates inactivation, whereas attachment to other mineral surfaces may protect viruses from inactivation [61, 111, 114]. Similarly, there have been contradictory reports regarding the influence of other microorganisms, such as bacteria, on virus inactivation [148]. Some studies have shown that viruses persist longer under sterile conditions, whereas the opposite effect has also been observed [148].

This observed variability in microbial growth and inactivation processes suggests that incorporation of these mechanisms into predictive models of microbial transport should be treated on a case–by–case basis.

3.7 Detachment of microorganisms is often overlooked

The release (detachment) of microorganisms from sediment grain surfaces can be of considerable importance in natural subsurface environments and engineered water treatment systems. Specifically, detachment of pathogenic microbes (e.g., *C. parvum*) from granular media can pose a significant risk to drinking water safety [50, 149]. Detachment of bacteria is also undesirable during *in–situ* bioremediation efforts, where the objective is to maintain a critical microbial mass at a specific location.

Unprompted release of microorganisms has been observed in laboratory and field–scale transport experiments involving bacteria, viruses and protozoa [5, 46, 54, 63, 64, 84, 116, 149]. In such studies, observations of "tailing" in measured breakthrough curves are attributed to microbial detachment, whereby an excessively long tail is indicative of very slow release [5, 114, 149]. Slow release of attached microbes over extended time periods may contribute significantly to long–distance transport, however few field–scale studies have actually monitored long–term changes in microbial concentration [58, 116, 149]. Zhang et al [149] observed extended tailing of bacteria at the South Oyster, VA site over a period of four months. In this study, microbial attachment and detachment rates were estimated by fitting a one–dimensional transport model (eqs 3 and 4) to the measured concentrations of suspended bacteria. Numerical simulations using the resulting model parameters revealed that detachment could substantially increase bacteria travel distances.

Although the occurrence of microbial detachment has been noted in several experimental investigations, it is often overlooked in efforts to model microbe transport behavior. For instance, detachment is not considered in the classical colloid filtration theory (eq 5), which is the most commonly used approach for predicting microbe migration and fate in saturated porous media [50,

146]. One explanation for this oversight is the deficiency in our current understanding of microbial detachment processes. Several physical, chemical and biological factors may influence the rate and extent of microbial detachment from sediment grain surfaces. For example, some studies indicate that a microorganism's detachment behavior may be linked to nutrient availability [50, 67, 92]. Others suggest that hydrodynamic shear and/or collisions with suspended particles may affect the degree of microbe release [50, 71, 80]. Research also shows that detachment may be a function of microbial residence time on the collector surface [69, 88]. In this case, the detachment process is described using a distribution in detachment rates. An improved understanding of these and other factors controlling microbial release is required before practical incorporation of this process into mathematical transport models.

4. Proposed Improvements to the Classical Filtration Theory

In the previous section, several issues were raised concerning current methods used to model microbe transport and fate in saturated porous media. Research suggests that the equilibrium sorption mechanism is not appropriate for describing microbial attachment under most conditions. The effects of microbe–specific surface features, physical straining, detachment, growth and inactivation are not well understood and therefore rarely considered in microbe transport models. As a consequence, the most widely used approach for evaluating microbial transport behavior has been the most simple; namely, colloid filtration theory. However, as indicated previously, there are many limitations related to the application of CFT. In light of these concerns, certain modifications to classical CFT have recently been proposed.

4.1 A distribution in particle deposition rates

An experimental investigation carried out by Albinger et al [3] was one of the first to show that predictions of colloid transport based on CFT were not in good agreement with laboratory observations. These researchers found that a broad range of adhesion affinities (α) was required to account for the observed filtration behavior of a monoclonal bacterial population. Other investigators have since suggested a number of different distribution functions (e.g., power-law, bimodal, and lognormal) to describe the measured profiles of retained microorganisms [10, 100, 135]. In these studies, it was proposed that a distribution in microbial deposition rates could arise from, amongst others, variations in microbial surface properties. More recent experiments conducted with model latex colloids in columns packed with glass beads (see *section 3.2*) indicate that distributions in particle-collector interaction energies could be the major cause of the observed breakdown of CFT [77, 130, 132]. Specifically, it has been shown that under generally *unfavorable* conditions for deposition, three different types of particle-collector interactions may occur: (i) particles may overcome a repulsive energy barrier to deposit in the primary energy well, (ii) particles may encounter a deep secondary energy well where they can be retained, and (iii) the presence of surface charge heterogeneities may provide opportunities for particle deposition in the primary energy well in the absence of an energy barrier [130, 132]. As described previously, these different attachment mechanisms can give rise to both "*slow*" and "*fast*" particle or microbe deposition rates in porous media. This variation in deposition rates – which is driven by the presence of particle/microbe heterogeneity – can be incorporated into CFT by considering a bimodal distribution in k_{att} [132]. Indeed, model predictions obtained using a proposed dual deposition mode (DDM) model were shown to be in good agreement with independent sets of experimental data obtained with model (latex) colloids [132] as well as biocolloids (e.g., oocysts of *Cryptosporidium parvum*) [133]. These findings suggest that modification of CFT to include a

bimodal distribution in deposition rates should be a valuable change with respect to improvement of the classical model.

4.2 Straining as a removal mechanism

Microbe removal by straining is generally considered irreversible and can be described mathematically using a depth-dependent first-order expression. Bradford et al [17] used such an approach to simulate the effects of straining on particle transport whereby they included a depth-dependent removal term in eq 4. That is, these researchers considered the general form of the microbial transport model (eqs 2 and 4) where they took into account the effects of physicochemical filtration (k_{att}), detachment (k_{det}), and straining. Results of their work indicate that a more realistic representation of the experimental data is obtained when the removal of particles by both attachment and straining are considered. The modeling approach implemented by Bradford et al [17] can readily be applied to extend classical CFT to include the effects of physical straining (in the absence of detachment):

$$\frac{\rho_b}{\varepsilon}\frac{\partial S}{\partial t} = k_{att}C - k_{str}\psi_{str}C \tag{8}$$

where k_{str} is the straining coefficient and ψ_{str} is a dimensionless depth-dependent straining function. The straining function may take a wide range of forms, but the following power-law function has been shown useful [17]:

$$\psi_{str} = \left(\frac{d_c + x}{d_c}\right)^{-\beta} \tag{9}$$

Here, β is a fitting parameter that controls the shape of the spatial distribution of retained colloids. When eqs 2 and 8 are combined and solved within the context of CFT, (i.e., steady-state

conditions and negligible dispersion effects), the resulting model can be used to interpret microbe transport behavior under the influence of straining and attachment.

4.3 Accounting for microbe mobility

Propelled by their flagella, motile bacteria swim in the direction of their long axis at a rate of approximately 35 diameters/s [12]. Certain motile cells are capable of sensing chemical gradients and moving towards more favorable conditions. These chemotactic bacteria may exhibit different transport behavior from their non-motile counterparts [26, 85], however well-controlled studies on the role of motility in porous media transport are scarce.

Barton and Ford [9] proposed a simple mathematical model which characterizes the transport of motile bacteria through porous media using two effective transport parameters — random motility and chemotactic sensitivity. Comparison of model calculations with previously published experimental data showed that the presence of the porous medium caused a reduction in the random motility of the microbe population consistent with theoretical predictions. Duffy et al [42] used cellular dynamics simulations (CDS) to examine the effect of local chemical gradients on chemotactic behavior in porous media. Results of their simulations indicate that chemotaxis may increase bacterial migration in response to microscale gradients and can also enhance bacterial residence time near a nutrient source. Although the models of Barton and Ford [9] and Duffy et al [42] provide useful insight on the role of motility in bacteria transport, the effect of fluid flow is neglected in these approaches.

More recently, Nelson and Ginn [89] developed a mathematical model of microbial transport which considers the influences of (i) cell motility, (ii) physicochemical interactions between microbes and collector surfaces, and (iii) convective flow. By integrating CDS with

particle trajectory analysis, these researchers calculate the value of the single-collector contact efficiency, η_0, with the effects of chemotaxis incorporated. Preliminary results suggest that, under certain conditions, chemotaxis may cause a change in the dependence of η_0 on porewater velocity [89]. However, it is important to note that this model does not consider the influences of sedimentation, hydrodynamic (viscous) interactions, and Brownian diffusion on microbial attachment. The two latter mechanisms can be significant for particles as large as a few micrometers for transport at low *Peclet* numbers or flow rates (such as in subsurface environments), thus including a broad range of motile microorganisms of environmental relevance [131].

5. Concluding Remarks

In an effort to develop improved models of microbial transport in natural and engineered systems, the factors controlling migration and removal of microbes in porous media have been studied extensively. Based on these studies, a number of different mathematical approaches have been proposed to model microbial behavior observed in traditional packed column experiments or field-scale investigations. Several of these modeling approaches have inherent weaknesses or inconsistencies which are often overlooked by users. For instance, the relevance of classical colloid filtration theory seems to be limited to mainly *favorable* deposition conditions in ideal systems. In certain cases, models of microbial transport have been modified to account for these limitations (e.g., several researchers propose that a distribution in deposition rates should be incorporated into CFT). Such adjustments to traditional modeling approaches should provide significant improvements to predictions of microbial transport in porous media. However, a great deal more research is necessary to develop a comprehensive model of microbial migration which

considers the wide range of transport and deposition behavior encountered in complex natural environments.

Future areas for fundamental research in this area have been identified and include: (i) inactivation kinetics of pathogens in soils, (ii) role of protozoan grazing in removal of bacteria, (iii) mechanisms of microbial detachment from sediment grain surfaces, (iv) interactions between cell/cyst surface biomolecules and mineral surfaces, and (v) the influence of physical and geochemical aquifer heterogeneity on microbial transport [45, 50, 126]. Ginn et al [50] indicate how biological processes may be affected by one another and it may be necessary to consider the interdependency of such processes in the development of new models. There may also not be a clear distinction between mechanisms that are typically considered physicochemical and those that are biological because these processes are often coupled in relation to microbial transport and deposition [50]. Additional well-controlled laboratory-scale studies could improve our fundamental understanding of these mechanisms and allow the design of more accurate and complete models of microbial transport in porous media.

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Notation

Symbols

C	aqueous phase (suspended) microbe concentration
C_0	bulk (influent) microbe concentration
D	hydrodynamic dispersion coefficient

d_{10}	grain diameter for which 10% of all particles in the volume distribution are smaller
d_a	arithmetic mean diameter of collector (sand grain)
d_c	diameter of spherical collector
d_g	geometric mean diameter of collector (sand grain)
d_p	diameter of particle
K_{eq}	equilibrium distribution coefficient
k_{att}	microbial attachment rate coefficient
k_{det}	microbial detachment rate coefficient
k_{str}	microbial straining coefficient
N_{Pe}	Peclet number; $N_{Pe} = vx/D$
R	retardation factor, $R = 1 + \rho_b K_{eq}/\varepsilon$
S	attached microbe concentration
T	absolute temperature
t	time
t_0	duration of microbe injection
U	approach (superficial) velocity of fluid
v	interstitial pore velocity; $v = U/\varepsilon$
x	distance

Greek Symbols

α	microbe attachment (collision) efficiency
β	fitting parameter for straining function
ε	porosity of a porous medium
η_0	overall single-collector contact efficiency
ρ_b	dry bulk density of porous medium
ψ_{str}	dimensionless depth-dependent straining function

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Figure Captions

FIGURE 1. (A) Measured breakthrough of bacteriophage PRD-1 (in 10^{-6} M Ca^{2+}) in a column packed with glass beads. Originally presented in ref [5]. (B) Measured breakthrough of bacteriophage MS-2 and NaCl (tracer) at a field site in the dune area of Castricum, Netherlands (Well 2). Originally presented in ref [116].

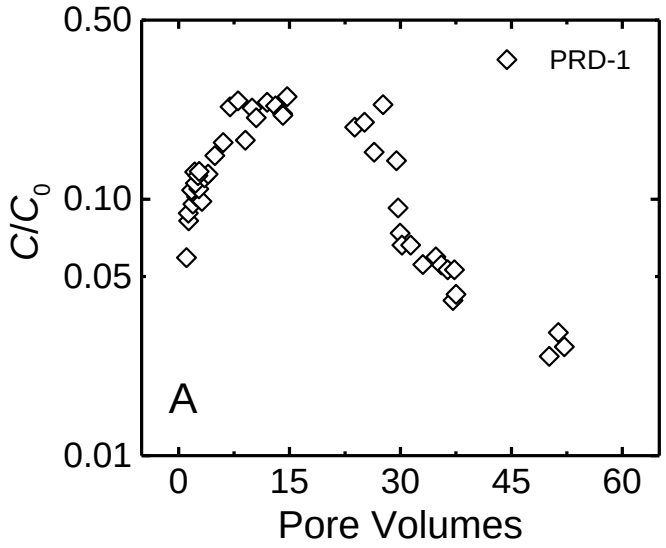
FIGURE 2. Comparison of measured retained particle concentration profiles (symbols) and predictions based on classical CFT using α_{BTC} determined from the corresponding breakthrough curve (dashed lines) for *favorable* deposition conditions. (A) Deposition of 3 μm carboxyl-modified latex particles suspended in 300 mM buffered KCl. Originally presented in ref [132]. (B) Deposition of 0.93 μm amine-modified latex particles in 1 mM buffered NaCl (approach velocity = 4 m/d). Originally presented in ref [77]. In both studies, particle deposition was examined using columns packed with glass beads.

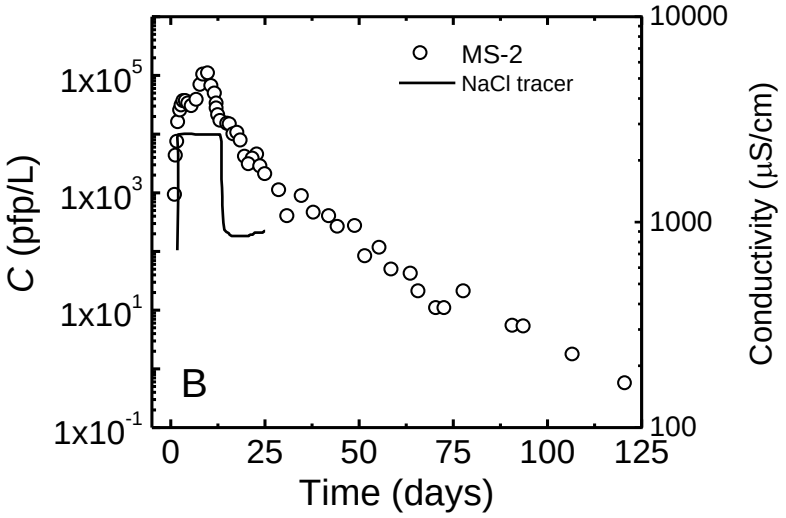
FIGURE 3. Comparison of measured retained particle concentration profiles (symbols) and predictions based on classical CFT using α_{BTC} determined from the corresponding breakthrough curve (dashed lines) for *unfavorable* deposition conditions. (A) Deposition of carboxyl-modified latex particles suspended in 3 mM buffered KCl. Originally presented in ref [132]. (B) Deposition of carboxyl-modified latex particles in 20 mM buffered NaCl (approach velocity = 4 m/d). Originally presented in ref [77]. In both studies, particle deposition was examined using columns packed with glass beads.

FIGURE 4. Deposition kinetics of viable, heat treated, and formalin treated *Cryptosporidium* oocysts onto a quartz surface as a function of solution ionic strength. The deposition kinetics are expressed as oocyst transfer rate coefficient, k_{RSPF}, and attachment efficiency, α. The capillary

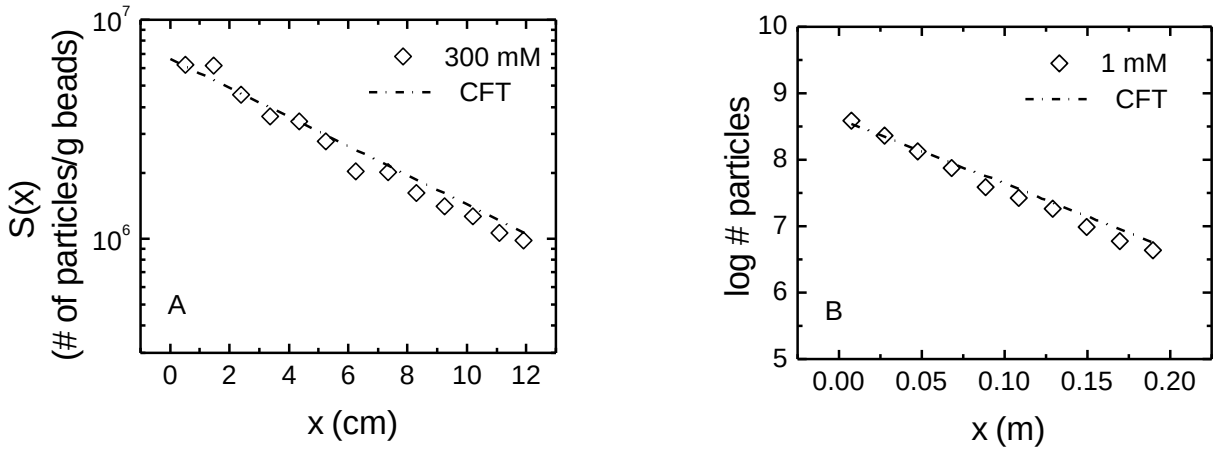
flow rate in the RSPF system was fixed at 9.0 mL/min (average velocity of 4.77 cm/s). Other experimental conditions were an unadjusted pH of 5.5 – 5.7 and a temperature of 25ºC (± 1ºC). Originally presented in ref [74].

FIGURE 5. Breakthrough curves for experiments conducted with model latex particles of increasing size (0.32, 1.0, 1.9, and 4.1 μm) suspended in DI water in a column packed with clean quartz sand. Other experimental conditions include approach velocity = 0.042 cm/s, porosity = 0.43, mean grain diameter = 0.21 mm, pH = 5.6–5.8, and temperature = 22-23°C. Originally presented in ref [134].



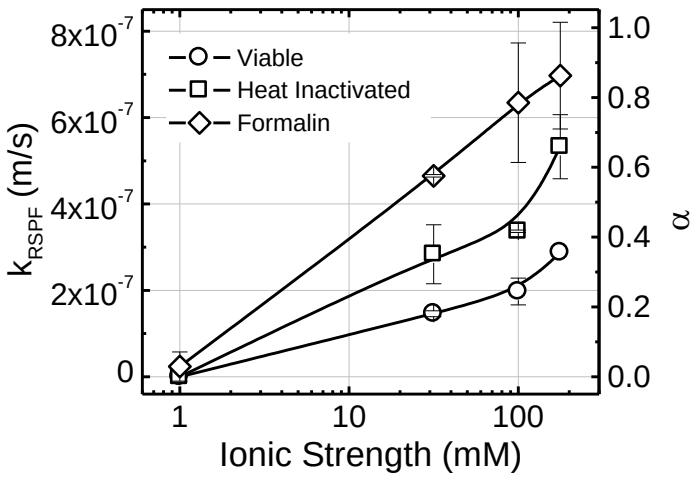


Tufenkji, FIGURE 1

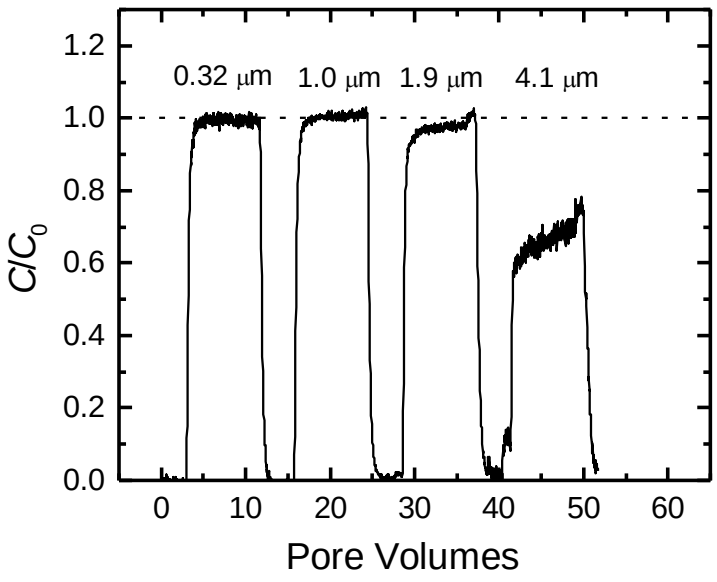


Tufenkji, FIGURE 2

Tufenkji, FIGURE 3



Tufenkji, FIGURE 4



Tufenkji, FIGURE 5