Bacterial transport in granular porous media: the effects of cell concentration and media pre-coating

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CONTRIBUTIONS OF AUTHORS

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ABSTRACT

Column transport experiments were conducted under saturated conditions to examine the effects of cell concentration and media pre-coating. Two strains of *E. coli* were used in the study; the commonly studied laboratory organism *E. coli* K12 D21 and a mutant of the waterborne pathogen *E. coli* O157:H7. Column experiments were conducted with both clean sand and sand that was pre-coated with bacteria. The influent concentration of the *E. coli* strains was varied over several orders of magnitude to examine the effect of cell concentration. Concentration dependent removal rates were observed for both organisms in both the clean and media pre-coated sand columns. It was also found that the media pre-coating either does not influence the transport behavior or it decreases the attachment efficiency. Although differences in transport are observed, these differences are not large enough to have a significant influence on the predicted travel distances.

RÉSUMÉ

Des expériences de transport par colonne ont été menées afin d'examiner les effets de la concentration des cellules et du pré-revêtement de média. Deux souches de bactéries ont été utilisées: *E. coli* K12 D12 et une souche mutante *E. coli* O157:H7. Les expériences par colonne ont été menées avec du sable propre et du sable qui a été préalablement enduit de bactéries. La concentration de l'influent en bactérie a été variée sur plusieurs ordres de grandeur pour examiner l'effet de la concentration cellulaire. Une dépendance du taux d'élimination à la concentration a été observée pour les deux souches de bactéries dans les deux types de sable. De plus, le pré-revêtement de média n'influence d'aucune façon le comportement du transport ni en réduit l'efficacité d'adhésion. Bien que des différences dans le transport ont été observées, celles-ci n'ont eu aucun effet significatif sur la prédiction de la distance à parcourir.

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LIST OF SYMBOLS

A	Hamaker constant
A_S	Porosity dependent parameter
a_P	Radius of the particle
C	Effluent concentration
C_0	Influent concentration
D_β	Diffusion coefficient in an infinite medium
d_c	Diameter of the collector grain
d_p	Diameter of the particle
f	Bed porosity
g	Acceleration due to gravity
k	Boltzmann constant
L	Bed depth
N_A	Attraction number
N_G	Gravity number
N_{Pc}	Peclet number
N_R	Aspect ratio
N_{vdW}	Van der Waals number
T	Absolute temperature
U	Fluid approach velocity
V_A	Van der Waals attraction energy
V_R	Electrical double layer interaction energy
V_T	Total interaction energy

List of Greek Letters

α	Attachment efficiency factor
ζ	Zeta potential
η_0	Single-collector contact efficiency
η_D	Single-collector contact efficiency for transport by diffusion
η_G	Single-collector contact efficiency for the transport by gravity
η_I	Single-collector contact efficiency for the transport by interception
μ	Absolute viscosity
ρ_f	Density of the fluid
ρ_p	Density of the particle

List of Abbreviations

CFT	Colloid Filtration Theory
CFU	Colony-forming units
DLVO	Derjaguin-Landau-Verwey-Overbeek
EPM	Electrophoretic mobility
ESD	Equivalent spherical diameter
LPS	Lipopolysaccharide
NOM	Natural organic matter
PV	Pore volume

CHAPTER 1: INTRODUCTION

1.1 OBJECTIVE

The main objective of this research was to study the effects of cell concentration and media pre-coating on bacterial transport. This was accomplished by conducting laboratory-scale column transport experiments with two different strains of bacteria: *E. coli* K12 D21 and a non-toxigenic mutant of *E. coli* O157:H7. To study the effect of cell concentration, the influent bacterial concentration was varied over several orders of magnitude. These bacterial solutions were then injected into the column and the transport behavior was analyzed. The influence of a bacterial media pre-coating was analyzed by first injecting a bacterial suspension into the column to coat the media. After the pre-coating process, a second bacterial strain was injected into the column with the influent cell concentration varied over several orders of magnitude. Another secondary objective was to compare two different detection methods; hence both spectrophotometric and direct colony counting techniques were utilized to analyze the column effluent. The data obtained in these experiments can be utilized to improve the accuracy of current bacterial transport models.

1.2 BACKGROUND

An understanding of bacterial transport in the subsurface is important to better protect drinking water supplies. Waterborne disease outbreaks have been reported on the global scale and the consequences are often deadly. A pathogen of particular interest is *E. coli* O157:H7 which has been implicated in several disease

outbreaks. For example, a serious outbreak of *E. coli* O157:H7 and *Campylobacter spp.* occurred in Walkerton, Ontario which caused 2300 illnesses and left seven people dead [1]. It is believed that the source of the contamination was manure runoff from a nearby farm [1]. Bacterial transport models provide a means of understanding and predicting pathogen transport behavior in natural subsurface environments. This allows regulators to develop laws to better protect our drinking water supplies and prevent future waterborne disease outbreaks. Not only are bacterial transport models useful in the protection of drinking water supplies, but other applications include riverbank filtration, the regulation of manure spreading and the development of bioremediation strategies [2-6]. For example, in riverbank filtration, the riverbed acts as a natural filter which removes contaminants such as natural organic matter, inorganic contaminants and pathogens [2]. The river water is pumped through the sand and soil deposits that form the riverbank to a pumping well which brings the filtered water back up to the surface [2].

A method of studying bacterial transport includes conducting laboratory-scale column experiments under different conditions to evaluate the effects of different parameters. In these experiments, a column is packed with media and a bacterial suspension is pumped through the column. Possible materials used as column media include glass beads and silica sand. The column effluent is collected and analyzed to determine the concentration and the extent of removal. The data obtained from these experiments is used to develop more accurate and reliable bacterial transport models. Current bacterial transport models are based on the colloid filtration theory (CFT) which was developed by Yao et al. [7]. This

model considers the forces and interactions acting upon the bacteria and sand grains to make predictions regarding the transport behavior. One of the most important mechanisms influencing bacterial transport is bacterial attachment to a sand grain. The extent and the type of attachment are controlled by the interaction energy which results between bacteria and the surface of the sand grain. Researchers are currently trying to improve existing bacterial transport models by studying different factors which can significantly influence bacterial transport. Some of these factors include: influent cell concentration, inorganic groundwater chemistry, cell surface properties, presence of organic matter, soil grain properties and media pre-coatings.

1.2.1 Colloid Filtration Theory

The transport of pathogens through porous media can be predicted through the use of colloidal filtration transport models. The term colloids refers to particles that have a particle size ranging from a few nm to a few μm such as macromolecules, clays, viruses, and bacteria [8]. The first transport model, the classic filtration model, was developed by Yao et al. [7] however this model does not take certain forces into account such as hydrodynamic interactions and van der Waals attractive forces [9]. To account for these limitations, recent computational approaches have been developed that incorporate more interactions. A correlation equation which takes more interactions into account was developed by Rajagopalan and Tien [10] (referred to as the RT equation), however, this correlation does not take into account the hydrodynamic interactions and van der Waals attractive forces on particles undergoing Brownian

diffusion [9]. Brownian diffusion refers to the random collisions between the colloidal particles and the surrounding fluid particles which causes the colloidal particles to flow along a random path [8]. Another correlation equation was recently developed by Tufenkji and Elimelech [9] (referred to as the TE equation) which takes into account the forces and interactions excluded by the previous correlation equations, thus providing a more accurate prediction [9]. The classic filtration model and the TE equation will be discussed in detail in the following paragraphs.

The classic filtration model was developed by Yao et al. and it is derived from a mass balance on the transport of a single colloidal particle traveling through a porous media [7]. The three main factors governing the transport of the particle include:

a) interception: colloid flowing along a stream line and colliding with a collector grain because of its size;

b) sedimentation or gravity: effects of buoyancy and fluid drag on the colloid; and

c) diffusion: Brownian movement of the colloid [7].

The integrated form of this general mass balance is:

$$\ln\frac{C}{C_0} = -\frac{3}{2}(1-f)\alpha\eta_0\left(\frac{L}{d_c}\right) \qquad (1.1)$$

where C is the effluent concentration, C_0 is the influent concentration, f is the bed porosity, α is the attachment efficiency factor, η_0 is the single-collector contact efficiency, L is the bed depth and d_c is the diameter of the collector grain [7]. The term α is a ratio between the number of collisions between a colloidal particle and a collector grain that result in attachment divided by the total number of collisions

[7]. Since α is a ratio, it must have a value between 0 and 1. It follows that if α=1, then all collisions result in attachment, whereas if α=0, then attachment does not occur. The term η_0 is a ratio between the rate at which colloidal particles collide with the collector grain divided by the rate at which colloids flow towards the collector grain [7]. This term measures the ratio of colloids that come into contact with a collector grain. An analytical expression for η_0 is represented by:

$$\eta_0 = \eta_D + \eta_I + \eta_G \tag{1.2}$$

where η_D is the transport by diffusion, η_I is the transport by interception, and η_G is the transport by gravity [7]. The original expressions for η_D, η_I, and η_G proposed by Yao et al. have been updated many times, most recently by Tufenkji and Elimelech in the development of the TE equation.

The TE equation is a correlation equation developed by Tufenkji and Elimelech [9] to predict the value of η_0, the single-collector contact efficiency which is defined as:

$$\eta_0 = 2.4 A_S^{1/3} N_R^{-0.081} N_{Pc}^{-0.715} N_{vdW}^{0.052} + 0.55 A_S N_R^{1.675} N_A^{0.125} + 0.22 N_R^{-0.24} N_G^{1.11} N_{vdW}^{0.053} \tag{1.3}$$

where A_S is the porosity dependent parameter which is defined as:

$$A_S = \frac{2(1-\gamma^5)}{2-3\gamma+3\gamma^5-2\gamma^6} \tag{1.4}$$

where $\gamma = (1-f)^{1/3}$; N_R is the aspect ratio; N_{Pc} is the Peclet number; N_{vdW} is the van der Waals number; N_A is the attraction number; and N_G is the gravity number [9]. Table 1.1 provides a summary of the definitions of the dimensionless parameters [9].

Table 1.1. Summary of Dimensionless Parameters [9]

Dimensionless Parameter	Definition	Summary of Variables in the Definition
N_R	$\frac{d_p}{d_c}$	d_p: diameter of the particle; d_c: diameter of the collector grain
N_{Pc}	$\frac{Ud_c}{D_\beta}$	U: fluid approach velocity; D_β: diffusion coefficient in an infinite medium
N_{vdW}	$\frac{A}{kT}$	A: Hamaker constant; k: Boltzmann constant; T: absolute temperature
N_A	$\frac{A}{12\pi\mu a_p^2 U}$	μ: absolute viscosity; a_P: radius of the particle
N_G	$\frac{2}{9}\frac{a_p^2(\rho_p-\rho_f)g}{\mu U}$	ρ_p: density of the particle; ρ_f: density of the fluid; g: acceleration due to gravity

This correlation equation takes into account both the hydrodynamic interactions and van der Waals attractive forces which were neglected in other correlations [9]. It also provides an accurate prediction of the value of η_0 when compared to the experimental results obtained in the laboratory [9].

There are currently no theoretical approaches to accurately predict the value of α, the attachment efficiency, therefore, it is necessary to conduct laboratory experiments to obtain this parameter [9]. The integrated form of the mass balance equation proposed by Yao et al. [7] can be rearranged as follows:

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-f)L\eta_0}\ln\left(\frac{C}{C_0}\right) \qquad (1.5)$$

In the laboratory, column experiments are conducted under many different conditions including various chemical conditions, with different collector grains, and with various types of colloids in order to determine the value of α for each set

of conditions. The experimental values of α and the transport models can then be combined to form a complete mathematical model to make predictions about the transport of pathogens through granular porous media.

1.2.2 Bacterial Attachment

One of the key mechanisms controlling the extent of bacterial transport through granular porous media is bacterial attachment or deposition onto soil grains. There are two main factors that affect the retention of bacteria in granular porous media: physical straining and physicochemical attachment [11]. Straining refers to the physical trapping of bacteria where the size of the pores is smaller than the size of the bacteria [11]. Physicochemical attachment occurs when the bacteria attaches to the surface of the soil or sand grain. When the pore size is larger than the size of the bacteria, attachment becomes the dominant factor affecting the removal of bacteria from the pore fluid [11]. The classic filtration model and the correlation equations for the single-collector contact efficiency are only related to bacterial attachment and they do not consider straining.

The extent of physicochemical attachment depends on the interaction energy between the colloidal particle and the collector grain. A theory developed by Derjaguin, Landau, Verwey, and Overbeek, known as the DLVO theory, defines the total interaction (V_T) between the colloidal particle and the collector grain as:

$$V_T = V_A + V_R \tag{1.6}$$

where V_A is the van der Waals attraction, V_R is the electrical double layer interaction, and all the terms are expressed as potential energy [8]. The van der Waals attractive force refers to the force that arises between closely spaced molecules due to the spontaneous electrical and magnetic polarization of the molecules [8]. In the case of bacteria attaching to soil grains, the van der Waals attractive force is calculated using the approximations developed for sphere-plate interactions. The electrical double layer interaction refers to the interaction of the double layers that surround the colloidal particle and the collector grain [8]. The strength of each of these forces defines the shape of the interaction energy profile which provides a theoretical explanation for physicochemical attachment. Figure 1.1 shows unfavourable and favourable potential energy profiles [8]:

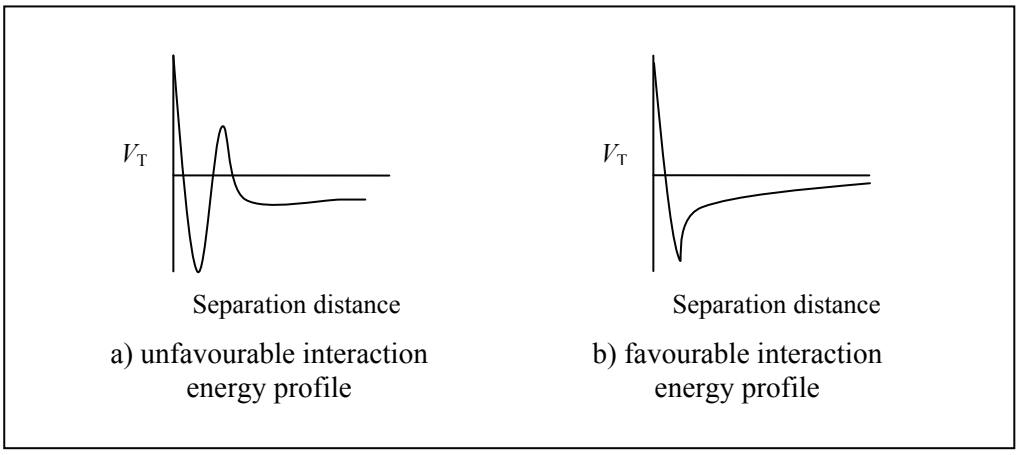

Figure 1.1 Interaction Energy Profiles between Bacteria and Media Grains [8]

In Figure 1.1, case a) represents unfavourable deposition conditions where repulsive interactions are present; and case b) represents favourable deposition conditions where repulsive interactions are only present at very close separation distances (i.e., Born repulsion) [8, 12]. These repulsive interactions are usually

made up of the electrical double layer repulsive interactions [12]. In unfavourable deposition conditions, the attachment efficiency factor, α, is less than 1, however, in favourable deposition conditions, α=1 [8]. Unfavourable deposition occurs in most groundwater systems [12]. Favourable deposition may occur in groundwater systems when there is a high ionic strength, a high level of hardness, or if the surface of the media grain is coated with a positively charged mineral such as iron, aluminum, or manganese oxides [12]. Unfavourable deposition is challenging to study because it has been found that the Colloid Filtration Theory does not provide accurate predictions regarding attachment when strong repulsive forces are present [13]. Updated models are currently being developed which provide more accurate predictions of bacterial transport in unfavourable conditions [13].

1.2.3 Factors Affecting Bacterial Attachment

Although the extent of physicochemical attachment is based on the total interactive forces between the colloid and the soil grain, the strength of these forces can be altered by changing various parameters of the system. For example, when the solution chemistry of the system is altered, the electrical double layer potential energy is also altered, however, the solution chemistry does not affect the van der Waals attractive potential energy [12]. When the interactive forces are altered then the extent of physicochemical attachment is also altered. There are many factors that affect the degree of bacterial physicochemical attachment to granular porous media. Some of these factors include: inorganic groundwater

chemistry, presence of organic matter, soil grain properties, cell surface properties, influent cell concentration and granular porous media coatings [14].

The inorganic chemical properties of groundwater can play a significant role in the bacterial physicochemical attachment onto collector grains, thus both ionic strength and pH are important factors to consider. The effect of ionic strength on bacterial attachment onto soil grains has been well studied and it is generally found that attachment increases with increasing ionic strength [14]. This behavior can be explained by the DLVO theory which describes the total attractive and repulsive interactions between the colloids and the media grains [12]. At high ionic strengths, there is a small double layer because the surface charges can be balanced with a smaller amount of counterions since the ion concentration at the surface is high [12]. When there is a small electrical double layer, interaction only occurs when the molecular surfaces are very close together, thus there is a reduction in double layer repulsion [12]. At high ionic strengths the van der Waals attractive forces dominate, thus attachment increases [12]. On the other hand, at low ionic strengths, there is a thick double layer because the surface charges must be balanced with a larger amount of counterions [12]. At low ionic strengths, the double layer repulsion is increased, thus attachment is commonly observed to decrease [12].

Another factor that affects the degree of bacterial physicochemical attachment to granular porous media is the concentration of natural organic matter (NOM). NOM comprises humic substances which are formed through the decay of plant and animal matter. It has been found that dissolved NOM can decrease the amount of bacterial attachment because the NOM competes with the bacteria

for attachment sites on the media grains [11]. Another possible explanation for the decrease in attachment is that the dissolved NOM adsorbs to the surface of the bacteria, thus increasing the negative charge of the bacteria [15]. Since the sand is also negatively charged, this increase of negative charge on the bacteria increases the repulsion between the sand grains and the bacteria, which decreases attachment [15]. In natural systems, there are numerous factors that overlap to control transport and attachment. It is important to study each of these factors both individually and in combinations in order to make accurate predictions concerning transport and attachment. In one study, Johnson et al. found that the presence of NOM slightly increased the attachment of bacteria on iron oxide coated quartz [15]. They suggest that the NOM increased the negative charge of the bacteria which increased the attraction to the positively charged iron oxide coated media [15].

It has been found that the physical properties of the media affect the attachment of bacteria. Some of these properties include particle size, surface roughness and surface charge. It has been found that smaller sand grains increase the degree of bacterial attachment because more surface area is exposed which provides more possible sites for attachment [11]. Fontes et al. suggest that particle size is the most important factor controlling the overall transport and attachment of bacteria when compared to other factors such as ionic strength and cell size [16]. It has been found that bacterial attachment increases when the grain surface is rough [11]. It is believed that this is caused by the reduction of shear forces which reduces detachment, and the increase in surface area which provides more possible attachment sites [11]. The surface charge of the soil grain is a major

factor controlling attachment. It is expected that a media surface charge that is more positive will have a higher bacterial attachment rate compared to a media surface charge that is more negative [11]. The surface charge of the soil grain depends on the ionization of its surface groups which are affected by factors such as ionic strength, NOM, pH, and different types of coatings [11]. This demonstrates the importance of studying the surface charge characteristics in relation to each of these factors in order to make accurate predictions about bacterial interactions and attachment.

The properties of the cell surface also can influence the extent physicochemical attachment. Some of these properties include: the presence of cell surface structures, the hydrophobicity of the cell surface, and the cell surface charge [17]. The surfaces of cells comprise structures such as outer membrane proteins, flagella, and extracellular lipopolysaccharides (LPS) which can affect attachment characteristics [18]. Walker et al. studied the effects of LPS on *E. coli* attachment to sand grains and although the LPS affected attachment, the exact attachment mechanism is still unknown because they did not find direct attachment correlations related to the LPS length nor to the charge of the LPS functional group [18]. The effects of cell hydrophobicity on bacterial attachment are also not well understood. For example, some studies have reported that hydrophobic microorganisms attach more effectively to hydrophobic media grains, however, other studies do not find correlations between hydrophobicity and attachment [11]. A recent study by Jacobs et al. investigating the effects of cell surface properties on attachment rates reported that hydrophobic microorganisms have a higher attachment rate than hydrophilic microorganisms

[19]. Jacobs et al. suggest that the differences in attachment between hydrophobic and hydrophilic microorganisms are due to the acid base interactions [19]. Although many studies have found a strong correlation with respect to the cell surface charge and the extent of physicochemical attachment to soil grains, a few studies have reported no correlations [11]. Where correlations exist, it was found that attachment decreases as the negative zeta potential of the cell surface increases [11]. Jacobs et al. also reported that microorganisms with low zeta potentials have lower attachment rates because of the strong electrostatic repulsions with the media grains [19]. Although it is known that cell surface properties influence attachment, the exact mechanisms are still unknown. This is currently one of the major challenges in developing accurate transport models and correlation equations [13].

The influent cell concentration had also been shown to have an effect on the transport of bacteria in the subsurface. In the literature, several studies have been reported where column experiments were conducted and concentration dependent removal rates were observed [20-27]. For example, in a laboratory column study conducted by Bai et al. with the organism *Pseudomonas*, it was found that as the influent concentration was increased over several orders of magnitude, the normalized breakthrough concentration also increased [20]. Thus at higher influent cell concentrations, there is less retention in the column and the attachment efficiency is lower. The possible explanations for this behavior include blocking [22, 23, 26] or straining [21, 24, 28]. Blocking occurs when bacteria already attached to the sand grain prevent the attachment of additional incoming bacteria [29]. Alternately, straining occurs when bacteria become physically

trapped between individual sand grains because the pores are too small to allow the bacteria to pass through them. Research has been conducted in the area of developing improved blocking and straining models [23, 24, 29].

The granular porous media coating can influence bacterial physicochemical attachment by altering the surface properties of the media. For example, an iron oxide coating on the media surface increases attachment by changing the surface charge of the media to make it more positive [11]. When the charge on the surface of the media grains becomes more positive, the interaction between a negatively charged bacterium and a positively charged soil grain becomes more favourable, thus increasing the probability that a bacterium will attach to the surface [11]. The effect of media coating was also studied by Foppen et al. where they compared *E. coli* attachment to several compositions of goethite coated quartz grains which is a mineral composed of iron oxide [30]. They found that when the amount of goethite coated quartz grains in the composition was increased, bacterial attachment also increased [30]. Bacterial attachment onto coated media surfaces can also be compared to the attachment of viruses and protozoa to these surfaces. A study conducted by Abudalo et al. showed that as the fraction of iron oxide coated sand grains increased, attachment also increased for both bacteriophage PRD1, a virus, and *Cryptosporidium parvum* oocysts, a protozoa [31]. These findings are consistent with the results obtained with the bacteria where attachment increases as the surface charge of the soil grains becomes more positive.

Another area of interest is the effect of a biological media pre-coating on the transport of bacteria. Although there has been little research focused on

bacterial media pre-coatings, some studies have considered the effects of a biofilm coating [32-34]. A biofilm is a structure of microorganisms and extracellular polymeric substances which can affect the transport behavior of bacteria. For example, Liu and Li studied the effects of *Pseudomonas aeruginosa* biofilm on the transport behavior of an *E. coli* strain [32]. It was found that the biofilm coating has an effect on bacterial transport, however biofilm grown from different strains of *Pseudomonas* had different impacts on the transport behavior of the *E. coli* strain [32]. One of the *Pseudomonas* biofilm coatings significantly increased the attachment of *E. coli* in the column, however the other coating did not have a significant influence on attachment [32].

CHAPTER 2: EFFECT OF MEDIA PRE-COATING AND CELL CONCENTRATION ON THE TRANSPORT OF TWO *E. COLI* STRAINS IN GRANULAR POROUS MEDIA

2.1 INTRODUCTION

The study of bacterial transport and retention in granular porous matrices is important in a broad range of environmental applications, including granular filtration in water treatment, design of riverbank filtration systems, regulation of land application of agricultural waste, treatment of waters impacted by urban stormwater, and implementation of bioaugmentation strategies in bioremediation [2, 35-37]. To better understand the influence of various environmental factors on bacterial transport, several laboratory-scale column studies have been reported in the scientific literature [13, 14]. Data from these investigations are often characterized using the classic colloid filtration theory (CFT) developed by Yao et al. [7] or modified versions of this traditional model. Although CFT implies that the extent of microbe removal is independent of the suspended microbe concentration, several laboratory studies have reported concentration dependent removal rates for microbes and nonbiological colloids transported in granular porous media [20-27]. In a study where the bulk concentration of *Pseudomonas aeruginosa* (*P. aeruginosa*) was increased from 1×10^{7} to 5×10^{11} cells/mL, the normalized breakthrough concentration demonstrated a gradual but significant increase [20]. Specifically, the extent of bacterial retention decreased with increasing bulk cell concentrations. This behavior has been reported in a number of investigations conducted using microorganism concentrations high enough to potentially result in blocking ($>\sim10^{7}$ organisms/mL) and experimental conditions representative of those in the subsurface. In contrast, several investigations conducted using markedly lower microorganism concentrations that would

preclude blocking (~10^{-2} to 10^{3} organisms/mL) and experimental conditions representative of rapid granular filtration during drinking water treatment have reported both increases and no apparent differences in microbial retention with increasing bulk microbial concentrations [38, 39]. Cumulatively, these studies suggest that when the influence of the bulk cell concentration is not considered in bacterial transport models, the extent of microbe migration in water saturated granular environments may be either under- or overestimated.

Many studies of bacterial transport are conducted in model laboratory systems using clean sands or glass beads as the granular matrix [18, 24, 40, 41]. However, in the natural subsurface or engineered systems, a wide range of biological or nonbiological materials (e.g., other microorganisms, organic macromolecules, clay particles) can attach onto the surfaces of the sand or soil grains. These attached materials can subsequently alter the transport and retention of additional bacteria. Yet, it is still unclear how these materials interact with and influence the transport behavior of new organisms introduced into the granular environment.

Although few researchers have considered the impact of individual attached bacteria on colloid and microbe transport, some studies have considered the influence of biofilms on the transport of particles and microbes in granular porous matrices [32, 33, 42]. Biofilms are complex structures of microorganisms in a matrix of extracellular polymeric substances. These studies have shown that biofilms can either enhance or hinder colloid transport depending on the properties of the system [32, 33, 42]. For example, Liu et al. [32] conducted laboratory column experiments to examine the interaction of a *P. aeruginosa*

biofilm coating on the attachment, growth and detachment of an *E. coli* strain in granular porous media. The biofilm coating had a significant influence on the transport behavior of *E. coli*, resulting in increased retention and survival. Detachment of the biofilm coating caused reintroduction of *E. coli* into the column effluent.

The goal of this study was to examine the influence of granular media pre-coating and bacteria concentration on the transport behavior of two strains of *E. coli*. Laboratory-scale column experiments were used to study the transport and retention of *E. coli* O157:H7 and *E. coli* K12 D21 in a clean sand matrix or a sand matrix that was pre-coated with individual cells of one of the bacterial strains. Experiments conducted at different influent cell concentrations of 10^5, 10^7 and 10^8 cells/mL provide insight into the effect of this parameter on bacterial transport behavior in granular aqueous environments representative of the subsurface.

2.2 MATERIALS AND METHODS

2.2.1 Selection and Preparation of Bacteria

The bacterial strains selected for this study were *E. coli* O157:H7 ATCC 43888 and *E. coli* K12 D21. The selected *E. coli* O157:H7 is a nontoxigenic mutant strain and *E. coli* K12 D21 is a well-characterized model laboratory organism that is resistant to streptomycin. The bacteria were maintained at -80°C in Luria-Bertani (LB) Lennox broth (20 g/L, Fisher) with 15% glycerol. A few days prior to use, the frozen cultures were streaked onto LB agar plates and incubated for 24 hours at 37°C. Colonies from the LB agar plates were used to inoculate 150 mL of

LB broth in a 500 mL baffled flask. For cultures of *E. coli* K12 D21, the liquid medium was also supplemented with streptomycin (100 mg/L) (Sigma, S9137-25G). The culture was incubated for 3 hrs at 37°C at 200 rpm. After the growth period, the bacterial cells were centrifuged (Sorvall RC6) for 15 min at 5860*g* in an SLA-1500 rotor (Sorvall) at 4°C. After centrifugation, the growth medium was decanted and the pellet was resuspended in a solution of 10 mM KCl. To remove all traces of the growth medium, the centrifugation and resuspension steps were repeated an additional time. The bacterial suspension was then maintained at a temperature of 4°C for 16 hrs before being used in an experiment. Prior to conducting a column transport experiment, the bacterial concentration in suspension was determined using a counting chamber (Hawksley Medical and Lab Equipment, Lancing, UK). The bacterial suspension was then diluted accordingly with 10 mM KCl to the desired target concentrations: 10^5, 10^7 or 10^8 cells/mL. The pH of the bacterial suspension was adjusted to 5.6 using KOH or HCl when needed.

The electrokinetic properties of the bacterial cells were characterized using laser Doppler velocimetry (Zetasizer Nano ZS, Malvern Instruments). The electrophoretic mobility (EPM) of the bacterial cells was measured at 25°C in a solution of 10 mM KCl using a cell concentration of 10^7 cells/mL. Bacterial zeta (ζ) potentials were calculated from the measured EPMs using the Smoluchowski equation [43]. The percentage of viable cells was quantified with a LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit (Invitrogen, L-13152) and found to be greater than 90% for both organisms.

The size of the bacteria was determined by analyzing images collected using an inverted fluorescent microscope operated in phase contrast mode. Image analysis software (ImageJ, NIH) was used to determine the arithmetic mean lengths of the major and minor axes of the cells and the resulting equivalent spherical diameter. At least 100 individual cells were analyzed and the cell size distribution was determined for each organism when suspended in 10 mM KCl at pH 5.6.

2.2.2 Preparation and Characterization of Granular Porous Media

The porous medium used in the transport experiments was high purity quartz sand (Sigma-Aldrich) with a mesh particle size of -50+70. Prior to conducting transport experiments, the sand was acid washed to remove impurities. The acid washing procedure involves soaking the sand in 12M HCl for 24 hrs, rinsing with deionized (DI) water until the pH reaches 5.6-6, then baking in a furnace. The acid washed sand was baked at 120°C for 1 hour then the temperature was raised to 800°C and the sand was baked for an additional 5 hours. The reported 256 μm average grain diameter of this sand was reported previously [44].

2.2.3 Bacterial Transport Experiments

Bench-scale column transport experiments were conducted to evaluate the transport behavior of the bacterial cells at varying cell concentrations and with different media pre-coating conditions. Column experiments were conducted by pumping (model 780230 syringe pump, KD Scientific) a bacterial suspension

through a glass column packed with clean quartz sand. The glass column had an inner diameter of 1.6 cm and the sand was wet packed with vibration to a height of 10 cm. Prior to bacterial injection, the column was equilibrated with 10 pore volumes (PVs) of a 10 mM KCl electrolyte solution. After equilibration, 3.5 PVs of the bacterial suspension were injected into the column. The pump flow rate was set to 0.7 mL/min, which corresponds to an approach velocity (U) of 5.8×10^{-5} m/s.

The bacterial concentration in the column effluent was analyzed using two methods: (i) on-line measurement using a flow-cell mounted in a UV-visible spectrophotometer (Hewlett-Packard model 8453) at a wavelength of 254 nm; and (ii) collection of several samples using a fraction collector (Spectra/Chrom® CF-1 Fraction Collector) and subsequent spread plating on agar plates (Difco™ R2A Agar) to obtain a direct colony count. Prior to plating the effluent samples, serial dilutions were prepared using test tubes containing sterile Maximum Recovery Diluent (Oxoid, CM0733). The agar plates were incubated overnight at 37°C and the individual bacterial colonies were counted to determine the bacterial cell concentration. Countable numbers of 20 to 200 CFU plate^{-1} were targeted [45].

Two separate series of experiments were conducted to evaluate the influence of media pre-coating and cell concentration on bacterial transport and retention. In the first set of experiments, each bacterial strain (*E. coli* O157:H7 ATCC 43888 and *E. coli* K12 D21) was injected into the clean packed column separately. These experiments were conducted using bacterial suspensions prepared at three different influent concentrations (C_0): 10^5, 10^7 or 10^8 cells/mL.

In the second set of experiments, the sand packed column was pre-coated with one of the strains of *E. coli* prior to injecting the second strain. First, 3.5 PVs of an *E. coli* O157:H7 suspension at a concentration of 10^7 cells/mL were injected into the column to pre-coat the granular media. Next, 3 PVs of the background electrolyte solution (10 mM KCl) were injected, followed by 3.5 PVs of an *E. coli* K12 D21 suspension at concentrations of 10^5, 10^7 or 10^8 cells/mL. In these experiments, the concentration of *E. coli* K12 D21 in the column effluent was confirmed using plate counts on agar plates prepared with streptomycin (100 mg/L). This ensured that the cell counts were not affected by the presence of *E. coli* O157:H7 that may have detached from the granular media. A series of experiments with column pre-coating were also conducted such that the *E. coli* K12 D21 (10^7 cells/mL) was injected to pre-coat the column followed by electrolyte and a final injection of *E. coli* O157:H7 at concentrations of 10^7 or 10^8 cells/mL. In these experiments, the concentration of *E. coli* O157:H7 in the column effluent was calculated as the difference between colony counts obtained on plates prepared with and without the antibiotic (only *E. coli* K12 D21 grew on the antibiotic plates, whereas both organisms grew on the antibiotic-free plates).

2.3 RESULTS AND DISCUSSION

2.3.1 Bacteria Characterization

The mean zeta potential of the bacteria suspended in 10 mM KCl at pH 5.6 was determined to be -2.4 mV and -38.2 mV for *E. coli* O157:H7 and *E. coli* K21 D21, respectively. Although the mean zeta potential of *E. coli* O157:H7 was

found to be near zero, aggregation was not found to be occurring. Furthermore, this result is in agreement with literature. For example, Castro et al. reported the zeta potential of *E. coli* O157:H7 and common surrogates [41]. They found that at 10 mM the zeta potential for these *E. coli* strains was slightly negative where all values were less negative than -2.5 mV [41]. *E. coli* O157:H7 was found to be equivalent in size (equivalent spherical diameter (ESD) of 1.0±0.14 μm) than *E. coli* K12 D21 (ESD=0.94±0.21 μm). These data are used later in the manuscript in the discussion of the bacteria transport experiments.

2.3.2 Influence of Cell Concentration

The effect of influent cell concentration was examined by conducting separate column transport experiments with the two selected strains of *E. coli*. Figure 2.1 shows a representative bacteria breakthrough curve obtained using both UV-vis spectrophotometry and direct plate counting measurements of bacteria concentration to evaluate the transport of *E. coli* K12 D21 in a clean sand column. The data presented in Figure 2.1 show that the two detection methods yield comparable results. Careful inspection of breakthrough curves obtained from column experiments conducted with the two bacteria at three different influent concentrations reveals that the direct plate count detection method was best suited for low influent concentrations (e.g., 10^5 cells/mL), whereas greater error was noted when using this technique at higher influent concentrations (e.g., 10^8 cells/mL). On the other hand, UV-vis spectrophotometry was best suited for experiments conducted at higher influent concentrations but was not appropriate

for low bacteria concentrations. Although both measurement techniques were used for all experiments, the data obtained by spectrophotometry was used for all calculations except for cases where the column effluent bacteria concentration was below the detection limit of the spectrophotometer. Also, the measurements obtained by spectrophotometry are used in all figures presented except where otherwise indicated. Each breakthrough curve presented in Figures 2.2, 2.5 and 2.6 represents the average result from at least two separate experiments conducted on different days. The specific number of experiments conducted is indicated in each figure. For ease of examination, error bars are included only at intervals of every 200 seconds on the breakthrough curves.

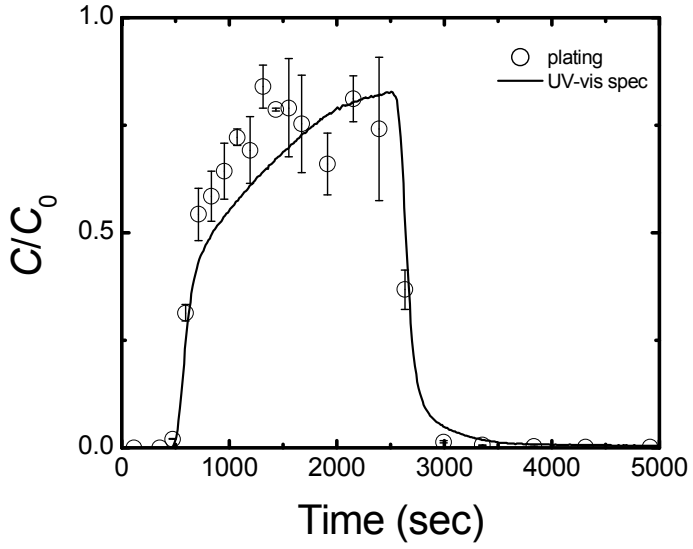

Figure 2.1 Breakthrough curves for column transport experiments with *E. coli* K12 D21 at an influent cell concentration of 10^7 cells/mL. The column effluent was analyzed with two measurement techniques: UV-visible spectrophotometry and direct plate counting.

Figures 2.2a and 2.2b show a summary of measured breakthrough curves for experiments conducted in clean sand packed columns with *E. coli* K12 D21 and *E. coli* O157:H7, respectively. The bacterial concentration in the influent suspensions was varied over several orders of magnitude (10^5, 10^7 and 10^8 cells/mL) to verify the influence of this parameter on bacterial retention behavior. Figure 2.2a shows distinct differences in the breakthrough behavior for each influent concentration of *E. coli* K12 D21 examined. The experiment conducted using the highest influent concentration (10^8 cells/mL) exhibits the highest normalized breakthrough concentration (C/C_0), followed by 10^7 cells/mL, then 10^5 cells/mL. The breakthrough curve for the experiment conducted at the lowest concentration is relatively flat, but the shape of the curve after ~1000 sec becomes more concave with increasing cell numbers. Similar results are noted for experiments conducted with *E. coli* O157:H7 (Figure 2.2b). The data presented in Figure 2.2b were obtained from UV-vis spectrophotometry measurements for the two higher concentrations tested. However, the experiment conducted with 10^5 cells/mL of *E. coli* O157:H7 has a breakthrough concentration which is below the detection limit of the spectrophotometer, thus this latter breakthrough curve was obtained by direct plate counts. The data in Figure 2.2b also show differences in the normalized breakthrough concentration obtained for each influent concentration; the highest values of C/C_0 were measured when *E. coli* O157:H7 was injected at 10^8 cells/mL, followed by 10^7 cells/mL, and finally 10^5 cells/mL. In the experiment conducted with 10^8 cells/mL of *E. coli* O157:H7, the breakthrough curve exhibits a distinct shape whereby the observed rate of change of C/C_0 increases with time.

Several other researchers have also noted changes in the shape of breakthrough curves when C_0 was increased [20, 21, 26]. The breakthrough curves presented in Figures 2.2a and 2.2b are consistent with observations from Brown et al. [46], who also noted such differences when studying the transport of *Sphingomonas* sp.; however, in that study the differences in the breakthrough curves were attributable to porous medium effects (e.g., sand grain size distribution) and experimental conditions (e.g., column flushing conditions). Those authors concluded that while changes in bacteria surface properties can occur over time, researchers should not be quick to attribute differences in bacteria breakthrough curves to differences in bacteria surface properties. While our study does not refute that result in either intention or outcome, the reproducibility of the breakthrough curves presented herein clearly indicates that a given bacterial strain can result in markedly different breakthrough curve characteristics.

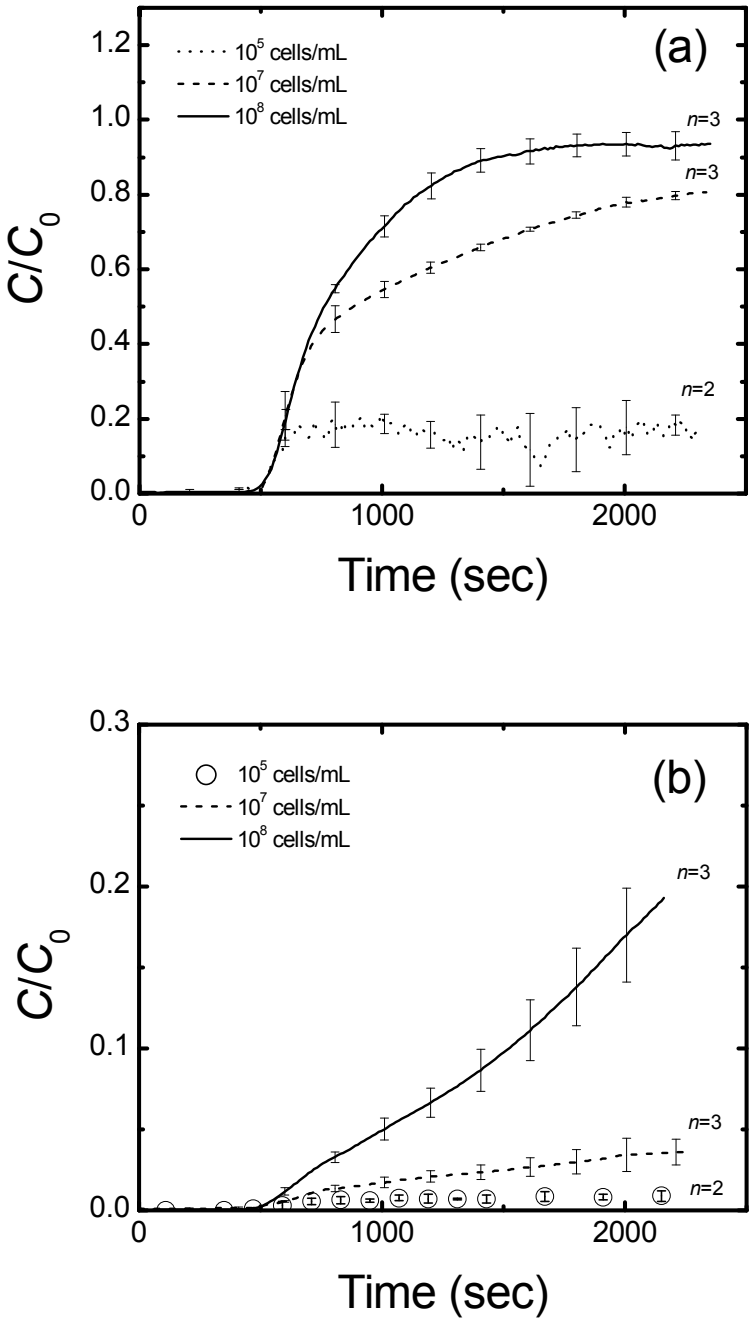

Figure 2.2 Mean breakthrough curves for the column transport of (a) *E. coli* K12 D21 and (b) *E. coli* O157:H7 at influent cell concentrations of 10^5, 10^7 and 10^8 cells/mL. When n=3, the error bars represent standard deviations; when n=2, the error bars represent the difference between the two replicates.

It is well known in the colloid literature that the concentration of colloids used in bench-scale column experiments can influence the transport behavior of nonbiological particles and microorganisms. Previous studies have reported a decrease in colloid retention with increasing particle or microbe concentration [20-24, 26]. This behavior is typically explained by considering the mechanisms of blocking [22, 23, 26] and straining [21, 24, 28]. Blocking occurs when colloids attached to the collector surface (i.e., sand grains) prevent the retention of additional colloids by an area larger than the attached colloids themselves [29]. When the bulk colloid concentration is increased, the number of available attachment sites remains the same, but since there are more microbes or particles in the system, the additional particles or cells are blocked from the collector (sand grain) surface. This phenomenon explains the observed higher breakthrough concentrations observed herein when cells are injected with a greater C_0 (Figure 2.2).

Another possible explanation for this observed behavior is straining, which occurs when colloids become physically trapped between sand grains, in pores that are too small to allow their passage. To describe the concentration dependent transport of colloids in granular media, Bradford et al. [21] proposed a modified straining model that assumed straining was hindered at higher particle concentrations as a result of repulsive interactions between suspended colloids and physically trapped (strained) colloids. Bradford et al. [47] reported that straining can be important for particle size to median grain size ratios (d_p/d_c) of 0.003 to 0.017. In the current study, the ratio d_p/d_c is approximately 0.0038, suggesting that physical straining of the bacteria and associated phenomena

resulting in the potential for hindered straining during transport through the packed granular medium may contribute to the observed outcomes.

The transport and retention behavior of different microorganisms or particles is typically quantified using an attachment efficiency (α) based on the classic filtration model developed by Yao et al. [7]. Although particle concentration can affect particle retention in granular porous media [48, 49], the classic clean bed filtration model suggests that particle removal is independent of influent particle concentration. It is a simple analytical solution to the one-dimensional advection-dispersion equation for the case of steady-state filtration and negligible hydrodynamic dispersion in which [50]:

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-f)L\eta_0}\ln\left(\frac{C}{C_0}\right) \qquad (2.1)$$

Here, d_c is the diameter of the collector (sand) grains, f is the packed bed porosity, L is the packed bed depth, η_0 is the single-collector contact efficiency, C is the effluent concentration, and C_0 is the influent concentration. The single-collector contact efficiency (η_0) was calculated using the correlation equation developed by Tufenkji and Elimelech [9]. It is not straightforward to calculate bacterial attachment efficiencies from experimental breakthrough curves that do not reach a steady-state; for the sake of comparison, we calculated α using the value of C/C_0 obtained from the clean-bed portion of each curve taking care to use the same time period in each experiment (PVs 1.5-1.7). Calculated values of the apparent attachment efficiency are presented in Figure 2.3.

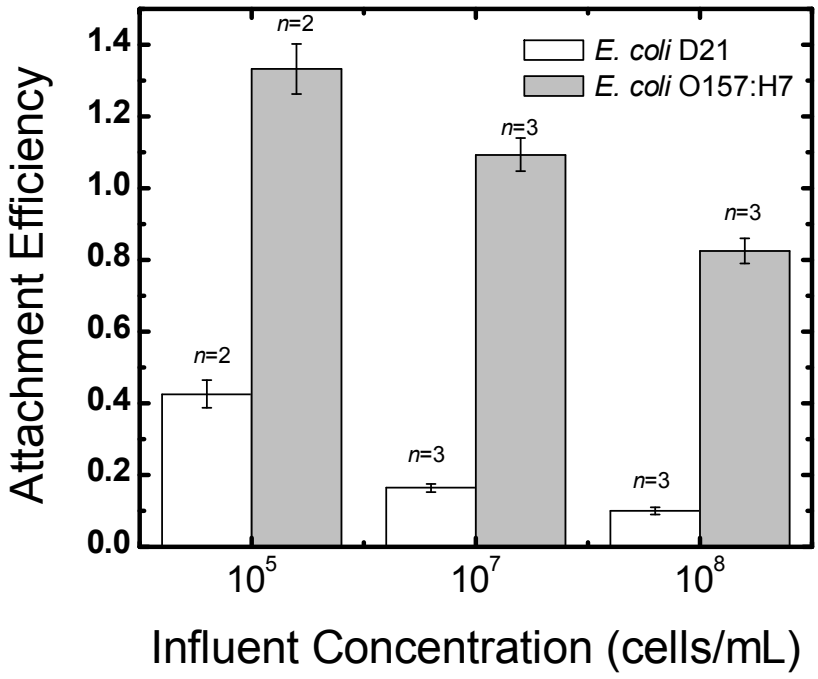

Figure 2.3 Calculated attachment efficiencies (α) for *E. coli* K12 D21 and *E. coli* O157:H7 at the influent cell concentrations of 10^5, 10^7 and 10^8 cells/mL. When n=3, the error bars represent standard deviations; when n=2, the error bars represent the difference between the two replicates.

The data presented in Figure 2.3 clearly show concentration dependent removal rates for both bacterial strains whereby the α values decrease with increasing C_0. It is also observed that the α value is higher for *E. coli* O157:H7 in comparison to *E. coli* K12 D21. This difference in attachment behavior is likely related to both electrostatic effects and blocking of each individual bacterial strain. For the low value of C_0 examined (10^5 cells/mL), the effect of blocking on bacterial retention is likely less relevant, as demonstrated by the relatively flat breakthrough curves measured under this condition (Fig. 2.2). Hence, the variation in attachment behavior between the two organisms at this low influent

concentration can be attributed to differences in electrostatic forces between each organism and the sand surfaces. At the pH of these experiments (pH 5.6), the sand surface will be mostly negatively charged. Likewise, most bacteria exhibit a net negative charge when suspended in aqueous media at this pH. Indeed, the mean zeta potential of *E. coli* O157:H7 was determined to be -2.4 mV and the mean zeta potential of *E. coli* K12 D21 was -38.2 mV when suspended in 10 mM KCl at the pH of our experiments. Hence, under these conditions, repulsive electrical double-layer interactions are expected to predominate during approach of the bacteria to the sand grain surface. Because the extent of repulsive electrostatic interactions is directly related to the surface potential of the bacteria and the sand surface, organisms with a greater absolute charge will experience greater difficulty in attaching to the sand surface [51, 52]. Our measurements show that *E. coli* K12 D21 has a more negative charge than *E. coli* O157:H7 and hence should experience greater repulsion upon approach to the sand surface. Hence, at the low value of C_0 examined (10^5 cells/mL), the data in Figure 2.3 show that *E. coli* K12 D21 has a lower attachment efficiency in comparison to *E. coli* O157:H7; predominantly due to differences in the relative electrostatic forces between the different types of microorganisms and the sand surfaces.

At the higher bacterial influent concentrations of 10^7 and 10^8 cells/mL, electrostatic effects are still important, however, blocking effects also have to be considered. Camesano et al. [23] demonstrated that the extent of blocking can depend on the surface charge of the colloid whereby the colloid with the greatest negative surface potential yielded the most blocking and the colloid with the smallest absolute potential produced the least blocking effects. This same trend

was also observed in our study in which the more negatively charged organism (*E. coli* K12 D21) appears to give rise to greater blocking than the less charged organism (*E. coli* O157:H7). The extent of blocking can be quantified by considering the ratios in the calculated apparent α values for experiments conducted with two different values of C_0 (Table 2.1). The ratios of α values for experiments conducted with *E. coli* K12 D21 are generally higher than the ratios determined for *E. coli* O157:H7, suggesting that the extent of blocking is more significant for the more highly charged organism (Table 2.1).

Also included in Table 2.1 are data from previous studies of the effect of changes in influent cell concentration on breakthrough behavior of bacteria in granular porous media at conditions consistent with groundwater environments. Specifically, we have summarized the results of previous investigations by calculating the apparent α values directly from the published figures using equation 1. Calculation of the α values directly from the breakthrough curves allows for comparison of previous results with those obtained in the current study. Calculated ratios of α values for our experiments conducted at different influent bacteria concentrations show that the apparent attachment efficiency can be up to four times greater when comparing results at $C_0=10^5$ versus $C_0=10^8$. In general, the calculated α ratios indicate that the underestimation of the bacterial retention rate becomes more important as the difference in C_0 between the experiments becomes greater. For example, the data of Bai et al. [20] obtained with *P. aeruginosa* show that the α ratio is near 1 when comparing experiments conducted using $C_0=10^7$ and $C_0=10^{7.7}$. This indicates that the bacterial transport behavior is

similar at these two conditions. In contrast, the α ratio is 13.1 when comparing experiments conducted using C_0=10^7 and C_0=$10^{11.7}$.

Table 2.1. Summary of Experimental Results from Clean Columns

Reference	Organism/Particle	Packed Media	Grain size (d_c) (μm)	C_0 (cells/mL)	α	α ratio	L_{99} (m)
Present study	*E. coli* D21	sand	256	10^5	0.43	α_5/α_7 = 2.6	0.28
				10^7	0.16	α_5/α_8 = 4.3	0.73
				10^8	0.10	α_7/α_8 = 1.6	1.21
	E. coli O157:H7	sand	256	10^5	1.3	α_5/α_7 = 1.2	0.09
				10^7	1.1	α_5/α_8 = 1.6	0.11
				10^8	0.82	α_7/α_8 = 1.3	0.15
Foppen et al. 2007	*E. coli* ATCC 25922	sand	180-212	1.8×10^8	0.88		0.40
				1.2×10^9	0.50	$\alpha_{8.2}/\alpha_{9.1}$ = 1.7	0.69
			75-90	1.4×10^8	0.12		0.46
				6×10^8	0.12	$\alpha_{8.1}/\alpha_{8.8}$ = 1.0	0.52
			38-45	1.5×10^8	0.065		0.19
				9×10^8	0.058	$\alpha_{8.2}/\alpha_9$ = 1.1	0.21
Bai et al. 1997	*P. aeruginosa*	sand	300-420	10^7	1.1		0.08
				5×10^7	0.94	$\alpha_7/\alpha_{7.7}$ = 1.2	0.09
				5×10^8	0.46	α_7/α_8 = 2.4	0.19
				10^9	0.31	α_7/α_9 = 3.6	0.28
				5×10^{11}	0.09	$\alpha_7/\alpha_{11.7}$ = 13.1	1.01
Bradford and Bettahar 2006	latex microspheres (1 m)	sand	360	8×10^7	0.03		2.8
				2×10^7	0.12	$\alpha_7/\alpha_{7.3}$ = 1.5	0.42
				10^7	0.18	$\alpha_7/\alpha_{7.9}$ = 6.6	0.18
			150	8×10^7	0.01		1.54
				2×10^7	0.06	$\alpha_7/\alpha_{7.3}$ = 1.0	0.28
				10^7	0.06	$\alpha_7/\alpha_{7.9}$ = 5.5	0.28
	latex microspheres (3.2 m)	sand	360	8×10^7	0.14		0.52
				2×10^7	0.38	$\alpha_7/\alpha_{7.3}$ = 0.8	0.19
				10^7	0.30	$\alpha_7/\alpha_{7.9}$ = 2.1	0.24
Camesano et al. 1999	CL microspheres	soil	127	8×10^7	0.085		0.72
				9×10^8	0.074	$\alpha_{7.9}/\alpha_{8.9}$ = 1.1	0.83
				10^{10}	0.033	$\alpha_{7.9}/\alpha_{10}$ = 2.6	1.86
				2×10^{10}	0.012	$\alpha_{7.9}/\alpha_{10.3}$ = 7.1	5.13
	CML microspheres	soil	127	9×10^9	0.096		0.64
				5×10^{10}	0.013	$\alpha_{10}/\alpha_{10.7}$ = 7.4	4.73
	P. putida KT2442	soil	127	6×10^8	0.088		0.70
				10^{10}	0.034	$\alpha_{8.8}/\alpha_{10}$ = 2.6	1.81

2.3.3 Effect of Media Pre-Coating on Bacterial Transport

In the natural subsurface environment, the surfaces of the soil or sand grains are not "clean". Rather, a wide range of biological and non-biological materials may adsorb or attach onto the collector surfaces and thereby influence bacterial transport in these systems. Examples of materials that may be present on grain surfaces include indigenous soil microorganisms (i.e., bacteria, viruses, protozoa), biological exudates (e.g., polysaccharides, proteins, complex lipids), clay particles, natural organic matter, etc. Although these materials are commonly present in natural subsurface environments, their influence on bacterial transport in these systems is not commonly considered in laboratory-scale studies. Hence, one of the main goals of this study is to examine the influence of media pre-coating with bacteria on the transport behavior of another organism. In these experiments, the influent concentration of the pre-coating strain was 10^7 cells/mL, whereas the injection concentration of the second organism was varied between 10^5 and 10^8 cells/mL. Table 2.2 provides a summary of the experimental conditions and results for the study of the effects of media pre-coating on bacterial transport. It can be noted from Table 2.2 that we did not conduct an experiment where the pre-coating organism was *E. coli* K12 D21 and the injection organism was *E. coli* O157:H7 (C_0=10^5 cells/mL). This particular experiment could not be conducted because the concentration of *E. coli* K12 D21 in the column effluent was too high to allow accurate enumeration of *E. coli* O157:H7.

Table 2.2. Summary of Pre-Coating Experiment Conditions and Results

Experiment	C_0 for *E. coli* K21 D21	C_0 for *E. coli* O157:H7	α	α ratio (clean/pre-coated)
1	10^5	10^7 (pre-coating)	0.15	2.8
2	10^7	10^7 (pre-coating)	0.16	1.0
3	10^8	10^7 (pre-coating)	0.12	0.87
4	10^7 (pre-coating)	10^7	0.87	1.3
5	10^7 (pre-coating)	10^8	0.81	1.0

Figure 2.4 shows a representative breakthrough curve for the pre-coated bacterial transport experiments. In this experiment, *E. coli* K12 D21 was the pre-coating organism (dashed line portion of the curve) and *E. coli* O157:H7 was the injection organism with an influent cell concentration of 10^7 cells/mL (solid line portion of the curve). A summary of the column experiments conducted with *E. coli* K12 D21 with a media pre-coating of *E. coli* O157:H7 is shown in Figure 2.5a. Figure 2.5b shows a summary of the column experiments conducted with *E. coli* O157:H7 with a media pre-coating of *E. coli* K12 D21. In Figure 2.5, only the second part of the experiment is shown for clarity (i.e., the pre-coating portion is not shown).

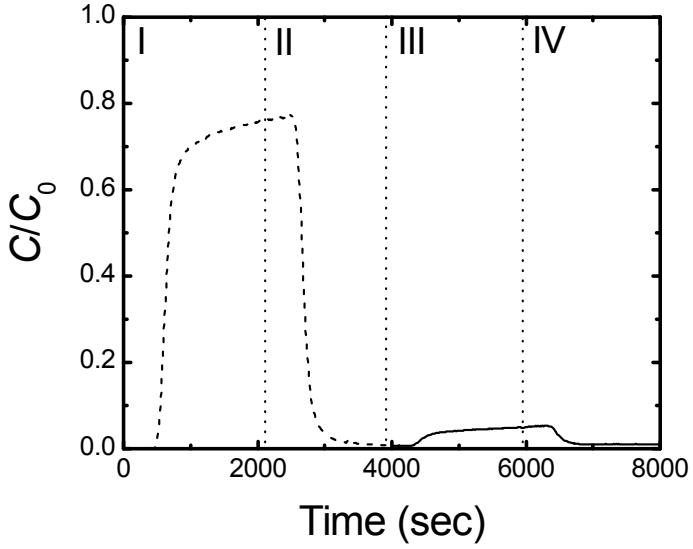

Figure 2.4 Representative breakthrough curve for the media pre-coating column transport experiments. Phase I: injection of *E. coli* K12 D21 at an influent cell concentration of 10^7 cells/mL; Phase II: injection of 10 mM KCl; Phase III: injection of *E. coli* O157:H7 at an influent cell concentration of 10^7 cells/mL; Phase IV: injection of 10 mM KCl.

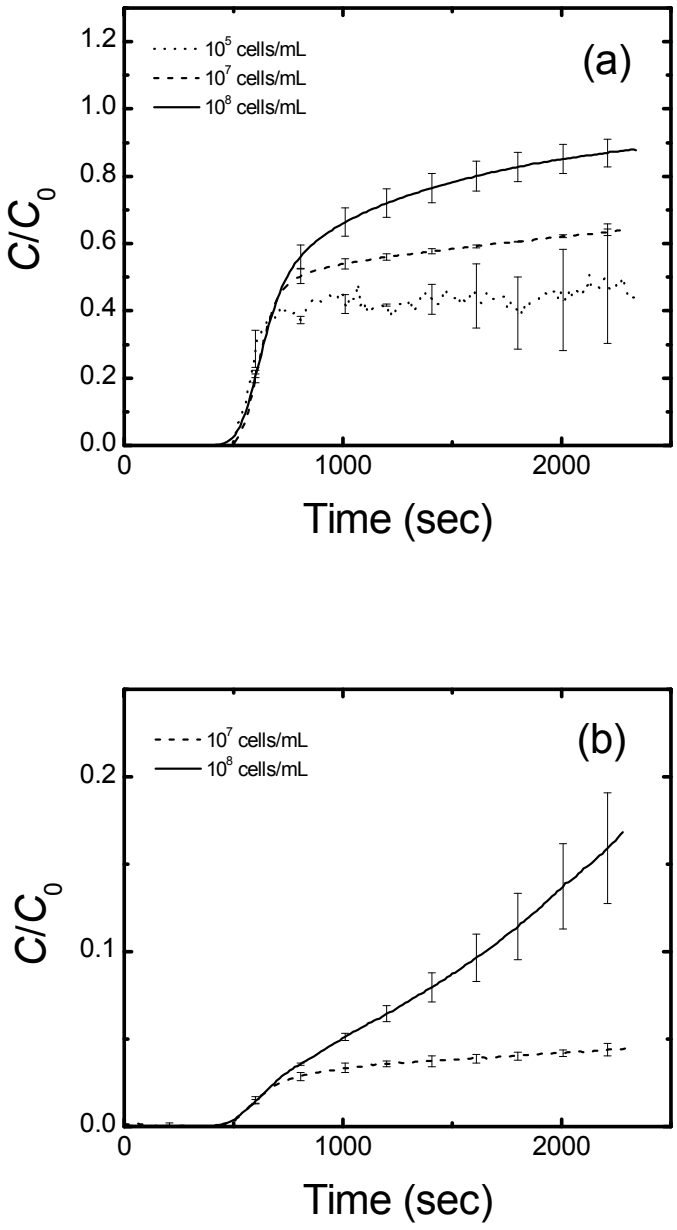

Figure 2.5 Mean breakthrough curves for the column transport of (a) *E. coli* K12 D21 at influent cell concentrations of 10^5, 10^7 and 10^8 cells/mL with the column media pre-coated with *E. coli* O157:H7 at an influent cell concentration of 10^7 cells/mL and (b) *E. coli* O157:H7 at influent cell concentrations of 10^7 and 10^8 cells/mL with the column media pre-coated with *E. coli* K12 D21 at an influent cell concentration of 10^7 cells/mL. Each experiment was repeated twice and the error bars represent the difference between the two replicates.

The results presented in Figure 2.5a show that there is a greater extent of *E. coli* K12 D21 retention when C_0 is lower. This result is similar to what we observed for the clean column experiments (Figure 2.2a) demonstrating that second-order effects (i.e., blocking) are also important when the medium is pre-coated with bacteria. When the granular medium is pre-coated with *E. coli* K21 D21 (Figure 2.5b), we observe more retention of *E. coli* O157:H7 at the lower influent concentration. It can further be noted in Figure 2.5b that when $C_0=10^8$ cells/mL, the error bars at the front end of the curve are relatively small, but become increasingly larger as the experiment progresses. This is also the case for the experiment with *E. coli* K12 D21 at $C_0=10^5$ cells/mL. Comparable behavior was previously reported by Brown et al. [46] where they observed good experimental reproducibility for the clean-bed region of the breakthrough curve, but not in the blocking region of the same curve.

Figure 2.6 shows a comparison of the average breakthrough curves for transport of *E. coli* O157:H7 ($C_0=10^7$ cells/mL) in the absence and presence of media pre-coating with *E. coli* K12 D21. Although the extent of retention is quite high in both cases, there are clear differences between the two experiments at the front end of the breakthrough curves. However, the two breakthrough curves seem to converge after approximately 2000 sec. Future studies conducted with longer bacteria injection times will allow us to better understand the relative importance of granular media pre-coating on bacterial transport distances. Because of the limited number of published controlled laboratory investigations examining bacterial transport in microbe pre-coated or biofilm pre-coated granular systems,

more studies are needed examining the influence of media coating during extended periods of bacteria injection.

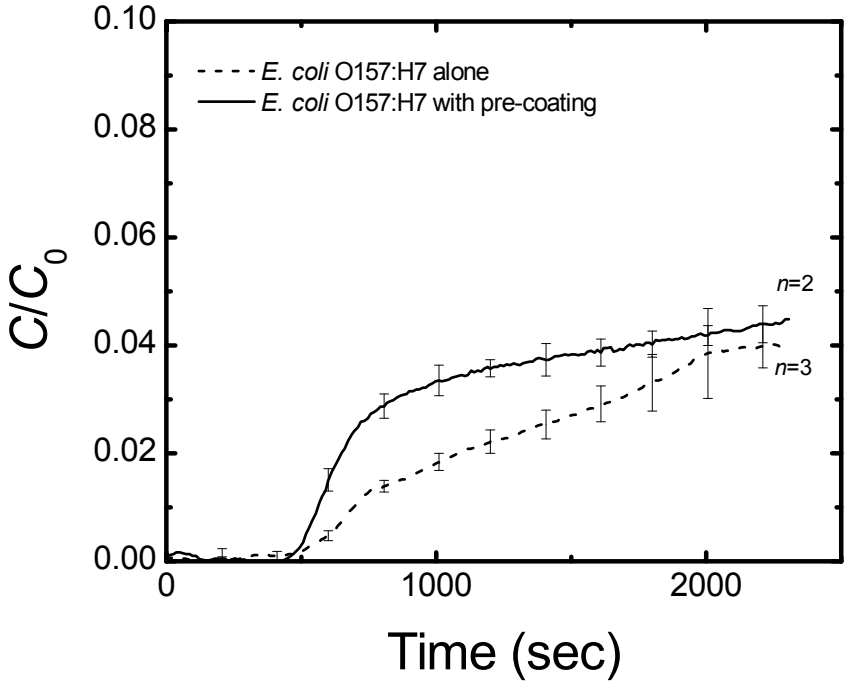

Figure 2.6. Mean breakthrough curves for *E. coli* O157:H7 at the influent concentration of 10^7 cells/mL. One curve represents the transport behavior when *E. coli* O157:H7 was injected into the column alone and the other curve represents the transport behavior when this organism was injected into the column after the column media was pre-coated with a solution of *E. coli* K12 D21. When n=3, the error bars represent standard deviations; when n=2, the error bars represent the difference between the two replicates.

To better understand the influence of media pre-coating on bacterial retention rates, values of the attachment efficiency (α) were calculated from the breakthrough curves in Figure 2.5 and are presented in Figure 2.7 and Table 2.2. Comparison of the bacteria attachment efficiencies in Figure 2.7 reveals that the influence of the media pre-coating on the bacterial retention behavior can be only

observed under certain conditions. When C_0=10^7 cells/mL or 10^8 cells/mL, no marked differences in the apparent attachment efficiency of *E. coli* K12 D21 in the clean or pre-coated column are observed (Figure 2.7a). However, at the lower influent concentration of *E. coli* K12 D21 (C_0=10^5 cells/mL), there is a significant difference in the apparent attachment efficiency of this organism. Specifically, the calculated α value was greater for transport of *E. coli* K12 D21 in the clean sand packed column. Likewise, in experiments examining the transport behavior of *E. coli* O157:H7 (Figure 2.7b), the effect of the media pre-coating on the bacterial retention behavior is not consistent at all influent bacteria concentrations. When C_0=10^7 cells/mL, the apparent attachment efficiency decreases when the sand is pre-coated with *E. coli* K12 D21; however, for C_0=10^8 cells/mL, α is the same in the presence and absence of pre-coating.

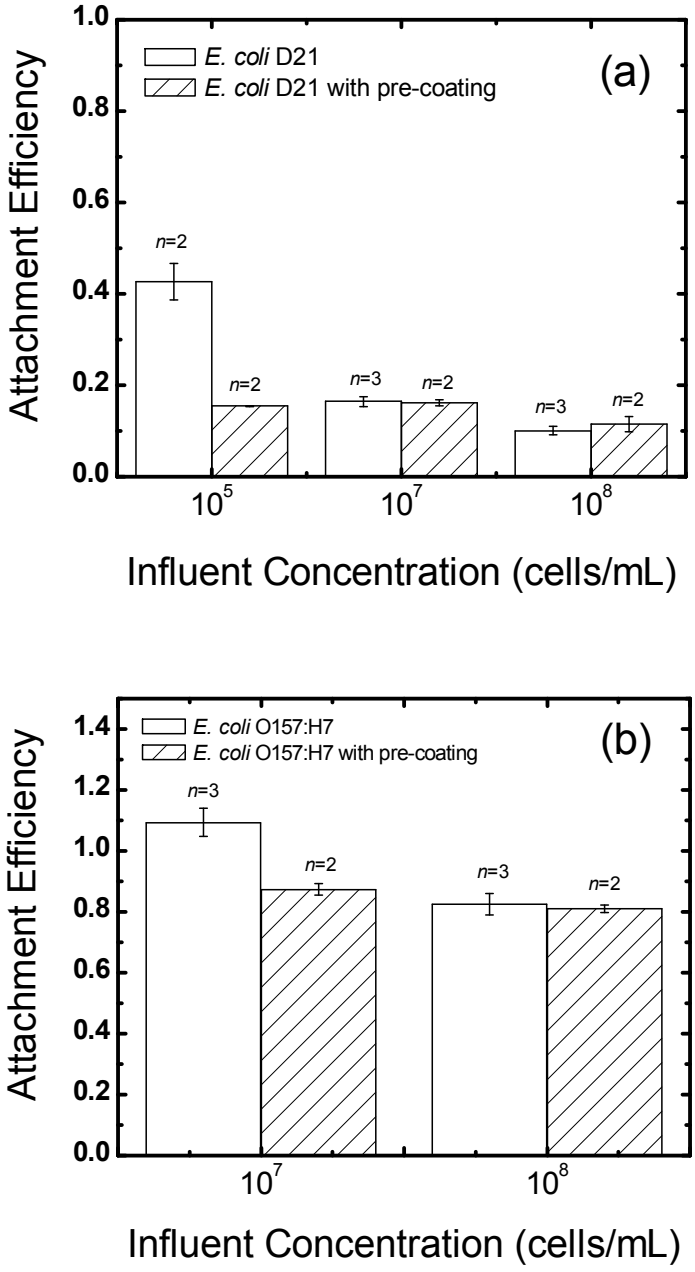

Figure 2.7. Comparison of calculated attachment efficiencies (α) for (a) *E. coli* K12 D21 at the influent cell concentrations of 10^5, 10^7 and 10^8 cells/mL and (b) *E. coli* O157:H7 at the influent cell concentrations of 10^7 and 10^8 cells/mL for both the media pre-coating experiments and the experiments where each organism was injected into the column alone. When n=3, the error bars represent standard deviations; when n=2, the error bars represent the difference between the two replicates.

Careful inspection of the transport behavior of both bacterial strains suggests that there is a critical concentration above which the media pre-coating does not influence the apparent attachment efficiency. Calculated α ratios comparing experiments conducted in clean sand columns and in pre-coated columns are presented in Table 2.2. These ratios are generally close to 1 for the higher values of C_0 indicating no significant difference in bacterial retention between the two treatments. For *E. coli* K12 D21, the α ratio is greater than 1 (ratio=2.8) when $C_0=10^5$ cells/mL, whereas for *E. coli* O157:H7, the influence of media pre-coating on the bacterial retention rate was notable only at $C_0=10^7$ cells/mL.

When the granular medium is pre-coated with one of the bacterial strains, we did not note any significant differences in the measured attachment efficiencies for different influent concentrations (Figure 2.7, hatched bars).

2.4 ENVIRONMENTAL IMPLICATIONS

The purpose of laboratory-scale column experiments is to generally develop a better understanding of the key factors controlling bacterial transport and fate in natural and engineered granular environments. The bacterial deposition rate or attachment efficiency evaluated from the results of such studies may be useful in estimating bacterial transport distances at larger length scales. To better understand the influence of changes in C_0 or column pre-coating on predicted bacterial transport distances, we used eq 1 to calculate the required filtration distance to achieve 99% removal of bacterial cells from the pore fluid. These data

are presented in Table 2.2 (L_{99}) for our study and previous studies reported in the literature. The analysis shows that the relative differences in transport distances predicted from data obtained using different C_0 are generally negligible. In the first three studies presented in Table 2.2, none of the experiments result in differences in predicted transport distances greater than 1 m. These relatively small variations in transport distance are not expected to be of major importance in predicting bacterial migration and fate in natural subsurface environments.

For simplicity, in this study, the bacterial deposition rate (or attachment efficiency) was considered to be constant for a given experimental condition. However, several studies have shown that microbes and nonbiological colloids can exhibit a wide distribution in deposition rates (or attachment efficiencies) [50, 53-55]. The results and analysis presented in Tables 2.1 and 2.2 suggest that variations in apparent α values resulting from mechanisms such as blocking may not be as significant as the variations in α that arise due to inherent population heterogeneities. That is, the range of α values determined from experiments conducted using different C_0 or with pre-coated columns is not as wide as the range of α values expected for a given monoclonal microbe population or even a microsphere population.

2.5 CONCLUSIONS

Pre-coating of the granular matrix with one organism can influence the transport and retention of another bacterial strain. The results show that media pre-coating either does not have an effect on the rate of bacterial retention or

causes a decrease in the bacterial attachment efficiency. The greatest effect of pre-coating was observed at lower influent cell concentrations, where there was a significant difference between attachment efficiencies measured in the clean sand packed column and the pre-coated granular matrix. As the influent bacteria concentration was increased, significant differences between the clean and media pre-coated columns were not observed.

The results of this study are in agreement with previously published research examining the role of bulk particle or cell concentration on colloid retention; namely, we observed decreased apparent deposition rates for higher influent bacteria concentrations. This behavior was observed for both organisms in the clean and pre-coated sand packed columns. Possible explanations for this behavior include processes such as blocking and straining. It was also found that the attachment efficiency for *E. coli* O157:H7 is higher than the attachment efficiency for *E. coli* K12 D21 for both the clean and media pre-coated columns. This difference in the attachment efficiency is attributed to electrostatic effects and blocking mechanisms.

When considered within the context of predicting bacterial transport at the field scale, the findings of this research (and previous studies) suggest that variations in influent cell concentration may not be a very important factor. Measured differences in bacterial attachment efficiencies for the two organisms studied here using influent concentrations spanning three orders of magnitude do not yield significant variations in predicted values of L_{99}. Other parameters, such as inherent population heterogeneities, have been shown to have a greater influence on the apparent rates of removal and corresponding transport distances.

CHAPTER 3: SUMMARY AND CONCLUSIONS

The experiments carried out in this thesis have furthered our knowledge of bacterial transport and the factors which influence this behavior. It was generally found that the influent cell concentration influences bacterial transport where there is more apparent attachment at lower influent concentrations. The apparent attachment efficiency gradually decreases as the influent cell concentration is increased. These concentration dependent removal rates were observed for both organisms and in both the clean and media pre-coated columns. Although there is a difference in the attachment efficiencies when different concentrations were tested, these differences do not appear to translate to a significant environmental impact. For example, when the expected travel distances to achieve 99% removal were calculated, a significant difference was not observed. It was also found that the bacterial media pre-coating does not have significant influence on the transport behavior. This media pre-coating either did not have an effect on the transport or it decreased the attachment efficiency. A significant difference was not observed between the attachment efficiencies of the clean and media pre-coated columns.

Differences in transport behavior are observed for both organisms where the attachment efficiency is higher for *E. coli* O157:H7 in comparison to *E. coli* K12 D21. This difference is attributed to the relative electrostatic charge of each strain where *E. coli* K12 D21 is more negatively charged in comparison to *E. coli* O157:H7. The difference between the two strains was more significant than the differences observed for different influent concentrations and media pre-coating conditions. These findings suggest that the charge of the organisms appears to

have a greater influence on the transport behavior than either the cell concentration or media pre-coating.

Two different detection methods were utilized in this study. The column effluent was analyzed with both spectrophotometric and direct colony plate counting techniques. It was found that the spectrophotometric measurements were more suitable for the higher concentrations because there was a smaller degree of error when compared to the plate counts. However, the direct colony plate counting method was more suitable for the lower concentrations because these low concentrations are below the detection limit of the spectrophotometer. Also at low cell concentrations, a smaller degree of error was observed between replicates in comparison to the higher concentrations. Although direct colony plate counting has a smaller error at low concentrations, there is still a notable discrepancy between replicates and triplicate plates. There is a need to develop new enumeration techniques to accurately quantify low concentrations of cellular matter.

In our study, the effects of influent cell concentration and media pre-coating were not found to have a significant influence on bacterial transport. Although the effects were not significant, these factors should still be considered in the development of more accurate and reliable bacterial transport models. Even though these parameters of cell concentration and media pre-coating did not have a significant influence in our system, perhaps these factors would have a greater effect if other system parameters were changed. For example, changing system parameters such as ionic strength, pH, flow rate and media type may alter the system such that cell concentration and media pre-coating could become

significant. Further research would be needed to test these other parameters to determine if different and significant effects are observed.

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