TRANSPORT AND FATE OF BACTERIA IN POROUS MEDIA

BY

Shiva Shojaei Tazehkand

Thesis

Submitted to Utrecht University

Master of Science
in
Environmental Hydrogeology

October 2008

This thesis has been examined and approved.

Thesis Advisors

Dr. Sharon L. Walker
Assistant Professor Department of Chemical and Environmental Engineering University of California, Riverside

Dr. Scott A. Bradford
Contaminant Fate and Transport Research Unit,
USDA-ARS U.S. Salinity Laboratory Riverside, CA.

Prof. S. Majid Hassanizadeh
Professor of Environmental Hydrogeology Department of Earth Sciences, Faculty of Geosciences, Utrecht University

ACKNOWLEDGEMENTS

I would like first to thank my supervisor, Dr. Sharon L. Walker for her highly motivating enthusiasm and valuable support of my research. I would not be where I am without her help, patience, and kindness especially during my hard pregnancy. As a major supervisor, she has shared knowledge, encouragement, direction, and trust with me.

I am also grateful to her group members, Nick, Gexin, and Berat for their assistance in lab works. My special thanks go to Dr. Scott Bradford and his wife Simon for helping and supporting me during my pregnancy and giving birth to my son. He was also instrumental in the success of this thesis. His knowledge of microbiology, scientific advice, and willingness to help me was invaluable.

I would like to extend my heartfelt appreciation and thanks to Prof. S. Majid Hassanizadeh my teacher, supervisor and supporter in Holland. I learned so much from him. I cannot thank him enough for his help, support and the hours he spent for me to pay attention to my concerns.

Thank you to Dr. Ruud Schotting for being a nice mentor and patient person in times of difficulty.

Many thanks to my parents for being more supportive than I could have imagined. I am grateful to my mother who taught me patience and gave me enthusiasm. Finally my very special thanks go to my beloved husband, Saeed, for standing beside me and his supportive role whether at university or at home.

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Figure 1. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mM) as a function of pore volumes. The cell separation method consisted of applying 15 min of high-speed centrifugation at 14000 g (repeated three times). Key experimental conditions were as follows: approach velocity, v = 0. 66 cm/min, porosity, = 0.43, pH =5.5-5.6 and temperature= 20-22 °C.

Figure 2. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mM) as a function of pore volumes. The cell separation method consisted of applying 15 min centrifugation at 3689 g (repeated three times). Key experimental conditions were as follows: approach velocity, v = 0. 66 cm/min, porosity, = 0.43, pH =5.5-5.6 and temperature= 20-22 °C.

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Chapter I

Literature Review

Introduction

Groundwater is a major source for drinking water. It has the potential to become contaminated with pathogenic microorganisms such as bacteria from land application of raw and treated waste water, septic wells, effluent from septic tanks, leaking sewage pipes and animal manure (Gerba et al., 2005). Contamination of drinking water sources occurs when wells are poorly constructed or when contamination sources are too close to wells. Public disease outbreaks occur when these waters are not treated properly prior to use. Fecal contamination has been detected in almost half of all drinking water wells in the United States (Macler et al., 2002). Fecal contamination includes both pathogenic and non-pathogenic microorganisms including bacteria, viruses and protozoa (Mawdsley et al. 1995). Microorganisms naturally inhabit the intestines of warm-blooded mammals and are consequently found in animal excreta (Craun et al. 1997; USDA 1992).

Groundwater can be protected from contamination with pathogenic bacteria through engineering solutions such as applying adequate setback distances between sources of contamination and production wells, with the soil serving as a natural filter. Surface water can also be treated effectively to remove pathogens by means of passage through the subsurface such as riverbank filtration (Schijven et al., 1996). These strategies rely on knowledge of potential microbial transport processes and conditions that are needed to achieve safe drinking water. In this regard, considerable research has been carried out for quantifying and modeling processes that govern the removal of bacteria by soil passage (Stevik et al., 2004; Foppen et al., 2006). Once the fate and transport of bacterial cells in porous media is understood, a better and more accurate estimate of the setback distances can be made for production of safe drinking water from groundwater. Moreover, an accurate knowledge of bacteria transport is vital to develop efficient bioremediation strategies where the bacteria cells should be delivered to the contamination site (Wilson et al., 1993). Hence, comprehensive knowledge of the transport of bacteria in subsurface environmes is essential for protecting groundwater resources.

Bacteria transport in porous media is affected by various physical and chemical processes including advection, diffusion, dispersion, interaction to solid phase, and biological factors. Biological processes such as growth, death, and parasitism can result in the increase or removal of bacteria in porous media (Dowd et al., 1997; Gordon et al., 2003). Bacteria transport through porous media often results in significant removal from the aqueous phase and the efficiency of the filtration depends on many factors, such as soil type, water chemistry, and bacteria type (USEPA 2001).

The objective of this chapter is to briefly review the major factors that affect the bacteria transport and fate in saturated porous media. Several reviews on the transport and fate of bacteria through the subsurface have been appeared (Stevik et al., 2004; Foppen et al., 2006; Tufenkji et al., 2006). The main purposes of this chapter is to review the major factors that affect these processes and summarize existing models, parameters, and their values used to describe and quantify the attachment and retention of bacteria during transport in aquifers under saturated conditions. This knowledge of retention and elimination of bacteria is needed to secure proper setting and design and operation of onsite systems to minimize the risk of pathogenic contamination of groundwater, and drinking water wells. This chapter is divided to some main parts, including transport and attachment sections and the factors affecting retention of bacteria during percolation through porous media.

1.1. Quantifying Bacterial Transport through Saturated Porous Media

Bacteria are transported through porous media by processes such as advection, dispersion and diffusion. Advection is the movement of bacterial cells with the bulk fluid flow (Schijven et al., 2000). Cells undergo advective transport moving with the pore water, whose velocity is governed by the hydraulic pressure gradient, porosity, and permeability distribution (Ginn et al., 2002). Bacteria that are in the center of the pore or in larger pores are advected at a considerably higher velocity than those close to the pore wall or in the small pores. This phenomenon creates dispersion of the bacteria because of variations in the fluid velocity field and the rfc of the paths through the porous media (Auset et al., 2004). In addition, random interactions among water molecules and bacteria can result in Brownian motion (Schwarzenbach et al., 1993). This is an important mechanism in transferring of bacteria to the surfaces of solid phase where they may possibly become attached.

Generally, the transport of bacteria in porous media is described by the advection-dispersion-adsorption equation (De Marsily 1986):

$$\frac{\partial C}{\partial t} + \rho_b \frac{\partial S}{\partial t} = \nabla \circ (D \nabla C) - \nabla \circ (\nu C) - k_a C + k_{\det} \rho_b S \qquad (1)$$

$$\rho_b \frac{\partial S}{\partial t} = k_{att} \theta C - k_{\det} \rho_b S \qquad (2)$$

where, C is the number concentration of suspended bacteria in the aqueous phase (L^{-3}), D is the hydrodynamic dispersion coefficient ($L2T^{-1}$), v is the pore water flow velocity (LT^{-1}),k_{att} is the attachment rate coefficient of bacteria (T^{-1}), k_{det} is the detachment rate coefficient of bacteria (T^{-1}) , θ is the porosity (-), S is the concentration of adsorbed bacteria on the sand (-), ρ_b is the bulk density of the saturated soil (ML^{-3}), and t is the time (T). Thus, bacteries that start their transport near the middle of the streamlines and in bigger pores are advected at a significantly higher rate than those along the solid–water interface or (SWI) and low velocity regions. This fact creates dispersivity and affects spreading of bacteris.

In porous media the dominant mechanism for retention of bacteria is adsorption occurring when the pores are larger than the bacteria (Sharmaet al., 1985). It is as an important mechanism influencing bacterial transport in porous media (Tan et al., 1992; McDowell et al., 1986; Gerba et al., 1975). Bacteria adsorption to surfaces is a two-step process (Stevik et al., 2004): i) transport of bacteria to surfaces by diffusion, interception, or gravitational sedimentation resulting in particle–matrix collisions, and ii) attachment of bacteria to the surfaces.

For the past 30 years, classical colloid filtration theory (CFT), originally conceptualized by Yao et al. (1971) has been used to describe the attachment of colloidal particles to a collector surface under saturated, steady-state conditions. According to this theory, a suspended particle may come into contact with a particle of the collector either by interception, sedimentation, or diffusion (Yao et al., 1971). In the CFT, the mass transport process is reflected in the single-collector contact efficiency,η, and the surface attachment step is described by the attachment (collision) efficiency, α, (Elimelech et al., 1995; Yao et al., 1971) . 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) (Unice et al., 2000).

The development of CFT by Yao (et al., 1971) was for water and waste water filtration processes where a spherical colloid was modeled as being filtered out of the bulk solution by those mechanisms. The attachment rate coefficient is related to the single collector efficiency,η, and collision efficiency sometimes refers to as sticking efficiency, α, as the following:

$$k_{att} = \frac{3}{2}\frac{(1-\theta)}{d_c}\alpha\eta v \qquad (3)$$

here, d_c is the average diameter of collector (grain size), [L], η is single–collector efficiency the fraction of particles that collide with the collector, α is the collision efficiency. The attachment rate coefficient is then used to describe the spatial distribution of colloid concentration in the packed bed, where steady-state conditions are assumed.

$$S(x) = \frac{t_0 \varepsilon K_{att} C_0}{\rho_b} \exp\left[\frac{-K_{att} x}{v}\right] \qquad (4)$$

η_D, η_I and η_G, represent theoretical values for the single-collector contact efficiency when the only transport mechanisms are diffusion, interception, and sedimentation, respectively (Yao et al., 1971; Tufenkji et al., 2004a).

$$\eta_D = 2.44 A_s^{1/3} N_R^{-0.081} N_{pe}^{-0.715} N_{vdw}^{-0.053} \qquad (5)$$

$$\eta_I = = 0.55 A_s N_R^{1.675} N_A^{0.125} \qquad (6)$$

$$\eta_G = 0.22 N_R^{-0.24} N_G^{1.11} N_{vdw}^{-0.053} \qquad (7)$$

where $A_s = (2(1-P^5)/2 - 3P + 3P^5 - 2P^{\wedge}$ (8)

and $P = (1-\theta)^{1/3}$ (9), $N_R = (a_p / a_c)$ (10)

for interception, a_c is the median of the grain size number distribution (m). The Peclet number ,N_{pe} is the sum of advection and diffusion as the following:

$$N_{p_e} = (\frac{V a_c}{D_B}) \qquad (11)$$

The van der Waals number characterizing the ratio of van der Waals interaction energy to the particle's thermal energy $N_{vdw} = (H / kT)$ (12)

where H is the Hamaker constant, assumed here to be constant at $6.5 \times 10^{-21} J$ (Walker et al., 2004). The attraction number representing the combined influence of the van der Waals attraction forces and fluid velocity on particle deposition rate due to interception.

$$N_A = (H / 12 \pi \varpi a_p^2 v \theta) \qquad (13)$$

The gravity number $N_G = (2ga_p{}^2(\rho_p - \rho_{fl})9\varpi v\,\theta)$ (14) for sedimentation where g is the gravitational acceleration constant (9.81ms^{-2}), ρ_p is the particle density (kgm^{-3}) and ρ_f is the fluid density (kgm^{-3}). R_c is the diameter of collector (L) and V is the velocity (LT^{-1}), and D is Diffusion (L2T^{-1}).

The single-collector efficiency, η_t is obtained by summing of the individual contributions of three mechanisms:

$$\eta_t = \eta_D + \eta_I + \eta_G \qquad (15)$$

1.2. DLVO Theory

As cells travel close to substrate surfaces, they are subject to attractive London-van der Waals and repulsive or attractive electrostatic forces (Stevik et al., 2004). The relative attractive and repulsive forces that exist between colloids (e.g., bacteria) and grain surfaces can be quantified by DLVO theory (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). The values of total interaction energy, electrostatic interaction energy and van der Waals (VDW) interaction energy are commonly made dimensionless by dividing by the product of the Boltzmann constant (kB=1.38 x 10-23 J K-1) and the absolute temperature in degrees Kelvin (TK).

DLVO interaction energy profiles are constructed by summing up the potential energies of double-layer repulsion or attraction, and VDW attraction to provide the total interaction energy between two surfaces of interest. VDW forces are the attractive forces effective at cell-substrate separation distances smaller than 50 nm (Marshall 1991) and occur when electron clouds form temporary dipoles. The fluctuating dipoles induce dipoles in surrounding molecules by distorting adjacent electron clouds, resulting in temporary attractions. It is linearly dependent on the value of the Hamaker constant. The Hamaker constant depends on the nature of the interacting materials (Swanton 1995). A double-layer electrostatic potential energy arises from the overlap of diffusive clouds of ions (double layers) that accumulate near charged surfaces to balance the surface charge. If the interacting surfaces are like-charged, the double-layer potential energy will be repulsive (Ryan et al., 1996). If the surfaces are oppositely charged, the double-layer potential energy will be attractive. In formulations of the double layer theory, potential energy is considered to be sensitive to variations in the surface potentials of the colloid and the collector, ionic strength of the solution, and colloid size (Ryan et al., 1996). Cells that are reversibly attached are held by temporary cellular bonds

which can be broken by changes in flow velocity, solution chemistry, or cell motility (Marshall 1980). Reversibly adsorbed cells do not come in direct contact with surfaces and are held at separation distances from surfaces by attractive or repulsive forces, depende on the some situations in an area termed the "secondary energy minimum" (Marshall 1980). This area is where cells do not directly contact surfaces but can remain in a state of reversible attachment (van Loosdrecht et al. 1990). Bonds between cells and surfaces are much more difficult to break down when cells are irreversibly attached (Mills 2003). Irreversible attachment occurs when repulsive forces are weak and cells can directly connect to surfaces in an area termed the "primary energy minimum" (van Loosdrecht et al. 1990).

For some systems (e.g., two surfaces of like-charged interacting across an interstitial fluid of relatively high ionic strength), the total interaction energy curve includes both a primary energy minimum, energy maximum or barrier, and secondary energy minimum, providing two discrete locations (i.e., surface separations) where bacteria deposition may occur (see Figure 1). The secondary energy minimum occurs at a greater separation distance than the primary; however, unlike the primary minimum, the secondary minimum is not always present. Cells can also become irreversibly attached because of cell surface structures or because of the excretion of extra cellular polysaccharides (Tufenkji et al., 2006). Attachment of cells is also referred to as adhesion, deposition or retention. As a bacterium approaches substrate surfaces, electrostatic forces increase. Gram-negative bacteria are generally negatively charged with some positively charged sites (Doyle et al., 1989) in natural groundwater conditions. The negative charge is due to phosphate, carboxyl and sulfate groups found in cell walls (Hancock 1991).

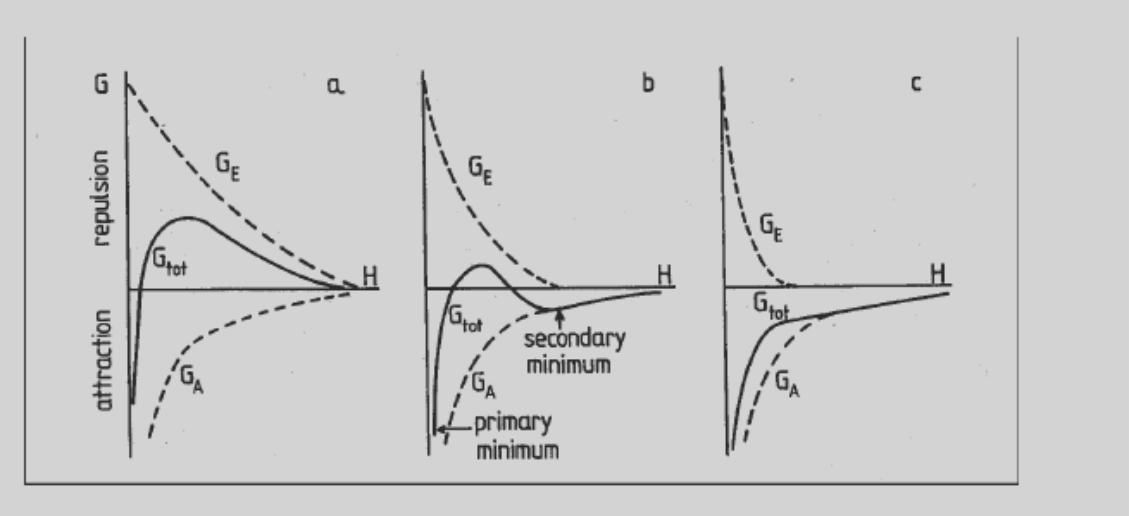

Figure1. Total Gibbs energy interaction potential (G_{tot}), van der Waals forces (G_A) + electrostatic forces (G_E) versus distance of particle (H) from a like-charged flat surface at (a) low ionic strength, (b) intermediate ionic strength and (c) high ionic strength (from van Loosdrecht et al. 1990). The particle is repelled at low ionic strength as a result of electrostatic forces, lies reversibly attached within the secondary minimum at intermediate ionic strength, and rests in an irreversibly attached state within the primary minimum at high ionic strength.

1.3. Factors affecting retention of bacteria in porous media

1.3.1. Effects of Solution Chemistry

1.3.1.a. Effect of ionic strength

Ionic strength plays a significant role in attachment of bacteria to the grain surface. Attraction of bacteria to the filter media surface depends on the thickness of double layer. The thickness of double layer is determined by ionic strength, decreasing with increasing ionic strength (Mills 2003). Thickness is a function of the concentration of the ions and the valency (Stevik et al. 2004). Increasing the ionic strength leads to a reduction in the thickness of the double layer (Gannon et al., 1991; Harvey et al., 1991), and the secondary minimum is forced closer to the media surface. As a result, cells may approach close enough to the surface for the VDW attraction to overcome the repulsion barrier.

This phenomenon is described by the DLVO theory. DLVO theory predicts that in groundwater at high ionic strength, the double layer is compressed and van der Waals forces dominate. Electrostatic repulsion is the dominant force in groundwater at low ionic strength (Rijnaarts et al. 1999).

Walker et al. (2004) while working with three E. coli strains found that an increase in the pore water ionic strength from 10-2 to 10-1.5M KCl results in increased retention of all strains. Fontes et al. (1991) and Mills (1994) also found that decreasing ionic strength by an order of magnitude decreased bacterial attachment rates to quartz sands. Similarly, Bolster et al. (2001) reported that decreasing ionic strength decreased attachment rates of bacteria to uncoated quartz sand. In contrast, (DeFlaun et al. 1990) reported that increases in ionic strength did not increase attachment of bacteria to sand. However this work was done in a complex electrolyte solution with mono and divalent

salts. At the field scale, (Mailloux et al. 2003) found that high ionic strength did not induce higher attachment rates for hydrophilic Comamonas sp. strain DA001.

1.3.1.b. Effect of pH

Another major factor that affects bacterial adsorption is pH. At higher pH, electrostatic repulsion increases, resulting in a decreased attachment rate and an increased detachment rate (Schijven 2001).

The influence of pH on bacterial deposition depends on the characteristics of the bacterial surfaces and ionic strength in the solution (Harvey et al., 1991). Two important factors, which can affect the zeta potential of bacteries, because of the dissociation of carboxylic and amino groups located on the bacterial cell wall, are pH and ionic strength. The importance of pH in determining the surface charge increases at low ionic strength (Kristiansen 1981; Goldschmith et al., 1973). Increasing in retention of cells has been found to occur when pH is decreased from 9.3 to 3.9 (Goldschmith et al., 1973). The retention of bacteria, however, was found to be unaffected within the pH range of 4.0 to 9.0 in experiments conducted by Stenstrom (Stenstrom 1989). The contradictory results, with respect to pH, are probably due to the different isoelectrical points and variability in charges around the iso-electrical point for different bacterial species. The pHiep, which is the iso-electric point at which charges are balanced, ranges from approximately 2 to 3.65 in Gram-negative bacteria (Mills 2003). At higher solution pH, Gram-negative bacteria are therefore negatively charged.

1.3.1.c. Effect of Organic Matter (Om)

Effects of organic matter are probably, responsible for considerable ambiguity in predicting bacterial retention. The major incidence of these materials can be seen in the form of humic acid and fulvic acid. Om can attach to the media and increase the cation exchange capacity, and alter surface charges and number of adsorption sites for bacterial adsorption (Lawrence et al., 1996; Huysman 1993). These materials, similar to bacterial polymers are negatively charged. They may reduce bacterial attachment by competing with bacteria for adsorption sites (Johnson et al., 1996; Harvey et al., 1989).They can also confer the negative charge to the surfaces which it coats. Dissolved organic

carbon may reduce the adhesion due to bacteria having a decreased tendency to contact and adsorb on particles where organic matter is abundant (Harvey et al., 1989).

Among dissolved and/or solid organic matter in the aquifer matrix, humic substances have the highest affinity to nonionic hydrophobic organic compounds (Ouyang et al., 1996). Dissolved organic matter can also be used to elute previously attached microorganism (Sobsey et al., 1980). Thus, detachment of microorganism may be strongly increased by dissolved or suspended organic matter. Dissolved organic matter may compete for the same binding sites as bacteria, and because they are usually present in higher concentrations than bacteria, they can decrease cells attachment. Dissolved organic matter, like surfactants, may also interrupt hydrophobic bonds between soil and microorganism, resulting in an increased detachment rate. At the same time, microorganism like bacteria and many organic materials contain hydrophobic groups on their surfaces. Therefore, once adsorbed, organic matter may provide hydrophobic binding sites for cells (Chi et al., 2004).

Chi et al. (2004) also found that the presence of solid organic matter may result in an increased attachment rate through hydrophobic binding. The hydrophobic adsorption effect will be most pronounced in soil with negatively charged grain surfaces.

1.3.1.d. Hydrophobicity

Hydrophobicity is defined as the distortion of polar water molecules around nonpolar molecules due to an inability to form hydrogen bonds. A hydrophobic surface is therefore repellant to water while a hydrophilic surface can bond with water molecules. Surfaces of bacteria are generally hydrophilic with hydrophobic sites (Gilbert et al., 1991; Van Loosdrecht et al., 1987a, b; Noda et al., 1986). Hydrophobicity varies for every bacterial strain. Hydrophobicity has been identified as an important factor in bacterial attachment to minerals (Stenstr6m 1989), and stainless steel (Vanhaecke et al., 1990). The negative charge of the cell wall is important in the attachment to polystyrene when the hydrophobicity decreased (van Loosdrecht et al. 1987a).

Hydrophobic groups on cell and substrate surfaces can decrease separation distances by removing water films between a cell and the surface (Marshall 1991). Hydrophobic bacteria have higher surface potentials than hydrophilic bacteria and attach to a greater extent than hydrophilic cells (van Loosdrecht et al. 1987a). Bacteria also become more hydrophobic during exponential growth phase (van Loosdrecht et al. 1987b; Walker et al. 2005).

In column experiments, McCaulou et al. (1994) observed slower attachment rates of hydrophilic bacteria than hydrophobic bacteria. They speculated that this was the result of the hydrophilic bacteria traveling farther prior to attaching. They also found that hydrophobic bacteria were more attracted to hydrophobic surfaces (sand coated with polymer to simulate organic carbon) than hydrophilic surfaces (quartz sand). Hydrophilic bacteria were also attracted to the hydrophobic surfaces but to a lesser extent than hydrophobic bacteria, and attachment rates of hydrophilic cells to hydrophilic surfaces were slightly higher than to hydrophobic surfaces. Alexander et al., (1991) found no correlation between hydrophobicity and bacterial transport in soil porous media.

Additionally, electrokinetic potential affects attachment and plays a bigger role as hydrophobicity decreases (van Loosdrecht et al. 1987b). In order to determine information about bacterial electrostatic attraction and repulsion, the electrokinetic potential (or zeta potential) of a cell is usually calculated from electrophoretic mobility and conductance (van Loosdrecht et al. 1987b). (van Loosdrecht et al. 1987b) reported that hydrophobic cells have high electrokinetic potentials, yet they speculate that charged groups cover less than 8% of cell surfaces.

2.3. Effects of Biological Factors

Researches have shown that biological factors have considerable affects on cell transport (Becker et al. 2004) and can help in irreversible attachment of bacteries to surfaces.

Biological factors involved in cell transport and retention include growth and survival (DeFlaun et al., 1990; Walker et al., 2005), cell concentrations (Camesano et al., 1998), cell size (Fontes et al. 1991), bacterial surface characteristics (McCaulou et al. 1994; Walker et al. 2004; Williams et al., 1996), and motility (Camesano et al., 1998).Many of these biological processes are also influenced by physical and chemicals conditions, and the changes in these conditions.

Growth is one of the important factors which can affect attachment rates. DeFlaun et al. (1990) reported that higher percentages of cells attached during exponential phase of growth (log- phase) versus stationary phase. In contrast, Walker et al. (2005), while working with E.coli D21g, found that cells in stationary phase were remarkably more adhesive than those in mid-exponential phase. They attributed it to the higher degree of local charge heterogeneity on the outer membranes of stationary phase cells, which results in decreased electrostatic repulsion between the cells and a quartz surface.

Harvey et al., (1991) found that although growth rates in the subsurface are much lower than in surface sediments, growth within aquifers is a factor over long time periods. Harvey et al., 1991 also indicated that survival of bacteria in the subsurface is affected by the length of time cells remain attached to surfaces and also by predation, starvation, competition and environmental conditions including groundwater chemistry and temperature.

The rate of adsorption has been suggested to increase linearly with cell concentration (Escher 1990). Lower cell concentrations, whether due to starvation or predation, may reduce the amount of bacteria transported through porous media (Gannon et al. 1991). In another study, Fletcher (1977) found increasing in the number of bacterial collisions with the media surface, by increasing in the bacterial concentration, and hence an increase in opportunities for adhesion. However, Johnson et al., (1996) found that as bacterial concentration increased and cells coverage on substrates increased, attachment rates decreased because attached cells blocked the attachment sites of surface. The process of attached cells preventing the attachment of other cells, termed the blocking effect, was stronger at low ionic strengths. Tan et al., (1994) observed greater bacterial breakthrough when cell concentrations were increased. This is an area needing further study.

Small cells usually move through pore spaces more easily than large cells (Gannon et al. 1991) because of fewer interactions between small cells and surface (Hendry et al. 1999). Smaller particles move more easily through pore spaces due to great numbers of potential flow paths (Sirivithayapakorn et al., 2003). Gannon et al. (1991) found that higher percentages of smaller cells than larger cells were transported through soil columns.

Cell surface characteristics previously investigated including hydrophobicity, cell surface charge, cell membrane proteins and the excretion of lippopolysaccharides (LPS) and extra cellular polymeric substances (EPS) (Navarre et al., 1999; McCaulou et al. 1994). Less than 0.1% of cell surfaces actually come into direct contact with a surface, therefore cell/surface interactions are believed to indirect (van Loosdrecht et al. 1990). Surfaces of cells can also change with environmental conditions (van Loosdrecht et al. 1989). The cell envelope is comprised of an inner and outer membrane and of peptidoglycan, which provides cell wall structure (Özkanca et al., 2002). The permeable outer membrane covers the peptidoglycan layer and is made up of proteins (including lipoproteins), phospholipids and lipopolysaccharide. These surface components contribute to cell surface hydrophobicity (Hancock et al., 1991) and are involved in attachment. Lipopolysaccharides,

which are nonpolar, may dominate in binding to hydrophobic surfaces, whereas polysaccharides, capable of polar or electrostatic interactions, may be involved in attachment of cells to hydrophilic surfaces (Williams et al., 1996). Fimbriae, proteinaceous surface structures can also be presented (0.2 to 2 μm long) are thought to be hydrophobic (Ward et al., 1980) and may also play roles in attachment (Doyle et al., 1989).

The LPS of E.coli D21g has a large number of negatively charged functional groups (i.e. three additional phosphate groups in the core region) (Coughlin 1et al., 1983; Gmeiner et al., 1980). These functional groups can dissociate and contribute to the net negative charge on the membrane surface under the examined solution chemistry. Thus, Walker et al., (2004) indicated that these phosphate groups will generate electrostatic repulsion which inhibits adhesion, at least at the moderate and low ionic strength conditions. Extracellular polysaccharides (EPS), excreted by bacteria are also involved in attachment (Doyle et al., 1989). These excretions can form "cement" that holds cells to surfaces.

Cell motility can also be involved in cell attachment. Motility is the means for some species of bacteria to move, in a process termed taxis. One mode of motility is the flagella, which can be up to 20 μm long. For example, movement may occur towards or away from areas which are either rich or deficient in nutrients, light or oxygen (chemotaxis, phototaxis, or aerotaxis, respectively). Motility may increase the chances of a cell arriving at a substrate, overcoming electrostatic repulsive barriers, and attaching to surfaces (Stevik et al. 2004; van Loosdrecht et al. 1989). Camesano et al., (1998) observed an increase in attachment of nonmotile bacteria but found a decrease in cell retention of motile bacteria when fluid velocities were decreased. They also reported that decreasing ionic strength by two orders of magnitude decreased retention of motile bacteria strains more than nonmotile strains.

In some cases, flagella have been found to be involved in actual cell attachment to substrates (McClaine et al., 2002). Flagella also facilitate bacterial adhesion as they protrude through the hydrophilic outer membranes of cells exposing hydrophobic sites on bacteria (Hancock 1991).van Schie et al., (1999) reported that flagellated motile cells, attached to surfaces while at log phase. At stationary phase, however, cells became nonmotile and attachment to surfaces was no longer observed. The relationship between motility and attachment remained unclear and also an area for further study.

3.3. Effects of Physical Factors

Straining and mechanical filtration represent the removal of microbes from solution by physical forces. Straining is the trapping of microbes in pore throats that are too small to allow passage (Corapcioglu et al., 1984; Griffin et al., 1968; Pekdeger et al., 1983) and is exclusively a result of pore geometry (Ginn et al., 2002; Harvey et al., 1991). Factors that control straining are grain size, shape and size of bacteria, and clogging of porous media (Stevik et al., 2004). It occurs typically if cell diameters are greater than 5% of the diameter of the pore throats (Harvey et al., 1991). However, straining has been shown to be more pronounced at the soil surface or at the boundary between different soil textures where the colloids encounter a new pore network (Bradford et al., 2003).

In the intricate geometries of natural porous media, there are many regions which are almost stagnant such as small pores formed in neighboring of grain-grain contact points and dead-end pores. These regions are also susceptible for particle straining. Depending on particles surface properties and flow regime, cells may tend to cluster and be retained at these sites (Bradford et al., 2006).

The grain size has a significant role in bacterial retention through the porous media (Ausland et al., 2002; Stevik et al., 1999). Fine sand, silt, and clay have pore sizes within the range of most bacterial cells. Straining can thus be mechanism in limiting the bacterial transport through these soils (Matthess et al., 1985). For sediments with uniform grain size, straining of most bacteria would not be predicted even for coarse silt (0.02-0.06 mm); however, most natural sediments have a highly non–uniform grain size distribution, and 10% of the pores in some heterogeneous sands are small enough to interfere with bacterial transport (Matthess et al., 1985). Bouwer et al., (1984) showed that straining occurs when the diameter of the suspended particles was larger than 0.2 times the diameter of grains constituting the porous media.

Shape and size of bacteria may also influence bacterial transport through a porous medium (Bitton et al., 1974; Lawrence et al., 1996). In experiment conducted with 19 strains of bacteria, Gannon et al., (1991) showed that cells with length shorter than 1 μm were transported more effectively than cells of 1 μm or longer. However, Corapcioglu et al., (1984) found that larger cells are removed more efficiently. Gannon et al., (1991) found that bacterial transport in porous media was strongly correlated to the cell size ($P = 0.01$).Weiss et al., (1995) investigated the effect of bacterial cell shape on the transport of 14 different strains in porous media. They suggested a preferential removal of rod- shaped cells during transport.

Size exclusion plays also significant role in bacterial transport. Size exclusion is a phenomenon where transported particles move faster than the mean pore-water velocity, and involves an increase in the transport rate due to the size or charge of the material conveyed (Ginn et al., 2002). Size exclusion results in bacterial and ion tracer breakthrough times that are different than those of a non-reacting tracer (Ginn et al., 2002). Field experiments have reported cell transport velocities as much as 70% greater than the mean pore-water velocity; apparently due to exclusion (Ginn et al., 2002). Identification of exclusion is made with tracers and particle breakthrough curves. However, the extent to which it plays a role is still a subject of research.

Many studies have shown that higher flow velocity results in a greater percentage of bacterial cells passing through the porous media (Camper et al., 1993; Trevors et al., 1990). However, this factor is also a subject of debate. Huysman (1993) observed that transport of bacteria in filters was much greater when water was applied at a rate of 4.7 than 0.8 cm/h. High flow rate increases the water movement through macro pores (Bouma et al., 1974; Thamos et al., 1974). Smith et al., (1985) showed that the retention of bacteria in porous media was inversely related to the rate at which water was applied to a filter.

Temperature is considered as a factor which impact on retention of bacteria in porous media. Hendricks et al., (1979) suggested that adsorption of bacteria was significantly greater at higher temperatures. Studies on marine Pseudomonads, showed that at a temperature of 3oC, the proportion of bacteria attached to polystyrene was decreased compared to that at 20oC (Fletcher 1977). A decline observed in attachment with decreasing temperature may have several causes: (a) enhancement in the viscosity of the bacterial surface macromolecules and of the liquid: (b) reduced chemisorptions and certain types of physical adsorption, and (c) changes in the physiology of the organisms (Fletcher 1977; Shaw 1970). Further work in this area is needed.

Summery

This chapter has focused on several important factors affecting transport and retention of bacteria in porous media. They have been summarized in three terms including chemical, biological, and physical factors. Chemical factors include (1) ionic strength, (2) pH, (3) organic matter and, (4) hydrophobicity. Biological factors, list (1) growth and survival of bacteria, (2) cell size, (3) cell concentration, (4) motility (5) bacterial surface characteristics , and (6) flagella. The last factors,

namely physical factors, are including (1) physical straining which is related to grain size, shape and cell size, and clogging (2) flow velocity (3) temperature, and (4) size exclusion.

Colloid filtration theory provides a helpful framework for predicting the transport of bacteria in porous media under saturated conditions; however its application is limited. We conclude, therefore one of the most important factors determining the attachment of bacteria in porous media is difference between surface charge of collector and bacteria. These charge differences depend on the solution chemistry, ionic strength, geochemical heterogeneity, bacteria surface heterogeneity, and the presence of lipopolysaccharides on the outer membrane of bacteria. Thus, an understanding of the mechanisms of bacterial deposition will also lead to greater insight into the transport of pathogenic microorganisms through soil.

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Chapter II

Transport and Fate of Bacteria in Porous Media: Coupled Effects of Chemical Conditions and Pore Space Geometry

Abstract

Experimental and theoretical studies were undertaken to explore the coupling effects of chemical conditions and pore space geometry on bacteria transport in porous media. The retention of *Escherichia coli* D21g was investigated in a series of batch and column experiments with solutions of different ionic strength (IS) and ultra pure quartz sand. DLVO calculations and results from batch experiments suggested that bacteria attachment to the sand surface was negligible when the IS was less than or equal to 50 mM. Breakthrough data from column experiments showed significant cell retention was strongly depending on the IS. This finding indicates that cell retention was dependent on the depth of the secondary energy minimum which increased with IS. When the IS of the influent solution was decreased to 1mM only a small fraction of the retained bacteria were released from the column. The remaining retained bacteria, however, were recovered from the sand that was excavated from the column and then placed in excess amounts of solution having the original IS. These observations suggest that the solution chemistry is not the only mechanism controlling bacteria retention in the porous media. Computational simulations of flow around several collector grains revealed another retention mechanism, which is dependent on both the solution chemistry and the pore space geometry. Simulations demonstrate that the pore space geometry created low velocity regions. The number of bacterial cells that may be transported to these relatively "immobile" regions will theoretically be dependent on the depth of the secondary energy minimum (IS). Once the bacteria are trapped in these immobile regions, reduction of the secondary energy minimum does not necessarily release them due to hydrodynamic constraints.

1. INTRODUCTION

An improved understanding of the mechanisms that govern the fate and transport of microbes in the subsurface is needed for a variety of purposes (Ginn et al. 2002). For example, predicting the fate and transport of pathogenic bacteria in groundwater requires knowledge on the processes controlling cell deposition in the porous media. Bioremediation and/or bio-augmentation strategies to clean up recalcitrant chemicals in subsurface environments could also be greatly improved by efficient delivery of specialized bacteria to targeted locations in the subsurface.

Considerable research has been devoted to understand the effects of the physical and chemical factors that control the transport and fate of bacteria in aquatic systems (e.g., see the review articles of Murphy and Ginn, 2000; Harvey and Harms, 2001; Foppen and Schijven, 2006). The factors investigated include solution chemistry (Jewett et al., 1995; Gannon et al., 1991; Martin et al, 1991), cell type (van Loosdrecht et al., 1987; Gannon et al., 1991), hydrophobic interactions (van Loosdrecht et al., 1987; Schafer et al., 1998), motility (Camesano et al., 1998; Vigean et al., 2003), surface charge characteristics (Gross et al., 2001), and surface features (e.g., lipopolysaccharides, fimbriae) (Herald and Zottola, 1989; Walker et al., 2004). Despite these attempts, the mechanisms governing the retention of bacterial cells in porous media are still not fully known.

Under controlled column experiments, the attachment of bacteria on solid surfaces is generally treated similar to that of inorganic colloidal particles. In these cases, classic DLVO theory (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948) is applied to explain the attachment behavior (Rijnaarts et al., 1995; Simoni et al., 2000; Poortinga et al., 2002). Specifically, DLVO theory states that the interactions between a collector surface can be expressed as the sum of the attractive van der Waals and electrostatic double layer interactions, which can be either attractive or repulsive. Since both the bacteria and collector surfaces are negatively charged in many natural environments, DLVO calculations indicate the presence of a repulsive force between the bacteria and solid surfaces. Despite these predictions, bacteria are often observed to be retained in porous media (Redman et al., 2004; Walker et al., 2004; Smets et al., 1999). There have been some attempts to resolve discrepancies between DLVO predictions and observed retention data by considering non-DLVO forces (e.g. Lewis acid-base interactions) into the so-called extended DLVO calculations (Simoni et al., 2000; Meinders et al., 1995; Azeredo et al., 1999). In some cases, these observed discrepancies were attributed to the existence of local surface charge heterogeneities on the sand grains, which are not accounted for by classical DLVO theory (Truesdail et al., 1998).

A growing body of evidence suggests that the secondary energy minimum can play a significant role to the retention of colloidal particles in saturated porous media (Tufenkji and Elimelech, 2004 and 2005; Franchi and O'Melia, 2003; Hahn et al., 2004). Redman et al. (2004) systematically examined the adhesion of *Escherichia coli* bacterial strain to quartz sand in the presence of repulsive interactions. Bacterial attachment increased with the solution ionic strength (IS), despite DLVO calculations indicating a sizable energy barrier to attachment. It was concluded in this work that bacteria deposition occurred in the secondary energy minimum. However, when the deposited bacteria were exposed to a very low solution IS, only a portion of the deposited cells in the column was recovered in the effluent. These authors were not able to provide a full explanation for the partial release of the bacteria after eluting the column with the low solution IS. The transport and retention behavior of inorganic colloids have also been observed to be highly dependent on solution chemistry under unfavorable conditions (Tufenkji and Elimelech, 2004, 2005; Bradford et al., 2007). Similarly, these studies have shown that once colloids are retained in the column at a given IS conditions, reducing of IS does not necessarily release all of the colloids. It is therefore possible that there are other mechanisms, beside physicochemical forces, controlling bacteria and colloid retention in the porous media.

Mass transfer of colloids to collector surfaces occurs via diffusion, interception, and sedimentation (Yao et al., 1971). Attachment involves collision with and subsequent retention of colloids on the grain surface. Therefore, once a colloid approaches the collector surface, attachment depends on the summation of forces and torques that act on the particle at this location (Cushing and Lawler, 1998; Bergendahl and Grasso, 2000; Torkzaban et al., 2007). The forces acting on the particle in the vicinity of the collector surface include physicochemical forces represented by DLVO calculations, hydrodynamics (drag and lift), gravity, buoyancy, and Brownian forces. Among these forces the DLVO and hydrodynamic drag forces are dominant forces under laminar flow conditions (Torkzaban et al., 2007). Under chemically favorable attachment conditions (i.e. attachment in the primary minimum of the DLVO profile), it may be reasonable to assume that hydrodynamic forces will have a negligible effect on attachment of particle due to the very large adhesive force between the bacteria and the collector. However, under chemically unfavorable conditions the hydrodynamic drag force acting on the colloids adjacent to the collector surface may have a substantial impact on the attachment efficiency of colloids to porous media (Torkzaban et al., 2007; Bradford et al., 2007).

The effect of hydrodynamic forces on bacteria deposition in porous media has received considerably less research attention as physicochemical forces.

In this study, the transport and retention behavior of a well-characterized *Escherichia coli* strain (*E. coli* D21g) in solutions with various IS were systematically investigated. Emphasis was placed on the coupled role of pore space geometry and system hydrodynamics on bacteria retention, and on whether the observed deposition behavior can be explained by classical DLVO theory and system hydrodynamics. To minimize charge heterogeneity effects, highly cleaned, ultra-pure quartz sand was selected as the porous medium. Following recovery of bacteria cell breakthrough data in the column experiments, the IS of the influent solution was decreased to 1mM and the column was flushed for several more pore volumes. Mass balance calculations for the bacteria were conducted from the breakthrough curve information and the final retained cells in the column. A series of batch experiments were also carried out to better deduce the role of the absence of pore structure. Moreover, computational simulations that considered the influence of hydrodynamic conditions and DLVO forces on bacteria retention to several sand grains were conducted. Analysis of the results suggests that the pore space geometry and the depth of the secondary energy minimum both play an important role in bacteria retention under unfavorable attachment conditions.

2. Material and Methods

2.1.Bacteria, Sand, and Electrolyte Solutions

Escherichia coli D21g which is a Gram-negative, non motile bacterial strain with low EPS on its surface was chosen for the experiments (Walker et al., 2004). Cells were grown in 200 ml Luria- Bertani broth (LB broth, Fisher, Fair Lawn, NJ) supplemented with 200 μl of gentamicin (Sigma, St. Louis, MO.0.03 mg/l) at 37 °C for 3 hours until reaching mid-exponential phase, at which time they were harvested for use. More details about the cell preparation protocols are given in Walker et al. (2004).

Ultra-pure quartz sand (Iota® quartz, Unimin Corp., NC) was employed as the porous media for column experiments. The sand medium grain size (d_{50}) was 205 μm with a coefficient of uniformity (d_{60}/d_{10}) equal to 1.26. Prior to use, the sand was treated with 12 N HCl (Fisher Scientific) for at least 24 *h* to remove any impurities, rinsed thoroughly with deionized water, baked at 800 °C for 8 h, and then boiled in deionized water for 1 *h* to rehydrate the sand surfaces.

Five different electrolyte solutions were prepared by addition of reagent-grade KCl (Fisher) to deionized water (Barnstead Thermolyne Corporation, Dubuque IA) to achieve IS of 1, 10, 30, 50, and 100 mM. The solution pH was measured to be 5.6 to 5.8. For the experiments discussed below, bacteria suspensions containing about 10^7-10^8 cells/ml were made using these various solution IS.

2.2.Electrokinetic Characterization and DLVO Calculations

The zeta potential of the bacterial cells was measured using a ZetaPals instrument (Brookhaven Instruments Cooperation, Holtsville, NY). The measurements were carried out five times for the four cell suspensions in terms of IS and the average values are given in Table 1. The zeta potential of the quartz sand at pH 5.5 and the ionic strengths examined in this study was estimated using results presented in Redman et al. (2004) (Table 1).

DLVO theory was used to calculate the total interaction energy (sum of London-van der Waals attraction and electrostatic double-layer repulsion) for bacteria upon close approach to quartz surfaces (assuming sphere-plate interactions) for the various solution chemistries (IS=1, 10, 30, 50, and 100 mM). Retarded London-van der Waals attractive interaction force was determined from the expression of Gregory (1981) utilizing a value of 6.5×10^{-21} J for the Hamaker constant (Simoni et al., 2000) to represent bacteria-water-quartz system. In these calculations, constant-potential electrostatic double layer interactions were quantified using the expression of Hogg et al. (1966) and zeta potentials (Table 1) in place of surface potentials.

2.3. Batch Experiment

Batch experiments were carried out to determine the potential for bacteria adsorption to the sand when pore structure does not exist (entire system in motion). These experiments were conducted by placing 10 g of sand and 10 ml of a known initial concentration of bacterial suspension into 15 ml centrifuge tubes with the temperature kept at approximately 20^{o}C. Four different solution ionic strengths were considered in the batch studies (10, 30, 50, 100 mM). The suspension and sand were allowed to equilibrate for 1 h by gently rotating the tubes end over end (15 rpm) on a tube rotator (Fisher Scientific). A control experiment without bacteria was also performed for measuring the background colloid concentration originated from the sand. The initial and final concentrations of bacteria in the suspension were determined using a UV/vis spectrophotometer at 240 nm (SP-890,

Barnstead International, Dubuque, IA) after setting the tube to rest for a few minutes. All experiments were performed in duplicate.

The concentration of the adsorbed bacteria to the sand grains in each tube was determined using the following equation:

$$S = \frac{(C_I - C_f) \times V}{M} \qquad (1)$$

where M is the total mass of sand in the tube (M, where M denotes units of mass), V is the volume of suspension added to the tube (L^3; where L denotes units of length), and C_I is the initial concentration of the bacteria in the liquid phase ($N_c\ L^{-3}$; where N_c denotes number of cells), C_f is the final concentration of the bacteria in the liquid phase ($N_c\ L^{-3}$), and S is concentration of bacteria on the solid phase ($N_c\ M^{-1}$).

2.4. Column Experiment

Column experiments were conducted using a glass chromatography column (Omnifit, Boonton, NJ). The sand was wet-packed in the column following the procedure detailed in Bradford et al. (2002) to produce a homogeneous packing. Prior to bacteria transport experiments, a solution containing 0.2 mM $NaNO_3$ (Fisher Scientific) was applied to the column using a syringe pump (KD Scientific Inc., New Hope, PA) to determine the dispersion coefficient and the porosity of the porous media. Porosity was also determined gravimetrically. Both approaches gave similar values of porosity of about 0.43. The tracer breakthrough curve was obtained by measuring the absorbance of the effluent at 204 nm by using a spectrophotometer. The column was flushed with about five pore volumes of the electrolyte solution to equilibrate the IS of the system.

A bacteria suspension was introduced into the column using a syringe pump set to provide an approach (superficial) velocity of 0.66 cm/min. The bacteria suspension was applied for four pore volumes (approximately 24 min), followed by bacteria-free solution of the same IS for several more pore volumes. An additional pulse of a bacteria-free solution with a low IS (1 mM KCl) was then applied to the column for several more pore volumes. The introduction of this low solution IS was continued until the cell concentration had decayed to low background levels.

Following this bacteria transport experiment stage, the total number of retained bacteria in the sand column was determined by placing the sand in a vial containing excess amounts of an electrolyte solution of the original IS that was used in the transport experiment. The vial was slowly

shaken for several minutes to liberate any reversibly retained bacteria (in the absence of a constant pore structure). The concentration of the bacteria in the excess solution was measured and the volume of solution and mass of dry sand was determined. In all experiments, the bacterial concentration was determined by measuring the absorbance at 240 nm with a spectrophotometer.

The HYDRUS-1D code (Simunek et al., 2005) was used to simulate the bacteria transport through the columns. Relevant aspects of this code are described below. The code numerically solves the Fickian-based advection-dispersion equation that accounts for bacteria deposition in the column:

$$\frac{\partial C}{\partial t} = \lambda v \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial z} - r_d \qquad (2)$$

where C is the concentration of bacteria in the liquid phase (N_cL^{-3}), λ is the dispersivity (L), v is the average pore water velocity (LT^{-1}), and r_d is the mass transfer rate of bacteria in the aqueous phase to the solid phase ($N_cL^{-3}T^{-1}$). The value of r_d is given by:

$$\rho_b \frac{\partial S}{\partial t} = r_d = nK_{dep}(1 - \frac{S}{S_{\max}})C \qquad (3)$$

where ρ_b is the soil bulk density (ML^{-3}), n is the porosity (-), K_{dep} is the bacterial deposition coefficient (T^{-1}), S is the concentration of deposited bacteria onto the sand (N_cM^{-1}), and S_{max} (N_cM^{-1}) is the maximum concentration of deposited bacteria on the sand. HYDRUS 1D is coupled to a non-linear least square optimization routine based upon the Levenberg-Marquardt algorithm (Marquardt, 1963) to fit model parameters to breakthrough curves.

2.5. Fluid Flow around Collectors and Cell Retention

Packed beds of granular media have often been represented by the single sphere-in-cell (SS) model (Happle, 1958; Payatakes et al., 1974). However, the effect of neighboring collectors on the fluid flow and colloid retention to the collector is not accounted for in this model. Here, we propose two additional simple models for representing the porous media, namely, two spheres-in-contact (TSIC) and two spheres- not-in-contact models (TSNIC). The spheres in these two models are placed in a cell, as for the single sphere-in-cell model. The diameter of the spheres in all three

models is 200 μm and in the case of TSIC and TSNIC models, the center line of the spheres is parallel to the direction of the fluid flow. The distance between the two spheres is zero in the TSIC model and 30 μm apart in the TSNIC model as illustrated in Figure 1. We believe that these two sphere models may capture more features of real porous media such as small pore spaces formed at grain-to grain contacts and role of neighboring grains than a single sphere model.

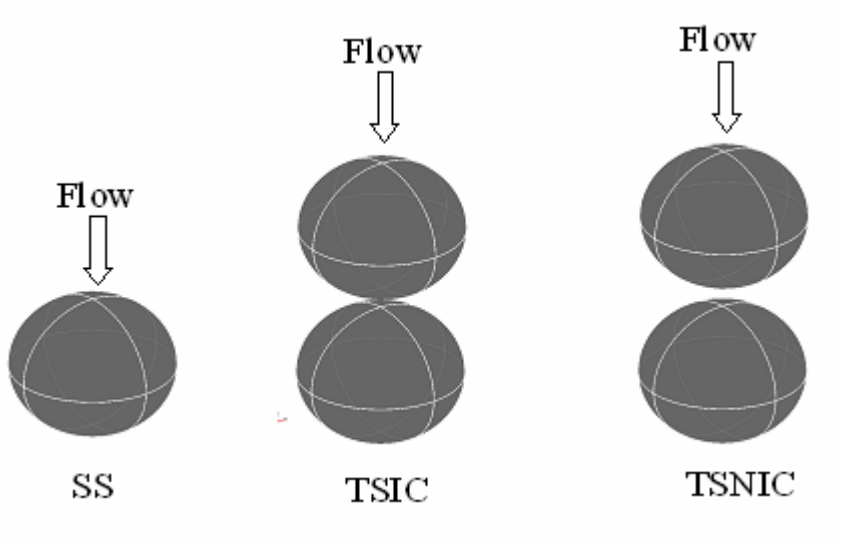

Fig.1. Schematic representation of porous media with three different models, namely, a single sphere (SS), two spheres-in-contact (TSIC), and two spheres- not-in-contacts (TSNIC). The spheres in each model are placed in a cell in order to account for porosity (in analogy to filtration theory). The spheres radii in all three models are 100 μm and the distance between the two spheres is zero in the TSIC model and 30 μm apart in the TSNIC model.

To compute the hydrodynamic forces acting on a bacterium near the collector surface, the fluid velocity field around the collector must be known. When the Reynolds number of the flow is sufficiently small, the inertia terms in the Navier-Stokes equations can be neglected. This is a reasonable assumption in porous media. It follows that the equations governing the motion of water flow around single or two sphere collectors is described by Stokes and continuity equations as:

$$\nabla p = \mu \nabla^2 v \quad , \qquad \nabla \bullet v = 0 \tag{4}$$

where $p[ML^{-1}T^{-2}]$ is the water pressure, v $[LT^{-1}]$ is water velocity, and μ $[ML^{-1}T^{-1}]$ is the fluid viscosity. The exact solution for the Stokes flow past these bodies in unbounded conditions has been obtained by many researchers for example Stimson and Jeffery (1926) and Cooley and O'Neill (1969) analytically solved the Stokes equation for two spheres when they are not in contact and when they are in contact, respectively. In the case of spheres bounded in a cell considered in this

work, the above equations were solved numerically in an axis-symmetrical coordinate system using the COMSOL software package (COMSOL, Inc., Palo Alto, CA) to determine the fluid velocity field around the collectors. The mesh was refined to submicron-sized elements near the surface to yield the fluid velocity at the center of bacteria in the vicinity of the collector. A no-slip boundary condition was imposed along the collector surface and a perfect slip boundary condition was set at the side boundaries of the cell around the collectors. To reflect typical column experiment conditions, the simulations were performed with an average water velocity of 0.66 cm/min which is equal to the average approach velocity in the column experiment. A fixed pressure difference between the inlet and outlet boundary of the cell was applied for each model in order to achieve the desired approach velocity. Once bacteria cells are transported to the collector surface, bacteria retention will depend on a balance of forces and torques that act on bacteria at this location (Cushing and Lawler, 1998; Bergendahl and Grasso, 2000; Torkzaban et al., 2007). A detailed description for examining the coupled effects of hydrodynamic and physicochemical conditions on colloid attachment to a collector has recently been described by Torkzaban et al. (2007). Only an abbreviated description is given below. It should be mentioned that the bacteria were assumed to be spherical in shape for the sake of simplicity in this analysis.

Interaction energies between bacteria and the solid surface are typically calculated using DLVO theory. The corresponding resisting (adhesive) torque ($T_{resisting}$; ML^2T^{-2}) for cells attached in either the secondary or primary minimum of the DLVO interaction energy distribution is represented by the net adhesive force (F_A, MLT^{-2}) acting on a lever arm (l_x, L) as:

$$T_{resisting} = F_A l_x \qquad (5)$$

The value of the F_A in Eq. [5] is estimated as Φ_{min}/h (Israelachvili, 1992); where Φ_{min} [ML^2T^{-2}] is the absolute value of the secondary or primary minimum interaction energy and h is the separation distance between the bacterium and the collector surface. The value of l_x is estimated using JKR theory (Johnson et al., 1971; Israelachvili, 1992; Soltani and Ahmadi, 1994; Bergendahl and Grasso, 2000; Torkzaban et al., 2007; Ahmadi et al., 2007) by assuming no direct physical contact between the bacteria and the collector surface.

Hydrodynamic forces will also act on cells that are in the vicinity of the solid- water interface as a result of water flow. When the water flow is laminar, the lift force acting on the cells perpendicular to the interface is negligible (Soltani and Ahmadi, 1994). The drag force that act on

the bacteria tangential to the interface, however, is significant and can be calculated using an equation developed by Goldman et al. (1967) and O'Neill (1968). The corresponding driving torque ($T_{driving}$; ML^2T^{-2}) acting on cells in the vicinity of the solid interface due to the hydrodynamic shear force is given as (Goldman et al., 1967; O'Neill, 1968; Sharma et al., 1992):

$$T_{driving} = 1.4 r_c F_D \quad (6)$$

where F_D (MLT^{-2}) is the drag force that act on the bacterium in the vicinity of the collector surface and r_c is the equivalent radius of bacterium.

Lifting, sliding, and rolling are the hydrodynamic mechanisms that can cause bacteria removal from an interface (Soltani and Ahmadi, 1994; Bergendahl and Grasso, 2000; Ahmadi et al., 2007). Rolling has been reported to be the dominant mechanism of hydrodynamic detachment from solid surfaces under laminar flow conditions (Tsai et al, 1991; Bergendahl and Grasso, 1998 and 1999). Rolling occurs when $T_{resisting}$ is overcome by $T_{driving}$ from hydrodynamic forces (Johnson, 1985).

3. Results and Discussion

3.1. DLVO Calculations

Table 1 shows that the zeta potential of the bacteria and sand becomes less negative as the IS increased due to compression of the thickness of the electrostatic double layer (e.g., Elimelech et al., 1998). This zeta potential information was used in conjunction with DLVO theory to calculate the total interaction energy for bacteria upon close approach to quartz surfaces for the various solution chemistries (IS=1, 10, 30, 50, and 100 mM). At an IS of 100 mM the DLVO calculations predict no energy barrier to bacteria deposition. In this case, bacteria cells may be strongly attached to the quartz sand in the primary energy minimum. At ionic strengths equal or less than 50 mM the DLVO calculations predict the presence of substantial repulsive energy barriers (ranging from 44 k_bT_k at 50 mM to over 2250 k_bT_k at 1 mM, where $k_b$is the Boltzmann constant and T_k is the absolute temperature) against bacteria deposition to the sand surface in the primary energy minimum. The bacteria may still interact with the sand surface, however, by the secondary energy minimum which increases in magnitude with IS. Table 1 provides the depth and separation distance for the secondary minimum at each IS.

Table 1. Electrokinetic properties of bacteria and quartz sand as well as secondary energy minimum depth (Φ_{min2}), repulsive energy barrier (Φ_{max}), and distance of secondary minimum from the surface (h).

Ionic strength(mM)	Zeta potential (mV)		Φ_{min2} (k_bT_k)	Φ_{max} (k_bT_k)	h (nm)
	Sand	Bacteria			
1	-38	-61	-0.09	2250	120
10	-22	-49	-1.27	262	32
30	-13.6	-38	-4.4	86	14.5
50	-12	-32	-7.5	44	10
100*	-11.2	-21	NB*	0	NB*

* No barrier to deposition and hence no secondary minimum

3.2. Batch experiments

The distribution coefficient (K_D; L^3M^{-1}) was determined from the measured linear adsorption isotherm to quantitatively compare the effect of solution IS on the bacteria attachment. The value of K_D was determined from Eq. (1) as C_f/S. The value of K_D was practically zero for all the experiments conducted at the solution IS of 10, 30, and 50 mM. This finding is consistent with the unfavorable conditions for attachment predicted by DLVO calculations, and also confirms the absence of chemically heterogeneities such as positively charged metal oxides on the sand surface. The K_D value for the experiment in IS of 100 mM, however, was 12 cm^3 g^{-1} of sand and therefore substantial attachment of cells occurred to the sand. This observation also shows qualitative agreement with DLVO calculations that predicted no energy barrier to attachment when the IS was 100 mM.

3.3. Column experiment

Bacteria transport experiments were repeated in duplicate at each IS. Summary information for these experiments is given in Table 2. Representative observed and simulated bacteria breakthrough curves for the various solution ionic strengths are presented in Fig.2. Here the normalized effluent concentration (C/C_0) is plotted against the number of pore volumes. The deposition of bacteria increased with the IS of the solution. Noticeably, more than 98% of the input bacteria were retained in the column at the highest IS (100 mM). When the IS was less than or equal to 50 mM, the effluent concentration increased slowly with time toward a steady state level. Potential mechanisms causing this behavior include: bacteria detachment from the sand and blocking/filling of available locations for bacteria deposition. Blocking/filling of available locations for deposition seems to be a more likely explanation, because detachment produced insignificant tailing in the breakthrough curves.

Table 2. Fitted model parameters for various solution ionic strengths. Also included in this Table are mass fractions of bacteria recovered during the bacteria transport (M_{tran}), during elution with the low solution ionic strength (M_{ell}), from the sand (M_{sand}), and the total mass balance (M_{total})

Ionic strength(mM)	k_d (min-1)	S_{Max}(No. of cell /gr sand)	M_{tran} %	M_{ell}	M_{sand} %	M_{total}
10*	0.041±0.01	0.4±0.07	75	6.6	23	1.05
10	0.06±0.01	0.35±0.08	76.5	4.2	27	1.08
30*	0.121± 0.07	1.7± 0.06	50	13.8	40	1.04
30	0.09±0.05	1.4±0.05	61	11.2	35	1.07
50*	0.29± 0.02	3.9± 0.7	22	26.7	55.6	1.04
50	0.25±0.06	4.1±0.3	26.5	28.6	55	1.1
100*	0.90± 0.13	-	<2	58.4	17.4	0.78
100	0.91±0.2	-	<2	60.4	20	0.81

* The presented breakthrough curves in Figure 2. are for these experiments.

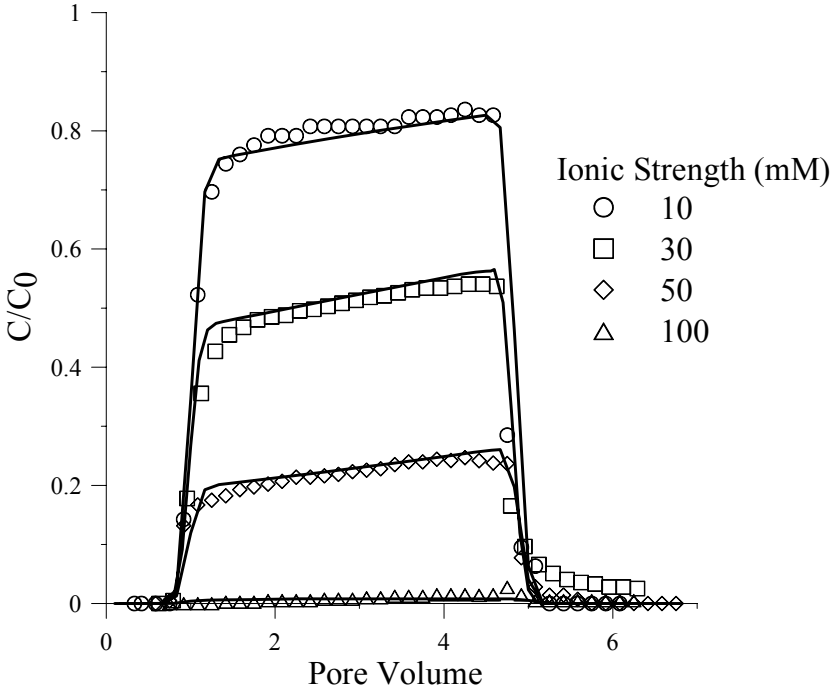

Fig 2. Observed (symbols) and simulated (lines) breakthrough concentrations of bacteria for different solution ionic strengths.

To quantitatively investigate the effect of IS on bacteria transport, the deposition rate coefficient (K_{dep}) and maximum concentration of deposited cells (S_{max}) were determined for each experiment by fitting the breakthrough data to the solution of Eq. (2) and (3). It should be mentioned that the dispersivity (λ) required in these simulations was estimated by fitting the solution of Eq. (2) to the tracer ($NaNO_3$) breakthrough data (data not shown). This value of λ was low (0.04 cm) as expected for a 10 cm column. For the bacteria transport experiments, the agreement between modeled and measured concentrations is generally good with the coefficient of linear regression (R^2) values ranging from 0.94 to 0.99. The value of K_{dep} and S_{max} increased with solution IS (Table 2). It is worth noting that because the slope of the rising limb of the breakthrough data in the 100 mM experiment was negligible, a unique value for S_{max} was not possible. The mass fraction of bacteria that was recovered in the effluent during the bacteria transport stage (M_{tran}) was also determined and decreased with increasing IS.

At first glance, it is tempting to attribute the increasing trend of bacteria deposition in the column with IS solely to solution chemistry and attachment. However, this hypothesis is not consistent with the observed negligible bacteria attachment that occurred in the batch experiments (except at 100 mM). To better determine the role of solution IS on bacteria deposition, additional information was collected in the column experiments. Specifically, following recovery of the bacteria breakthrough data each column was flushed with several additional pore volumes of 1 mM solution before excavating the sand to determine the deposited cell mass.

Figure 3 present the recovered bacteria concentrations in the effluent as a function of pore volumes after flushing the column with 1mM solution. The corresponding bacteria breakthrough curves for the various solution ionic strengths were presented in Figure 2. The observed cell peak upon change of solution IS has typically been ascribed to release of colloids that were associated with the solid surface via the secondary minima (Franchi and O'Melia, 2003; Hahn and O'Melia, 2004) and the primary minima (Ryan and Elimelech, 1996). In fact, the release of previously deposited colloidal particles by lowering the solution IS has been used as supportive evidence for particle deposition in the secondary energy minimum (Redman et al., 2004; Tufenkji et al., 2005). Table 2 presents the mass fraction of introduced bacteria that were recovered during elution with the low solution IS (M_{ell}). Notice that just a fraction of the input bacteria were recovered in the effluent when the 1 mM solution was flushed through the columns. M_{ell} data ranges from about 5% when the bacteria were deposited with 10 mM solution to 60% when the cells were deposited with 100 mM solution. In other words, in the experiments conducted using 10 and 100 mM solutions 85 to 40% of the retained cells, respectively, were not released when the IS of the eluting solution was lowered to 1 mM. It is worthwhile to mention that when the bacteria transport was conducted in a solution with IS of 1mM, about 99% of the introduced bacteria was recovered in the effluent (data not shown). Furthermore, the remaining bacteria in the column after elution with the low solution IS were recovered when the pore structure was destroyed by excavation and suspending the sand in excess amounts of solution at the same IS as the original bacteria transport experiments. Table 2 also shows the mass fraction of cells that were recovered from the sand (M_{sand}), as well as the total mass balance (M_{total}) for the column experiments. When the IS was less than or equal to 50mM, the total mass balance was very good (between 104 to 108%). This finding indicates that the bacteria were not irreversibly attached to the quartz grain in the primary energy minimum.

All of the above observations strongly indicate that the solution chemistry plays an important role in bacteria deposition in the porous media. Many other researchers have reported a similar dependence of colloid transport on IS under unfavorable attachment conditions (e.g., Li et al., 2004; Tufenkji and Elimelech, 2004 and 2005a; Bradford et al., 2007). However, the batch experiments and the low IS elution results discussed above also provide evidence that solution chemistry is not the only mechanism controlling the deposition. In particular, recovering the remaining deposited bacteria by suspending the sand in a solution indicates that the pore structure may play an important role in the bacteria retention process.

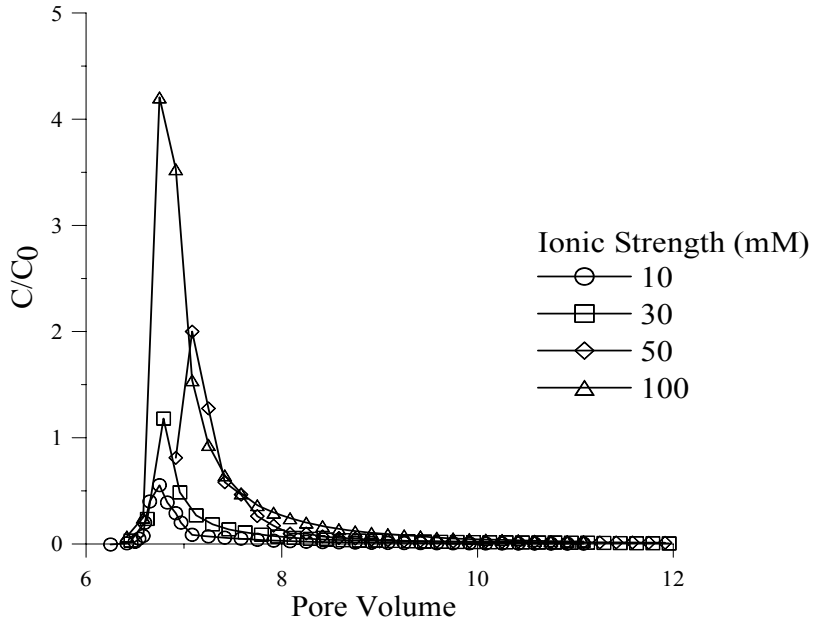

Fig.3. Observed effluent concentrations of bacteria obtained from the column when the influent was switched to the low solution IS of 1mM. In these experiments the bacteria transports were conducted with different solution ionic strengths (the main breakthrough curves are presented in figure 2).

3.4. Analysis of Resisting and Driving Torques

To further examine the underlying mechanisms that control bacteria retention, an analysis of the resisting (from adhesive force) and driving (from drag force) torques on collector surfaces was conducted. Figure 4 presents the calculated distribution of the tangential component of drag force that acts on bacterium in the vicinity of collectors from the three different porous media models, namely: single sphere (SS), two spheres-in-contact (TSIC), and two spheres-not- in-contact

(TSNIC). It should be noted that the presented distribution of the drag force is just for the top collector in the two spheres models. The magnitude of the tangential component of the drag force along the collector surface is plotted versus normalized distance (L/L_{max}), which is defined as the distance along the collector surface from the front to the rear stagnation point (L) divided by half the collector perimeter (L_{max}). As expected, the value of the drag force (and therefore $T_{driving}$) is dependent on the location along the collector surface. It is zero at the front and rear stagnation points (zero velocity), and increases with the distance from these two points until it reaches a maximum value at the collector midpoint. Moreover, it is observed that values of the hydrodynamic drag force at locations near the rear stagnation point in the SS model are significantly higher than those associated with the TSIC and TSNIC models. The magnitude of drag force near the rear stagnation point also changes in a nonuniform fashion with L/L_{max} for the TSIC and TSNIC models. This observation will be explained in detail later on in the manuscript.

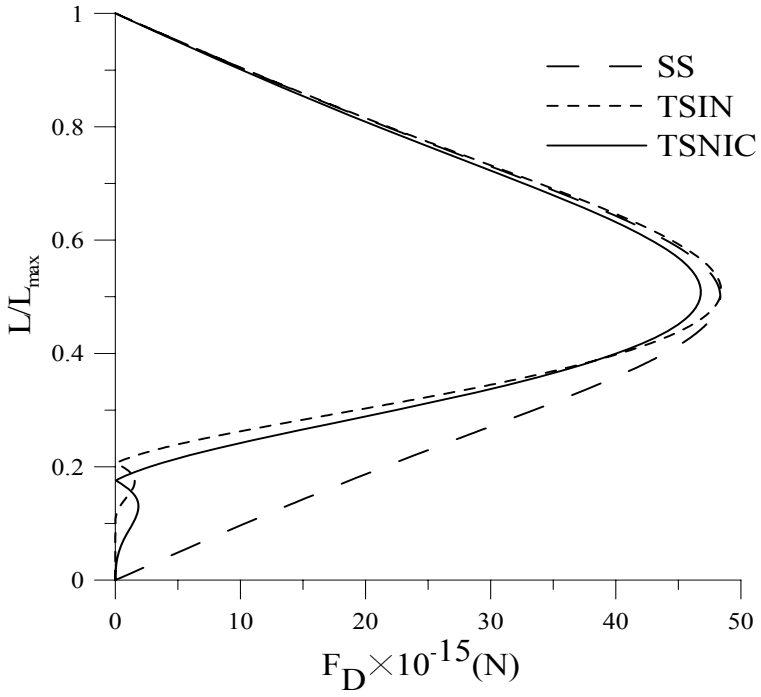

Fig.4. The calculated distribution of the tangential component of drag force that acts on a bacterial cell (assumed spherical shape) in the vicinity of the collectors for three different models of porous media. The average water velocity is 0.66 cm/min. The distribution of tangential drag force along the collector surface is plotted versus normalized distance (L/L_{max}), which is defined as the distance along the collector surface from the front to the rear stagnation point (L) divided by half the collector perimeter (L_{max}).

Figure 5 shows a plot of the fraction of surface area of the spherical collectors that may contribute to bacteria attachment (S_f) as a function of IS for the SS, TSIC, and TSNIC models. Assuming rolling as the principal mechanism for bacteria detachment, attachment may occur on the gain surface when $T_{resisting} > T_{driving}$. For all the models the value of S_f is unity when the IS reaches 100 mM, because attachment occurred in the primary minimum. The SS model predicts that the value of S_f is virtually zero for the three ionic strengths of 10, 30, and 50 mM (with a maximum of 0.1% for 50 mM), and implies that bacteria attachment will be negligible. Under these unfavorable attachment conditions ($T_{resisting} < T_{driving}$), bacteria that collided with the collector will be swept away by hydrodynamic shear. This result is in agreement with the results of batch experiments that indicated negligible attachment for ionic strengths less than or equal to 50 mM.

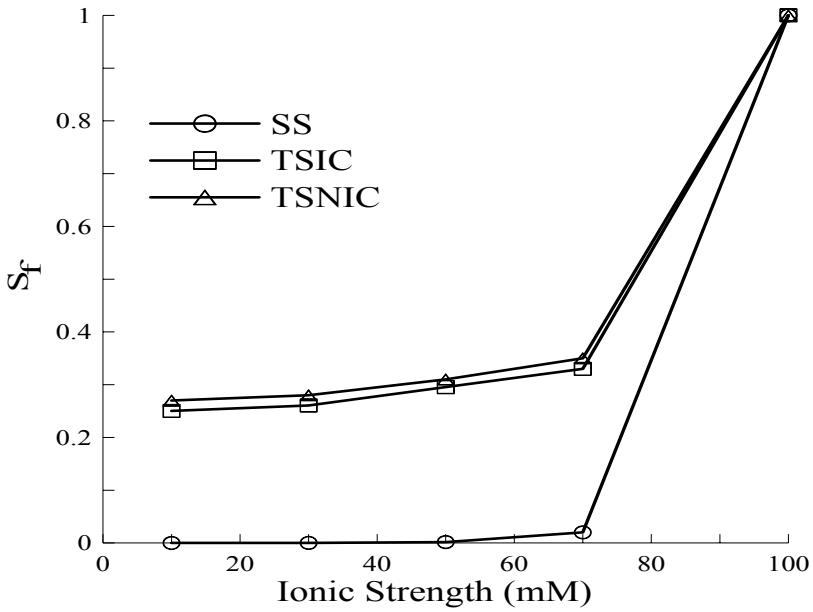

Fig.5. Plot of S_f as a function of ionic strength for three different models (SS, TSIC, TSNIC) in the presence of an average pore water velocity of 0.66 cm/min.

In contrast to the SS model predictions, the values of S_f for the TSIC and TSNIC models are significantly higher for 10, 30, and 50 mM solutions and increases very slowly with increasing IS. This observation suggests that lower drag forces occurred in some regions of the TSIC and TSNIC

models than the SS model. In fact, Figure 4 demonstrates that lower velocities regions did occur near the rear stagnation point of the top sphere in the TSIC and TSNIC models. These regions may therefore contribute to greater bacteria retention even at low IS because $T_{resisting} > T_{driving}$.

The torque balance calculations provide an explanation for the enhanced bacteria retention in the column experiments compared to the batch studies. However, the torque balance calculations shown in Figure 5 indicates that S_f will increase very slowly with increasing IS when the IS was less than or equal to 50 mM. In contrast, Figure 2 indicates that colloid retention behavior was a strong function of solution IS over this same IS range. Moreover, the torque balance calculations predict that the value of S_f is negligible for all three models when the IS of 1mM was considered in these calculations. This implies that if the resisting and driving torques (hydrodynamic and physicochemical forces) were the main mechanism for bacteria retention, the retained bacteria should have been released upon introduction of low solution IS. In contrast, the elution experiments shown in Figure 3 indicate that only a fraction of the retained bacteria were released after flushing the column with 1 mM solution (Table 2). This apparent discrepancy suggests that the underlying mechanism for bacteria retention also depends on other factors that were not considered in this analysis.

3.5. Immobile Zones and Cell Retention

Additional flow simulations where conducted to examine the fluid flow field near the rear stagnation point of the SS, TSIC, and TSNIC models. Figure 6 shows the streamlines and normalized velocity arrows in regions near the rear stagnation point for these models under conditions of steady flow at a low Reynolds number. As expected, flow in the SS model tends to follow the collector surface and no separation of the fluid streamlines from the collector surface is observed (Figure 6a). In contrast, the pore space geometry of the TSIC and TSNIC models causes fluid streamlines to separate from the collector surface, and form immobile regions near the gap between the two grains. In these immobile regions, where water does not mix with the bulk solution, water rotates in an infinite set of nested ring vortices (Figures 6b and 6c). This behavior of Stokes flow around two spheres in contact or close to each other has theoretically been demonstrated (e.g. Davis et al., 1976 and 1977) and experimentally visualized by Taneda (1979). Indeed, the unusual behavior of the tangential component of the drag force shown in Figure 4 for the TSIC and TSNIC models can be explained by

the complex flow field (nested ring vortices) in the gap region between the two spheres. It should be mentioned that immobile regions in porous media, especially unsaturated conditions, have been used to explain a variety of observed transport behavior without recognizing the exact system hydrodynamics (Toride et al., 2003; Pardilla et al., 1999).

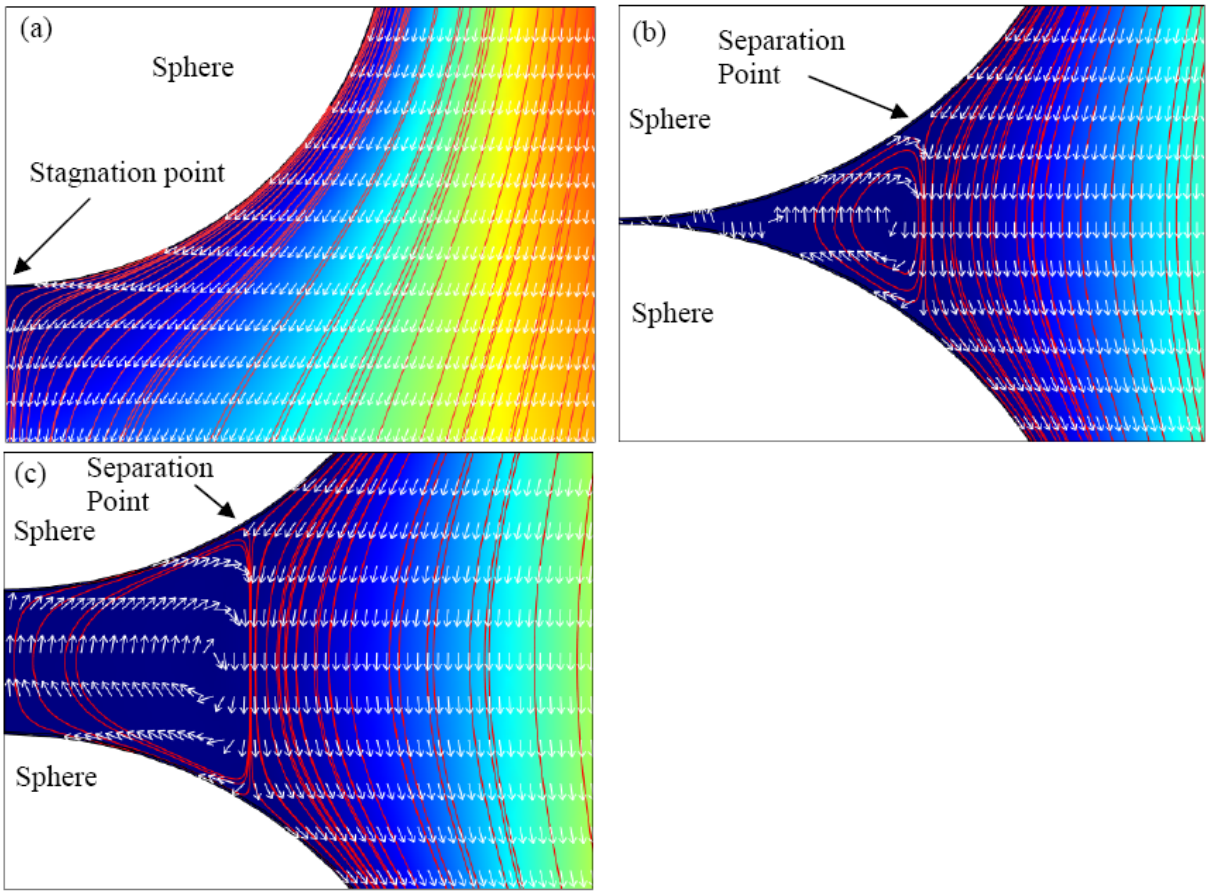

Fig.6. illustrates the velocity field near the rear stagnation point of the SS model (a) near the gap between the two spheres in TSIN (b) and TSNIC (c) models. In case of TSIC and ISNIC models, separation of streamlines (red lines) form the collector occurs and as a result an immobile region within the gap between the two spheres is formed. The white arrows are the normalized water velocity indicating that water rotates with a very low velocity in the immobile regions.

The insight obtained from studying the flow structure near the contact point in TSIC model and around the gap between two spheres in the TSNIC model may clarify the mechanism of bacteria or in general colloid retention in porous media. When the porous media is static (e.g. column experiments), the pore space geometry will produce many hydrodynamically-disconnected (immobile) regions (Figures 6b and 6c). Once the bacterial cells enter these regions, they will be trapped even under unfavorable attachment conditions. The only way that colloids may escape these

immobile zones is through Brownian diffusion. This important finding provides a reasonable explanation for the observations that just a fraction of the retained bacteria were released after flushing the columns with low IS solution (Table 2). It also provides an explanation for differences in bacteria retention in batch and column experiments. During batch experiments the porous media is in constant motion which leads no immobile regions in the system. Under these conditions, bacteria retention may only occur when the resisting torque (physicochemical forces) is greater than the driving torque (hydrodynamic forces); i.e., the 100 mM batch experiment.

It should be mentioned that the magnitude of velocity in the immobile zones (hydrodynamically-disconnected regions) is very small. This results in small drag forces in this region, and the bacteria cells therefore have a great chance to attach to the collector surface (Figure 5). Even if the conditions are not favorable for bacteria attachment to the solid surface in these regions (e.g. $T_{resisting}<T_{driving}$), the cell will still continue to circulate. It is also likely that bacteria-bacteria interactions will be enhanced in immobile zones because the number of bacteria collisions may drastically increase relative to that in bulk solution. As bacteria accumulation continues and starts to fill the immobile zones, the pore space geometry will become a limiting factor in bacteria retention. Indeed, fluorescent microscopy and x-ray microtomography studies have demonstrated that colloids accumulate in the narrow region of the pore spaces near the contacts of irregularly shaped sand grains under unfavorable attachment conditions (Bradford et al., 2005, 2006, Xu et al., 2006; Li et al., 2006; Yoon et al., 2006). In these studies, pore-space constrictions apparently served as locations for colloid retention, whereas few colloids appeared to be immobilized far from the grain-to- grain contacts. It should be noted that colloid retention in the smallest regions of the pore space such as those formed near grain to grain contact points has been referred to in the literature as straining (Hill, 1957; McDowell-Boyer et al., 1986; Cushing and Lawler, 1998; Bradford et al., 2006).

3.6. Coupling of Factors in Cell Retention

Figure 2 indicates that colloid retention behavior was a strong function of solution IS. This dependency can be explained by considering mechanisms of bacteria transfer to hydrodynamically-disconnected immobile regions. According to conventional filtration theory, bacteria may collide with grain surfaces via sedimentation, interception, and diffusion (Yao et al., 1971). Bacteria cells that are weakly associated with the solid-water interface via the secondary energy minimum,

however, experience significant hydrodynamic forces due to fluid flow that may result in bacteria detachment via rolling or sliding (Tsai et al, 1991; Bergendahl and Grasso, 1998 and 1999; Torkzaban et al., 2007). Some of these weakly associated bacteria can be translated and/or funneled by fluid drag forces to immobile regions (e.g., grain-to-grain contact points) and be retained in these straining sites (Bradford et al., 2007). Increasing the IS results in a greater depth of secondary energy minimum (see Table 2) which likely leads to larger numbers of bacteria (rolling or sliding along the solid surface) and entering the immobile zones. Indeed, recent experimental evidence by Kuznar and Elimelech (2007) demonstrates that colloids captured in the secondary energy minimum can be translated along the collector surface via hydrodynamic forces and be retained in regions near the rear stagnation point. These findings provide a plausible explanation for the observed dependence of bacteria retention on solution IS shown in Figures 2 and 3. In particular, the coupled affect of solution chemistry, pore structure, and system hydrodynamics on bacteria retention.

4. Conclusions

Experimental evidence in this study has demonstrated that solution chemistry and bacteria attachment are not the only factors and mechanisms responsible for bacteria retention. In particular, the data indicates that pore space geometry also played an important role in bacteria retention. The first piece of indirect evidence are results from batch experiments (where the pore structure was eliminated) which found no significant bacteria attachment to the sand when the IS was less than or equal to 50 mM. Second, reversing the IS in column experiments to a very low value (1mM) only resulted in release of a fraction of the retained bacteria that were deposited under higher IS conditions. Finally, column mass balance indicates that good recovery of the retained bacteria was achieved when the pore structure was destroyed by suspending the sand in excess amounts of solution of the same IS that was used in the transport experiments.

Computer simulation results also confirm the importance of the system hydrodynamics. The special pore space geometry such as those formed near grain to grain contact points was found to produce hydrodynamically-disconnected regions. These immobile regions with an infinite set of nested ring vortices can significantly contribution to bacteria retention. These computer simulations could explain differences in retention behavior in batch and column experiments, as well as the incomplete recovery of retained colloids when the columns were flushed with a low IS solution.

The observed increasing amount of bacteria retention with increasing IS was explained by considering the mass transfer of bacteria (rolling along the interface) to the immobile regions of the pore medium. In particular, increasing the IS results in a greater depth of the secondary energy minimum (see Table 2) which likely leads to greater numbers of bacteria being transported along the solid surface by hydrodynamic forces to these regions (straining locations). Hence, bacteria retention in porous media is a coupled process that strongly depends on solution chemistry, pore structure, and system hydrodynamics.

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Chapter III

The influence of cell preparation methods on *E.coli* D21g surface chemistry and transport in saturated porous media

Abstract

The effect of cell preparation methods on the surface chemistry and deposition of *Escherichia coli* D21g was investigated over a range of ionic strength conditions. The cell preparation methods, which were considered, included filtration and centrifugation (at various speeds and for different durations). For a given ionic strength condition, it was found that cells prepared by filtration were more negatively charged and hydrophobic than cells prepared by centrifugation. Increasing the force imposed or duration of centrifugation produced cells with increasing zeta potentials (less negative) and decreased hydrophobicity. Column transport experiments were also conducted with ultra pure quartz sand using the same solution chemistries and *E. coli* D21g. The retention of these cells was quantified by fitting a first-order deposition rate coeffecient (k_d) and the maximum deposited concentration (S_{max}) to the collected effluent concentration curves. The results demonstrate that the rate of the deposition of *E. coli* D21g increased with increasing force and duration of centrifugation, and was lowest in the case of filtered cells. Moreover, the results show that the influence of cell preparation methods was more pronounced in lower ionic strength solutions.

1. Introduction

Bacteria cell surfaces play a crucial role in their interaction with porous media in the environment (Pembery 1999). The ability of cells to adhere to abiotic surfaces can be attributed to the physiological state of the organism (Zvyagintsev, 1977; Fletcher, 1977) which has recently been associated with the physicochemical properties and metabolic behavior exhibited by their cell surface (Grasso, 1996, Dufrene, 1996, Walker, 2005). Physicochemical interactions between the bacterial cell surface and the porous medium surface have been reported to govern the adhesion of cells (Smets et al., 1999). Most experimental procedures (e.g. cell surface analysis, bacterial adhesion experiments) require that cultures of organisms are harvested in some way before experimentation. The purposes of such procedures are to concentrate the cells and to clean the cells from their high nutrient environment.

Harvesting protocols may affect the physiochemical nature of and/or the metabolic behavior of the bacteria, creating artifacts in the measurement of adhesion and other bacterial properties (Pembery et al., 1999). Some research has indicated that variation in the surface physiochemical properties in vitro may completely change the attachment mechanisms of an organism compared to its behavior in the natural environment (Marshall, 1994). Exposure to such parameters as different ionic strength (IS), temperature, centrifugation protocol, freezing and drying are just some of the processes that need to be considered for their ability to cause experimental artifacts with bacteria (Bell et al., 2005). Specifically, the effects of centrifugation on a bacterium's capacity to attach to substrates are of particular concern because centrifugation is commonly used in cell preparation procedures (Bell et al., 2005). Few systematic studies of the effects of centrifugation on cell surface characteristics have ever been reported (Gilbert 1991; Makin 1996; Pembery et al., 1999). In the work conducted by Pembery et al. (1999), it was found that the net surface charge of *E.coli* and *S. epidermidis* reduced when the cells were subjected to a high speed centrifugation (15000 xg). In contrast, the charge characteristics of *Psychrobacter sp.* strain SW8 remained unchanged after the centrifugation. It has been reported that centrifugal separation of the cells from their suspending medium subjects them to enormous centrifugal forces, which in centrifuge tubes is translated to high hydrostatic pressures (Gilbert et al., 1991).

Filtration is also a separation method commonly used in the field of aquatic microbiology for a variety of objectives including bacteria harvesting (Kepner et al., 1994). In contrast with

centrifugation, it is reported that filtration does not greatly affect cellular behavior (Bell et al., 2005). In spite of this advantage, it is seldom utilized in routine studies and experiments, in part due to the relatively longer time required for filtering the cell suspension.

The aim of this research was to investigate how the cell harvesting methods (centrifugation vs. filtration) influence the surface chemistry of bacteria (*Escherichia coli* D21g) under well controlled solution chemistry conditions. We also compared the deposition trends of bacteria in a packed bed column that had been either harvested by filtration or various modes of centrifugation. Different speeds (i.e. 3689 and 14000 g) and duration (10 and 15 minutes) of centrifugation were investigated. In addition to the deposition experiments, the nature of the cell surfaces was characterized using techniques such as the microbial partition to hydrocarbon (MATH) test, and measurements of the cell surface electrophoretic mobility to capture the changes in the nature of the cell surface due to the preparation method.

2. Materials and Methods

2. 1. Porous Media Preparation

Ultra pure quartz sand was used as the porous media. The sand was prepared in advance to ensure that sand grain sizes were uniform and the grain surfaces were free of organic matter and metal oxides. The sieved sand (Unimin Corporation, Spruce Pine, NC) had an average diameter (d_{50}) of 205 μm. In order to remove chemical heterogeneities that could have influence the bacteria deposition behavior, prior to doing experiments, the sand was soaked in 12N HCl (Fisher) at least 24 hrs. The sand grains were then was thoroughly washed, baked in an oven at 800 ^{o}C for 8 hrs, and again rehydrated by boiling in deionized water (Barnstead Thermolyne Corp., Dubuque, IA) for 1 hr.

2. 2. Bacterial Cell Preparation and Growth

Escherichia coli D21g a Gram-negative, non motile bacterial strain was chosen for experimentation. It is reported that this bacterium has minimal lipopolysaccharides (LPS) (Gmeiner et al., 1980; Walker et al., 2004), and negligible amounts of extra- cellular polymeric substances (EPS) (Razatos et al., 1998). A pre-culture of bacteria was prepared by inoculating 5 mL of Luria-Bertani broth (LB Broth, Fisher Scientific, Fair Lawn, NJ) that had been supplemented with 0.03 mg/L gentamycin (Sigma, St. Louis, MO). The pre-culture was incubated on a rotary shaker table

overnight (12-18 hrs) at 37^{o}C and then added to 200 mL LB liquid media containing 0.03 mg/l gentamycin and incubated at 37^{o}C until reaching mid-exponential growth phase.

2.3. Bacterial Separation Methods

2.3.1. Centrifugation

Cells were separated from the growth media using a centrifuge device (Fisher accuspin * 3R centrifuge). In the standard case the cell suspension was centrifuged for 15 min at 3689 g (Swing Bucket Rotor 7500 -4339) to separate the cells from the growth media. The medium was decanted and the pellet was resuspended in a 10 mM KCl solution by a vortex (Auto Touch Mixer Model 231, Fisher) for about 1 minute. To ensure all traces of growth medium were removed, the process of centrifuging, decanting, and resuspending in the electrolyte solution was repeated twice more, so that the cells were subjected to a total of 45 minutes of centrifugation. During the centrifugation process, the temperature was maintained at 4^{o}C. The electrolyte solution used during this rinsing process was prepared with deionized water and reagent-grade KCl (Fisher Scientific) with no pH adjustment (pH 5.6-5.8). To better elucidate the effect of centrifugation on bacterial surface, the same procedure as discussed above was used, although the centrifugation time was 10 min instead of 15 min. In this case, the total time of centrifugation was 30 min. To study the impact of centrifugation speed, the cell suspension was spun at a higher speed (14000 g) for 15 min following the same rinsing protocol.

2.3.2. Filtration

Cells were also separated from the LB media by filtering through a 0.45μm membrane (Fisher Scientific, Pittsburgh, PA) and back-washing with 30 mL of electrolyte solution (10 mM KCl). The back-washing procedure was repeated twice to remove any trace of the growth medium. To recapture cells retained on the membrane, the membrane was removed and placed into the centrifuge tube. Then 15 mL of KCl solution (10 mM) was added to the tube. The tube was vortexed a few seconds to remove the bacterial mass from the membrane. Completion of this cell separation method lasted about one hour because filtration was a slow process as cells blocked membrane pores and reduced the flow across the membrane.

2.4. Bacterial Characterization

2.4.1. Electrophoretic Mobility

The zeta potential of cells was determined as follows: Pellets were diluted in KCl electrolyte solution to a final concentration of 10^7-10^8 cells/mL. Electrolyte solutions were prepared with deionized water and KCl (3.16, 10, 31.6 and 100 mM) with no pH adjustment (5.6-5.8). Electrophoretic mobility measurements were conducted at 25^{o}C using a ZetaPALS analyzer (Brookhaven Instruments Cooperation, Holtsville, NY). Briefly, the micro electrophoresis chamber was filled with a bacterial suspension. This device calculates the zeta potential from measured electrophoretic mobility's using the Smoluchowski equation (Hiemenz et al., 1977). Zeta potential measurements were determined in triplicate for cells in the various ionic strength solutions and harvested with each cell separation method.

2.4.2. Hydrophobicity.

Cell surface hydrophobicity was quantified using the Microbial Adhesion To Hydrocarbon (MATH) test, following a procedure described by Walker et al. (2005) and Pembery et al., (1999). Briefly, 4 mL samples of a cell suspension (optical density of 0.2-0.25 in 10 mM KCl at 546nm) (Perkin Elmer UV/VIS spectrophotometer, Irvine, CA) were transferred to individual test tubes, each of which contained 1 mL of n-dodecane (laboratory grade, Fisher Scientific). The test tubes were vortexed at full speed for 2 min and then left to stand for 15 min to allow phase separation. Partitioning of the bacterial suspension was expressed as the percentage of cells adsorbed by the hydrocarbon phase. The mean percentage of partitioning of an organism into the n-dodecane phase was calculated by using triplicate samples.

2. 5. Column Experiments

Bacterial transport experiments were carried out in glass chromatography columns packed with ultra pure quartz grains. An adjustable bed height column (Omnifit USA, Toms River, NJ) which had a 1.5 cm inner diameter was wet packed by allowing the quartz grains to settle in deionized water while the column was agitated. Solutions were pumped (Model 200 syringe pump, KD Scientific Inc., New Hope PA) to the column at a constant flow rate of 1.26 mL/min. The approach (superficial) velocity (*U)* and the column length *(L)* in the column experiments were 0.66 cm/min and 10 cm, respectively. Porosity values ranged from 0.43-0.45 which was determined gravimetrically. Prior to each experiment, the packed column was equilibrated by flushing through

several pore volumes of deionized water followed by 6 pore volumes of the background electrolyte solution. The ionic strength of the electrolyte solution ranged from 30-100 mM KCl, the pH was between 5.6-5.8, and maintained at room temperature (22-25^{o}C). Bacterial cells (10^{7}-10^{8} cell/mL) harvested with a given separation method injected into the column for approximately 4 pore volumes (equivalent to 24 min) followed by background solution of the same ionic strength for an additional 5- 6 pore volumes. The cells were detected in the effluent using a spectrophotometer (Perkin Elmer UV/VIS spectrophotometer, Irvine, CA) at a wave length of 240 nm.

2.6. Numerical Modeling.

The HYDRUS-1D code (Simunek et al., 2005) is a finite element model for simulating the one-dimensional movement of water, heat, and multiple solutes in variably saturated media. The code numerically solves the Richards' equation for saturated-unsaturated water flow and advection-dispersion equations for the nonlinear equilibrium and kinetic reactions between solutes (here bacteria cells) and porous media. The model parameters can be obtained by optimization of the breakthrough curve and/or deposition profile information to experimental data. The transport of bacteria through the sand column is described using the one-dimensional form of the advection-dispersion equation that accounts for bacteria deposition in the column:

$$\frac{\partial C}{\partial t} = D\,\frac{\partial^2 C}{\partial z^2} - v\,\frac{\partial C}{\partial z} - r_d \qquad (1)$$

where C is the number of bacteria per unit volume of the aqueous phase (NL^{-3}), D is the dispersion coefficient (L^2T^{-1}), v is the average pore water velocity (LT^{-1}), z (L) is the vertical direction, t (T) is time, and r_d is the deposition rate of bacteria ($NL^{-3}T^{-1}$) given by:

$$\frac{\rho_b}{n}\frac{\partial S_d}{\partial t} = r_d = k_d \psi_s C \qquad (2)$$

Where ρ_b is the soil bulk density (ML^{-3}), n is the porosity (-), k_d is the bacterial deposition rate coefficient (T^{-1}), ψ_s is a dimensionless deposition function for deposited bacteries (-), and S_d is the concentration of deposited bacteria in the column (N_cM^{-1}).

A simple and flexible form for ψ_s is used in the model to account for time-dependent deposition behavior according to the Langmuirian blocking approach (Adamcyzk, 1994) as:

$$\psi_s = \left(1 - \frac{S_d}{S_{\max}}\right) \qquad (3)$$

Where, S_{max} is the maximum concentration of deposited bacteries ($N_c M^{-1}$). When the value of S_{max} is large, then this term approaches a value of 1 and time dependent deposition behavior becomes irrelevant.

3. Results and Discussion

3.1. Bacterial Deposition

The effects of the various cell harvesting methods on the transport and deposition of *E.coli*.D21g in porous media was studied by conducting a series of column experiments at several solution ionic strengths (30, 50, and 100 mM). The transport experiments were repeated in duplicate at each condition examined. Figures 1-4 present representative measured and simulated bacteria breakthrough curves (BTC) obtained from the column experiments. Figures 1, 2, 3, and 4 depict the breakthrough data for cell harvested using high-speed centrifugation (14000 g for 15 min), 15 min centrifugation (3689 g), 10 min centrifugation (3689 g), and filtration, respectively. In these figures the normalized effluent concentration (C/C_0) is plotted versus the number of pore volumes passed through the column. After about 1 pore volume, the introduced bacteria break through the column and are detected in the effluent. Subsequently, the normalized effluent concentration gradually increases toward steady-state conditions, except for the IS=100 mM experiments. After about four pore volumes, the influent was switched to a bacteria-free solution with the same ionic strength for an additional 5-6 pore volume. The breakthrough concentrations decreased after about one pore volume from the end of cell suspension injection.

No significant BTC tailing was observed in Figures 1-4, which indicates that release or detachment occurring was negligible in these experiments. For a given cell harvesting method, increasing the IS resulted in lower peak effluent concentrations and greater mass retention of the bacteria in the column as expected due to compression of electrostatic double layer (EDL) (Redman et al., 2004).

To quantitatively compare the results breakthrough data the solutions of the equations (1) and (2) (simulated using the HYDRUS-1D model) were fitted to the measured data by optimizing the model parameters. As it is observed, application of the advection-dispersion equation that accounts for first-order kinetic bacteria deposition in the column produced a satisfactory fit to all the breakthrough data. The values of linear regression (R^2), which is a measure of the goodness of model fit were always greater than 0.95. The values of k_d and S_{max} which are presented in Table 1 can also be used for comparing the effect of each harvesting method and IS on the deposition behavior of the bacteria. Table 1 also presents the bacteria mass fractions that were recovered in the effluent for all the experiments (M_{eff}).

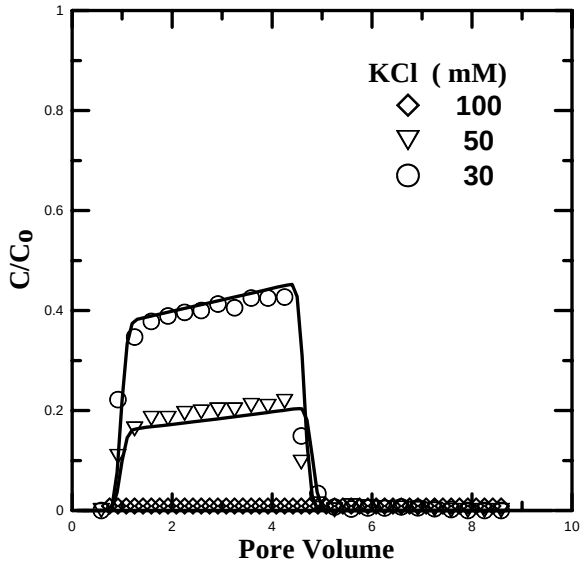

Figure1. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mM) as a function of pore volumes. The cell separation method consisted of applying 15 min of high-speed centrifugation at 14000 g (repeated three times). Key experimental conditions were as follows: approach velocity,v = 0. 66 cm/min, porosity, = 0.43, pH =5.5-5.6 and temperature= 20-22 °C.

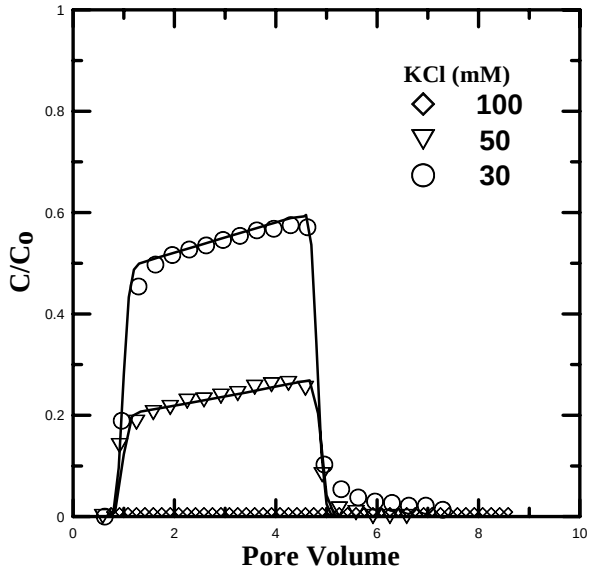

Figure2. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mM) as a function of pore volumes. The cell separation method consisted of applying 15 min centrifugation at 3689 g (repeated three times). Key experimental conditions were as follows: approach velocity,v = 0. 66 cm/min, porosity, = 0.43, pH =5.5-5.6 and temperature= 20-22 °C.

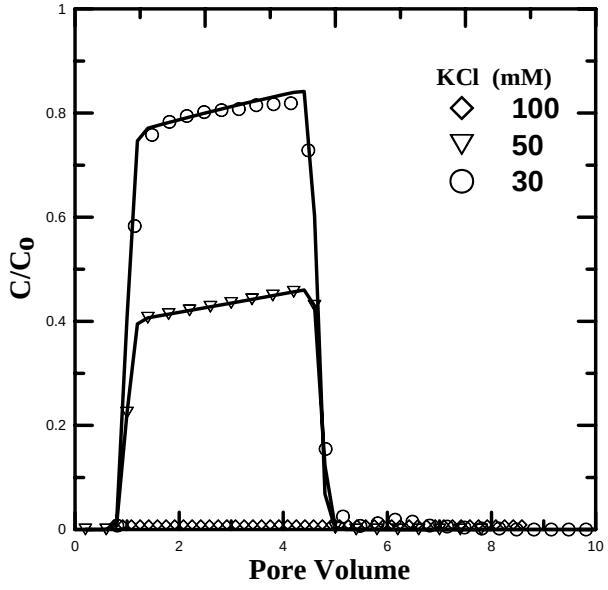

Figure3. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mM) as a function of pore volumes. The cell separation method consisted of applying 10 min centrifugation at 3689 g (repeated three times). Key experimental conditions were as follows: approach velocity,v = 0. 66 cm/min, porosity, = 0.43, pH =5.5-5.6 and temperature= 20-22 °C.

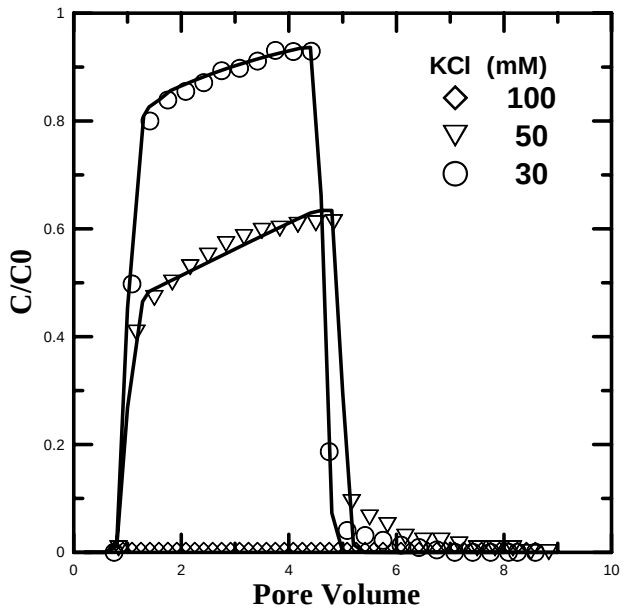

Figure4. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mM) as a function of pore volumes. The cell separation method consisted of filtration. Key experimental conditions were as follows: approach velocity,v = 0. 66 cm/min, porosity, = 0.43, pH =5.5-5.6 and temperature= 20-22 °C.

Table1. Fitted model parameters,and M_{eff} obtained using different separation methods. The parameter $S_{max}^{*}= S_{max}/N_{ic}$; where N_{ic} is the number of colloids in a unit volume of influent suspension.

Cell Preparation method	IS (mM)	k_d (min^{-1})	S_{max}^{*} (gr^{-1})	M_{eff}
High-speed	30	0.16±0.08	4± 0.2	38.5
		0.19±0.04	3.7±0.5	33.3
	50	0.31± 0.05	7± 0.9	17.4
		0.42±0.06	7.4±0.7	13.3
	100	1. 0± 0.09	-	<1
		0.95±0.08	-	<1
15 min	30	0.121± 0.07	1.7± 0.06	59.6
		0.09±0.02	1.4±0.03	64.7
	50	0.29± 0.01	3.9± 0.7	27.4

		0.256±0.01	4.1±0.3	23.8
	100	0.904± 0.13	-	<1
		0.83±0.002	-	<1
10 min	30	0.04± 0.001	0.5± 0.02	71.3
		0.07±0.07	0.4±0.06	74.6
	50	0.15± 0.02	3.6± 0.08	43.7
		0.19±0.08	3.2±0.01	34.9
	100	0.85± 0.1	-	<2
		0.9±0.02	-	<2
Filtration	30	0.03± 0.01	0.16± 0.03	84.3
		0.03±0.01	0.12±0.09	79.6
	50	0.13± 0.05	1.1± 0.06	59.5
		0.11±0.04	0.9±0.02	65.3
	100	0.83± 0.01	-	<2
		0.89±0.05	-	<2

For a given IS, filtered cells always resulted in the highest breakthrough levels than the experiments conducted with centrifuged cells. As a result, filtered cells also showed the lowest deposition rate coefficients and the highest values of M_{eff} (Table 1). In the experiments conducted with cells harvested by centrifugation the following trends can be observed at each solution IS. The lowest M_{eff} and higher values of k_d and S_{max} were obtained for the high-speed centrifugation, followed by low speed centrifugation for 15 min, and then low speed centrifugation for 10 min. Decreasing the centrifugation speed and duration produced higher effluent concentrations and lower values of k_d and $S_{max.}$ It should be noted that the influence of cell harvesting method on deposition behavior was most pronounced at lower ionic strengths.

3.2. Electrophoretic Mobility.

The average zeta potentials of cells, harvested with the different separation methods at different solution ionic strengths are presented in Table 2. For all cell regardless of harvesting method zeta potential became less negatively charged with increasing in IS as expected due to compression of the electrostatic double-layer (Ryan and Elimelech, 1996). Cells with the least negative zeta potential were those prepared by centrifugation, while the most negative zeta potential was found for filtered cells. Cells became less negatively charged as the speed and duration of centrifugation increased (Table 2). These results are consistent with the trend of cell retention in the column experiments.

The zeta potential measurements are in agreement with previous studies in which it was demonstrated that centrifugation can alter the surface charge of bacteria. For example, Pembery et al. (1999) reported that harvesting by high-speed centrifugation (15000 g) generally reduced the net surface charge of *E.coli*. This effect was attributed to removal of materials from the cell surface and thus generating a new and very different micro-environment interface. It is hypothesized herein that centrifugation causes some negatively charged macromolecules such as LPS or EPS to be removed from (leave exposed surface) or folded on the cell surface due to the shear forces during centrifugation. The presence of macromolecules like LPS, and trace amount of EPS on the outer membrane, generate the net negative environment on the cells (Walker et al., 2004). For cells prepared by the filtration method, the cells are more negatively charged presumably because of the presence a great amount of intact macromolecules (e.g., LPS).

3.3. Hydrophobicity

E.coli has previously been reported to be hydrophilic (Noda and Kanemasa, 1986). However our results show that this parameter is also sensitive to preparation methods. Results for the MATH test, which was used to determine cell surface hydrophobicity, in 10 mM KCl solution for the various cell separation methods are as follows: 25.9±0.39 for the 15 min high-speed centrifugation at 14000 g, 31.6±2.34 for the 15 min centrifugation at 3689 g, 45.4±1.27 for the 10 min centrifugation at 3689 g, and 52.87±0.83 for the filtration. Cells harvested by filtration were observed to be more

hydrophobic (i.e. greater partitioning into dodecane) than the cells harvested by centrifugation. Cell hydrophobicity also depended on the centrifugation speed and duration. Cell hydrophobicity tended to increase with decreasing speed and duration of centrifugation. In overall, our results show that centrifugation may cause alterations in the hydrophobicity of the cell. This implies that, an increase in the length of time and speed which cells are exposed to centrifugation cause the cell to become more hydrophilic, probably due to changes in conformation of loosely bound surface macromolecules as discussed in section 3.2.

Table 2. Zeta potential (mV) measurements of *E.Coli.* D21g at different IS and separation methods (High-speed, 15 min, 10 min centrifugation, and Filtration).

IS(mM)	Sand	High- Speed	15 min	10 min	filtration
3.16	-30.8	-46.3±1.4	-51.3±1.8	-69.0±0.4	-86.5 ±0.6
10	-22.2	-41.6±0.5	-45.9±4.1	-57.0±1.6	-66.4±1.5
31.6	-13.6	-29.1±1.6	-35.7±1.7	-49.8±1.8	-51.4±0.2
100	-11.6	-19.1±0.6	-21.0±1.6	-22.0±1.0	-24.4±1.1

Note: Zeta potential measurements of sand were estimated from Redman et al. (2005)

The roll of cell hydrophobicity in cell attachment has been recognized in previous research (Noda and Kanemasa, 1986; Van Loosdrecht et al. 1987b). It is reported that there is a positive relation between the bacterial hydrophobicity of some strains and their adhesion to negatively charged substrate. Despite of this fact, Gilbert et al. (1991) refined the general hypotheses by van Loosdrecht et al. (1987a) and arrived at an important conclusion that for relatively hydrophilic organisms, such as *E. coli*, the hydrophobicity does not have a dominant effect on adhesion relative to the charge properties. Scholl et al. (1990) and Scholl and Harvey (1992) also concluded that for relatively hydrophilic organisms, the major factor controlling the initial adhesion of bacteria is the surface charge of the minerals in the aquifer. Therefore, based on these findings and support from the literature, the role of hydrophobicity on cell retention in the column experiments is likely to be negligible and the observed retention behavior is attributed to the surface charge of the cells and quartz.

4. Implications of Bacteria Preparation Methods on Transport and Retention

As noted previously *E.coli* is a Gram negative bacterium with an outer membrane wall containing membrane-bound protein, LPS and trace amounts of EPS. It is well documented that the net surface charge of *E. coli* in natural aquatic systems is negative (Foppen and Schijven, 2006). The negative charge of *E.coli* D21g originates from the exposed surface molecules which are primarily LPS (Coughlin et al.2002, Gmeiner et al., 1980). LPS contains two parts. The first part includes a lipid containing three fatty acids and a Glycerol, and the second part or core region contains polysaccharide attached to lipid A by KDO (Ketodeoxyoctonate) (Rietschel et al., 1994). The second part of LPS is responsible for the negative charge of D21g due to the presence of three additional phosphate groups in the core region (Coughlin et al.2002, Gmeiner et al., 1980). These functional groups can easily ionize and provide the net negative charge on the membrane surface under the different solution chemistry conditions (Walker et al., 2005).

Experimental evidence in this study demonstrates that cell preparation protocols have a significant impact on cell surface properties. Our results show that the time and speed of centrifugation cause the cells to become less negatively charged, leading to more retention in the porous media. This conclusion is drawn from the results obtained from column experiments and numerical modeling which indicate an increase in k_d and S_{max} as the time and speed of centrifugation increased (Table 1). If the separation methods did not have an effect on cell surface charge, the column breakthrough curves should have been the same in the experiments conducted under similar solution IS. The zeta potential measurements also demonstrate the effect of centrifugation on the cell charge characteristic (Table 2).

The impact of centrifugation on the cell surface properties has also been observed by Gilbert et al. (1991) and Pembery et al. (1999). Gilbert et al. (1991) showed that harvesting cells by centrifugation subjects cells to huge centrifugal forces, which in centrifuge tubes produces high hydrostatic pressures. Moreover, the cells are subject to high shear stresses as they are propelled within the solution during the centrifugation. It is hypothesized herein that when cells are exposed to centrifugal forces, some macromolecules on the cell surface are removed from or folded on the surface due to this stress.

It is worth noting that under all solution conditions and preparation methods examined, both the bacterial cells and the quartz grains have a net negative zeta potential. Therefore, repulsive electrostatic interactions should inhibit cell deposition on the sand surface and continue to be the dominant interaction mechanism involved in cell attachment. However, a clear trend of increasing

deposition with increasing IS, time and speed of centrifugation is observed (Figure 3). To gain further insight into the mechanism responsible for deposition, DLVO theory (Derjaguin, and Landau, 1941; Verwey and Overbeek, 1948) was used to calculate the total interaction energy as a bacterial cell approaches a quartz grain. The total interaction energy, that is, the sum of attractive van der Waals and repulsive electrostatic interactions, was calculated by modeling the bacteria-quartz grain system with a sphere-plate interaction. Repulsive electrostatic double layer interaction energies were determined using the constant surface potential interaction expression of Hogg et al. (1966) with zeta potentials utilized in place of surface potentials. The retarded van der Waals attractive interaction energy was calculated using the formula given by Gregory (1981). A value of 6.5×10^{-21} J was used for the Hamaker constant (Simoni et al., 2000).

As listed in Table 3 for solutions with ionic strength less than or equal to 50 mM, DLVO calculations predict the presence of a substantial repulsive energy barrier to bacterial deposition ranging from 120 kT at 50 mM for the high-speed centrifugation, to over 400 kT at 10 mM for the filtration method. Because surface chemical heterogeneities are probably negligible for the highly pure quartz sand, the huge energy barriers suggest that it is unlikely that the bacterial cells will deposit in the primary energy minimum at the quartz surface. However, at solution ionic strength of 100 mM, the interaction energy calculations indicate no energy barrier to deposition, suggesting that cells, regardless of harvesting method, may be depositing in a primary minimum. Calculations of the total interaction energy predict the presence of a secondary energy minimum at a greater separation distance than that of the energy barrier. Therefore, bacteria approaching a quartz grain would first experience an attractive force before encountering the significant repulsive energy barrier. Therefore, cells unable to overcome the energy barrier may remain associated with the quartz grain within the secondary energy minimum unless they had sufficient diffusive or hydrodynamic forces to escape (Hahn and O'Melia, 2004). Table 3 shows that the magnitude of the secondary energy minimum increases with ionic strength and speed and duration of centrifugation. In particular, the depth of the secondary minimum ranges from 0.07 kT at 10 mM for the filtration method, to 12 kT at 50 mM for high-speed centrifugation (Table 3).

As indicated in Table 3, sizable energy barriers exist to inhibit bacterial deposition at most of the examined ionic strengths. However, experimental evidence shows a clear trend of the deposition rate increasing with ionic strength and time and speed of centrifugation. The increasing k_d and S_{max} parallels with the increase in calculated secondary energy minimum depths with increasing ionic

strength and time and speed of centrifugation. It is therefore possible that bacterial deposition is not occurring in the primary energy minimum at the grain surface, but is influenced by the secondary energy minimum. Indeed, the significant contribution of the secondary energy minimum to the retention of colloidal particles in saturated porous media is now established (Tufenkji and Elimelech, 2004 and 2005; Redman et al., 2004; Franchi and O'Melia, 2003; Hahn and O'Melia, 2004; Torkzaban et al., 2007). Therefore, it is proposed that as a result of centrifugation bacteria cells become less negatively charged and this results in an increase in the magnitude of the secondary energy minimum interactions. Consequently, centrifuged cells are deposited in porous media to a greater extent than those harvested by filtration.

Table 3. Total interaction energy parameters (secondary minima, energy barrier, and the separation distance) obtained from DLVO calculations assuming planar quartz surfaces and spherical bacteria cells.

Cell preparation Method	IS (mM)	secondary minima (k_bT_k)	energy barrier (k_bT_k)	Distance (nm)
High	3.16	-0.203	745	56
	10	-0.845	359	25.5
	31.6	-3.99	67.4	9.5
	100*	NB	NB	0.5
15 min	3.16	-0.199	798	57
	10	-0.583	385	35.5
	31.6	-3.69	88.6	10
	100*	NB	NB	0.5
10 min	3.16	-0.187	902	60
	10	-0.778	430	27
	31.6	-3.29	118.47	11
	100*	NB	NB	0.5
Filtration	3.16	-0.178	946	62
	10	-0.749	451.51	27.5
	31.6	-3.25	120	11.5
	100*	NB	NB	0.5

NB: There are no energy barriers and secondary energy minimum in this IS and the DLVO theory predicts the existence of a primary minimum

Conclusion

The effects of cell preparation methods on bacteria surface characteristics and transport and fate were studied. Multiple-step centrifugation increased the adhesion of cells to sand, presumably due to decreasing the repulsive force between the bacteria and sand. Cells isolated with high–speed centrifugation had the most adhesive behavior than that of the other methods. The enhancement of cell adhesion caused by centrifugation could be attributed to changes in LPS conformation on the cell surface due to shear forces during centrifugation. The filtration method appeared to have no and/or the least impact on changes of cell surface characteristics.

The results of this systematic study illustrate that protocols for the preparation and harvesting of microbial cells, used for experiments or cell analysis, will have a significant impact on surface characteristics and deposition behavior. Hence, it is essential that the effects of cell preparation protocols on microbes be ascertained in every investigation pertaining to laboratory experiments and that protocols remain consistent between comparable experiments. This may guarantee that cells are subjected to the least disruptive preparation methods and the results reflect the nature of the true microbial cell surface as logically as possible.

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Chapter IV

Summery and conclusions

The research presented in this thesis was mainly aimed to explore the fundamental mechanisms involved in the transport and fate of bacteria in saturated porous media.

This thesis contains of three chapters. Chapter 1 is a literature review of fate and transport of bacteria in porous media and the investigations which have been done in this field. This chapter has focused on several important factors affecting transport and retention of bacteria in porous media. These factors have been summarized in three terms including chemical, biological, and physical factors. Chemical factors include (1) ionic strength, (2) pH, (3) organic matter and, (4) hydrophobicity. Biological factors, list (1) growth and survival of bacteria, (2) cell size, (3) cell concentration, (4) motility (5) bacterial surface characteristics , and (6) flagella. The last factors, namely physical factors, include (1) physical straining which is related to grain size, shape and cell size, and clogging (2) flow velocity (3) temperature, and (4) size exclusion.

The objective of chapter 2 was to explore a couple effects, hydrodynamic situation and chemical condition of porous media, in retention of bacteria. In this chapter, the fate and transport of *E.coli* D21g was investigated in column and batch experiments and under unfavorable attachment condition. Our results showed that the retention of bacteria in the soil column increased as ionic strength of solution increased and when the chemical conditions were favorable for attachment. The results of DLVO calculation and batch experiments indicated a sizable energy barrier to attachment when the ionic strength was less than or equal to 50 mM. Experimental evidence in this study has demonstrated that solution chemistry and bacteria attachment are not the only factors and mechanisms responsible for bacteria retention. In particular, the data indicated that pore space geometry also played an important role in bacteria retention. Therefore, we conclude that the pore structure plays an important role in deposition of bacteria. This process is interrelated with solution chemistry.

The last chapter, namely chapter 3, described the effect of cell preparation methods on *E.coli* D21g and its attachment behaviors to grain surface. In this chapter, we compared two separation methods, centrifugation versus filtration. The retention of these cells was quantified by fitting a first-order deposition rate coeffecient (k_d) and the maximum deposited concentration (S_{max}) to the collected effluent concentration curves. Our results demonstrated that the rate of the deposition of *E.*

coli D21g increased with increasing speed and duration of centrifugation, and was lowest in the case of filtered cells. Moreover, the results show that the deviation between the cell preparation methods is more pronounced in lower ionic strength solutions.

Further development in understanding of bacterial transport processes in subsurface porous media under various conditions will require extensive research efforts, both at the basic and practical levels. Progress in this respect can only be made by additional studies from laboratory experiments conducted under carefully controlled chemical and physical conditions.