Coupled effects on the transport and deposition of biocolloidal particles in saturated porous media

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Environmental Engineering

Department of Civil and Environmental Engineering

University of Alberta

Abstract

The transport and deposition of biocolloidal particles in porous media is a crucial phenomenon in understanding environmental challenges from pathogen contamination to bioremediation of soil and water. The fate and transport of biocolloidal particles in porous media is influenced by many factors ranging from the bulk solution condition (e.g. ionic strength and oxygen tension), microbial particle's surface property (e.g. surface charge and extracellular polymeric substances (EPS)) to porous media property (e.g. surface charge). This dissertation is focused on exploring the role anaerobic conditions have on microbial cell transport in packed bed columns and the mechanisms involved in the transport process. In addition, this dissertation evaluated the filtration of a recently developed surrogate for pathogenic *Cryptosporidium* and elucidated the mechanisms affecting its removal.

The body of work was constructed in three individual parts.

In the first part, experiments were performed in glass bead packed bed columns under strictly anaerobic conditions to examine the effects of solution ionic strength (1, 10 and 100 mM) on the transport of three microbial species (*Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*) in an enriched anaerobic trichloroethene (TCE) dechlorinating mixed culture. Experimental results interestingly indicated that regardless of the microorganisms' distinct surface properties, they showed similar travel ability with normalized breakthrough concentrations (C/C_0) of 0.95, 0.84, and 0.56 at 1, 10 and 100 mM, respectively. The microorganisms also exhibited almost equal sensitivity to solution ionic strength changes, possibly due to interactions and the heterogeneity among different

microbial species in the culture. When using this culture for in situ bioremediation of TCE contaminated sites, success can be readily achieved when the microbial species in the culture travel in a similar fashion.

In the second part, experiments were performed in glass bead packed bed columns under aerobic and strictly anaerobic conditions to examine the effects of solution ionic strength (1, 10 and 100 mM) and oxygen tension on the fate of the facultative *Pseudomonas aeruginosa*. Experimental results indicated that growth under aerobic or anaerobic conditions altered the surface properties of *P. aeruginosa* through bacterial surface substrates (e.g. 1.19 and 1.23 times higher protein and polysaccharides content under anaerobic conditions than under aerobic conditions). This significantly influenced the transport and deposition of *P. aeruginosa* in the saturated porous media as lower normalized breakthrough concentrations were observed under aerobic conditions (C/C_0 = 0.89, 0.61 and 0.44 at 1, 10 and 100 mM, respectively) as compared to anaerobic conditions (C/C_0 = 0.90, 0.74, 0.54 at 1, 10 and 100 mM, respectively). When predicting the travel trajectory of *P. aeruginosa* through natural aquifers, practitioners need to be aware that deposition rates obtained under aerobic conditions are not the same under anaerobic conditions. As such, research exploring the transport ability of a broad range of species under anaerobic conditions needs to be further developed.

In the third part, experiments were performed in quartz sand packed bed columns under aerobic conditions to examine how a *Cryptosporidium* surrogate (glycoprotein modified microspheres) transport is affected by variables such as flow velocity, alum addition and humic acid (HA) adsorption. Experimental results showed increased normalized breakthrough concentrations of the glycoprotein modified microspheres with

increased flow rate (C/C_0 of 0.006, 0.017 and 0.030 at 4.6, 10.9 and 21.8 m/day, respectively), possibly due to increased hydrodynamic forces. Addition of 5 mg/L alum significantly increased the removal of the modified microspheres (C/C_0 of 0.003, 0.003 and 0.010 at 4.6, 10.9 and 21.8 m/day, respectively), while the adsorption of 1 mg/L HA on the sand collector induced the opposite effect (C/C_0 of 0.062 and 0.015 as compared to 0.017 and 0.003 at 10.9 m/day). The combined effects of alum and HA for *Cryptosporidium* surrogate removal seemingly eliminated the effects of alum in filtration. The utilization of this surrogate can satisfy the need for studying the filtration of *Cryposporidium* as their surface properties and structures resembled well to those of the viable *Cryptosporidium*, while eliminating personnel health hazards.

Preface

This thesis is an original work by Huixin Zhang under the supervision of Dr. Yang Liu and Dr. Ania Ulrich. Chapter 3 of this thesis has been published as Zhang, H., Ulrich, A.C., Liu, Y., 2015. Retention and transport of an anaerobic trichloroethene dechlorinating microbial culture in anaerobic porous media. Colloids and Surfaces B: Biointerfaces, 130, 110-118.

The surrogates used in Chapter 5 of this thesis were prepared in the lab of Dr. Ravin Narain in the Department of Chemical and Materials Engineering at University of Alberta.

Acknowledgement

Completing the PhD program is probably the most challenging part of my life in the past years. It has been a great privilege to study and research in the Department of Civil and Environmental Engineering at University of Alberta. I would like to take this opportunity to thank all the persons who helped me making it possible to complete my PhD program.

First and foremost, I am extremely grateful to my supervisors, Dr. Yang Liu and Dr. Ania Ulrich, for their continuous support, motivation, and patience throughout my research. They have been great mentors for me, and they are great examples to follow. I would not have come to today without their invaluable guidance, inspiration and suggestion. It truly was one of the greatest experiences in my life. Words cannot express my gratefulness for their contribution to my personal and professional growth.

I would like to thank Dr. Hongbo Zeng in the Department of Chemical and Materials Engineering for the measurement of zeta potential. Kind collaboration of Dr. Ravin Narain for preparing the surrogates used in Chapter 5 is appreciated. In addition, I thank a number of technicians and research assistants for their advice and support: Elena Dlusskaya, Emily Cao, Jela Burkas, Chen Liang, and Maria Demeter in the Department of Civil and Environmental Engineering at University of Alberta. Thank you to all my colleagues from both labs. I would especially like to thank Lisa, Xiaohui, Zhiya, Jiaming, Weibing, Jeff, Yinan, Christina, Melissa, Simmon and Dena for helping and teaching me everything.

I also would like to thank Dr. Ian Buchanan at the Department of Civil and Environmental Engineering and Dr. Ravin Narain at the Department of Chemical and Materials Engineering from University of Alberta for serving as my examination committee members and their valuable suggestions on my dissertation. Special thanks to Dr. Gordon Chua at the Department of Biological Sciences from University of Calgary for being the external examiner for my final defense and his valuable comments on my work.

I would like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the Helmholtz Alberta Initiative (HAI) and EPCOR Water Services Inc. for providing generous funding for my research.

I have had great support from many friends and family through the years. Last and most importantly, I would like to thank my parents and my sister, for their steadfast encouragement and unconditional love over the years. I can never fully express how lucky I feel to have them in my life.

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List of Symbols and Abbreviation

α	Collision efficiency
ε_0	Dielectric permittivity in a vacuum
ε_r	Relative dielectric permittivity of water
ζ	Zeta potential
η	Single collector efficiency
κ	Inverse Debye length
λ	Characteristic wavelength of the dielectric
ρ_b	Porous medium bulk density
ψ_c	Surface potential of collector
ψ_p	Surface potential of particle
a_p	Colloidal particle radius
A	Hamaker constant
C/C_0	Normalized column breakthrough concentration
f	Porosity
h	Separation distance from approaching particle to collector surface
k_d	Deposition rate coefficient
L	Length of column
S(X)	Particle deposition concentration in column bed
t_0	Duration of continuous particle injection
U	Approach (superficial) velocity
V_A	Energy of van der Waals attraction
V_R	Energy of electrical double layer interaction
V_T	Total interaction energy
X	Column depth
AGW	Artificial Groundwater
AGP	Alpha-1-acid Glycoprotein
ANOVA	Analysis of Variance
CFT	Colloid Filtration Theory
CFU	Colony Forming Units

CV	Coefficient Variation
DCE	Dichloroethene
DGGE	Denaturing Gradient Gel Electrophoresis
DLVO	Derjaguin-Landau-Verwey-Overbeek
DNA	Deoxyribonucleic Acid
DNAPL	Dense nonaqueous phase liquid
DO	Dissolved Oxygen
EB	Energy Barrier
EDL	Electrical Double Layer
EPS	Extracellular Polymeric Substances
HA	Humic Acid
HC	Hydrocarbon
IP	Isoelectric Point
IS	Ionic Strength
LB	Luria-Bertani
LPS	Lipopolysaccharides
NOM	Natural Organic Matter
OTU	Operational Taxonomic Unit
PAH	Polycyclic Aromatic Hydrocarbons
PCE	Tetrachloroethene
PM	Primary Minimum
PR	Protein
PS	Polysaccharides
qPCR	Quantitative Polymerase Chain Reaction
SEM	Scanning Electron Microscopy
SM	Secondary Minimum
TCE	Trichloroethene
TOC	Total Organic Carbon
UV	Ultraviolet
VC	Vinyl Chloride
XPS	X-ray Photoelectron Spectroscopy

Chapter 1. Introduction

1.1 The transport of colloidal particles in porous media

Clean drinking water is one of the most pressing global environmental and health problems of our time. About 30% of fresh water comes from groundwater (Gleick 1993), therefore, groundwater contamination often leads to serious drinking water outbreaks. For example, an outbreak of *Cryptosporidium* in Brush Creek, Texas, USA occurred when untreated groundwater was consumed, which affected 1,300 to 1,500 people in July 1998 (Bergmire-Sweat et al. 1999). In May 2000, an outbreak of *Escherichia coli* O157:H7 occurred in a groundwater supply in Walkerton, Ontario, Canada, which led to seven deaths and over two thousand local residents became ill (Mclaughlin 2000). It was estimated that this outbreak in Walkerton caused them an economic impact of around $155 million including fixing the water supply system, lost business revenues, and spending on household and corporate bottled water and disinfection equipment, according to a CBC News report (2010). But these are just a few examples. From 1971 to 2006, 54% of reported drinking water outbreaks were caused by the consumption of untreated groundwater (31%) or deficiencies in groundwater treatment (23%) (Craun et al. 2010). These groundwater outbreaks were commonly caused by the transport of parasites (*Cryptosporidium sp.*, *Giardia intestinalis*), bacteria (*E. coli* O157:H7, *Salmonella spp.*) and viruses (norovirus, Hepatitis A) to the water supply systems (CDC 2013, Craun et al. 2010).

Chemical contamination of groundwater systems through inappropriate waste disposal is also a large contributor to the problem. Residential consumption of trichloroethene contaminated water in Woburn, Massachusetts has led to an unusually high incidence of childhood leukemia and other illnesses during the mid to late 1970s (Costas et al. 2002).

Even today, trichloroethene is still listed as a possible human carcinogen by the US Environmental Protection Agency (EPA). In situ bioremediation is generally accepted as the least expensive remediation option based on a review performed by McDade et al. in 2005. They reported that bioremediation had the lowest cost per treatment volume of $\$29/yd^3$, as compared to thermal ($\$88/yd^3$), chemical oxidation ($\$125/yd^3$) and surfactant/cosolvent ($\$385/yd^3$) when treating dense nonaqueous phase liquid (DNAPL) sites (McDade et al. 2005). To improve bioremediation efforts an understanding of microbial transport in subsurface environments will only help.

As outlined above, an understanding of the transport and deposition behaviors of colloidal particles in saturated porous media is of considerable interest in natural and engineered systems. A thorough grasp of particle filtration is essential for predicting the fate and transport of microbial particles, such as bacteria, viruses and protozoa in a subsurface environment (Liu et al. 2007, Tufenkji and Elimelech 2004a, Tufenkji et al. 2002). Particle transport and retention is also the basis of the granular filtration process in water and wastewater treatment (Yao et al. 1971).

Transport of colloidal particles from the bulk fluid to the vicinity of a filter grain is typically governed by three mechanisms: interception, gravity sedimentation, and Brownian diffusion. Interception occurs when a particle moving along the flow streamline comes into contact with the collector grain due to its finite size. Gravity sedimentation refers to the settling of particles that are heavier than the surrounding fluid onto the collector surface. Brownian diffusion happens when particle movement is caused due to the random bombardment of molecules in the solution. These mechanisms were

described by Yao et al. in 1971 when they presented a conceptual model for water and wastewater filtration processes (Yao et al. 1971).

Yao et al. (1971) developed the classic colloid filtration theory, which predicts that particle concentration in the fluid phase is represented by first order kinetics with a spatially and temporally constant colloid deposition rate coefficient. Based on this, the concentration of suspended and retained particles in porous media is considered to decrease exponentially with travel distance. Yao's theory, can determine the deposition rate coefficient from the fraction of colloids recovered from the effluent of packed bed columns or aquifers and can evaluate the deposited particle concentrations in the packed bed column (Tufenkji and Elimelech 2004a, Tufenkji et al. 2003).

However, a growing number of studies have demonstrated deviations between retained particle profiles in packed bed column experimental data and the log-linear decrease pattern as anticipated from Yao's theory, thus, implying that Yao's theory failed to adequately predict transport behaviors under certain conditions. More recently, the predicted log-linear exponential decrease, the hyperexponential and power law decreases became the most commonly observed patterns (Tong and Johnson 2007, Tufenkji et al. 2003). Studies have revealed that various factors can cause the observed discrepancy, including but not limited to heterogeneity in the microbial population and on collector surfaces, straining, block and release of previously attached particles, and unfavorable conditions for deposition calculated from the classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Baygents et al. 1998, Bradford et al. 2002, Silliman et al. 2001, Tufenkji et al. 2003).

For efficient removal or deposition, particles transported to the collector surfaces in porous media should have effective contacts with the collector grains through irreversible initial attachment, i.e. have a favorable condition for deposition. In order to explain the effects of electrolyte concentrations on the initial adhesion of bacterial cells onto solid surfaces in aquatic systems, the DLVO theory has been used as a qualitative model, but also in quantitative ways to calculate adhesion free energy changes involved in initial microbial adhesion. The use of DLVO theory for explaining bacterial adhesion was first suggested by Marshall et al. (1971). Specifically, DLVO theory states that the total interaction energies existing between a colloidal particle and 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. However, it fails to yield a universal application (Hermansson 1999).

As discussed above, the interactions between particles and collectors in the porous media were found to be affected by various factors. Therefore, the factors affecting the transport and deposition behaviors of particles in porous media can be roughly divided into these three components: (1) For biotic and abiotic particles, their size, shape, motility, concentration, surface properties (e.g. zeta potential and hydrophobicity) and surface structures (e.g. lipopolysaccharides and extracellular polymeric substances) (Camesano and Logan 1998, Liu et al. 2007, Seymour et al. 2013, Walker et al. 2004) can lead to distinct transport ability; (2) Change of solution chemistry (ionic strength, chemical composition) and bulk flow rate can also change the retention of particles in packed bed columns (Camesano and Logan 1998, Chen and Walker 2012); and (3) Lastly, different porous media characteristics, such as grain size, surface roughness, and saturation of

porous media also differentiate the transport of particles from others (Bradford et al. 2007, Chen et al. 2010a, Chen and Walker 2012). Aside from all the work previously reported, understanding microbial transport is still an essential task because of the varying nature of particles in different systems. For example, changes in the environment, such as bulk solution ionic strength, dissolved oxygen concentration, and bulk flow rate may cause different travel behaviors for the same colloidal particles. This becomes further complicated when looking at different colloidal particles. Our understanding of transport and deposition of colloidal particles in these environments needs to be elucidated.

The research described in this dissertation will help close this gap in understanding. As such, this thesis primarily focused on evaluating the fate and transport of different microorganisms under anaerobic conditions and their response to changes in solution ionic strength. Anaerobic conditions of natural aquifers are rarely considered in past research yet are commonly encountered when microorganisms travel in the subsurface. Considering the subsurface redox environment requires special attention since bacteria may respond differently to various dissolved oxygen concentrations and alter their travel habits. To elucidate this effect an anaerobic dechlorinating culture containing mixed microbial species and a pathogenic facultative *Pseudomonas aeruginosa* were utilized to respectively represent colloidal particle transport during in situ bioremediation of trichloroethene and pathogenic transport in a natural aquifer. In addition, this dissertation evaluated the filtration of glycoprotein modified microspheres as surrogates for *Cryptosporidium* under various effects.

1.2 Objectives

The overall objective of this body of work was to evaluate the transport and deposition of microbial particles under various coupled conditions (redox condition, solution ionic strength, etc.) that broadly represent natural and engineered environments, and elucidate the DLVO interactions involved in the transport process. Three individual projects were constructed and the specific objectives, research questions, hypothesis, and the novelty of this work follow:

1. Explored the transport of an enriched trichloroethene (TCE) dechlorinating culture under strictly anaerobic conditions and compared the motility of *Dehalococcloids, Geobacter,* and *Methanomethylovorans* under different ionic strengths.

   Research questions: (1) In what way will the three microbial species in the mixed microbial consortium transport and deposit in anaerobic porous media? (2) Are they equally sensitive to changes of solution ionic strength?

   We hypothesized that the three species would have different travel abilities in the packed bed columns under the same condition, because of their different cell surface zeta potentials. Cells with high zeta potentials (absolute values) are prone to travel longer distance and deposit less on collector surfaces (Chen and Walker 2012, McEldowney and Fletcher 1986). In addition, we hypothesized that increasing solution ionic strengths would lead to more cell deposition in the columns because higher ionic strengths have been shown to retard the transport (Chen and Walker 2012). For **the first time**, the transport of a mixed, strictly anaerobic TCE degrading culture in a flow-through porous media was assessed.

2. Compared the fate and transport of facultative *P. aeruginosa* under aerobic and anaerobic conditions and explored the effects of aerobic and anaerobic growth, together with ionic strength on the mobility of *P. aeruginoas* in packed bed columns.

   Research questions: (1) How will *P. aeruginosa* transport in porous media under aerobic and anaerobic conditions? (2) Does *P. aeruginosa* respond the same to changes in solution ionic strength under aerobic and anaerobic conditions? (3) What differences will be induced when *P. aeruginosa* grows under different oxygen tension?

   We hypothesized that growth of *P. aeruginosa* under aerobic and anaerobic conditions would introduce different surface properties of *P. aeruginosa.* It has been shown that *P. aeruginosa* grown under anaerobic conditions reduces the formation of the B-band polysaccharides and adheres less on hydrophilic glass surfaces (Sabra et al. 2003, Schobert and Jahn 2010, Singh et al. 2000). The reduced ability to adhere was hypothesized to increase the transport of *P. aeruginosa* in glass bead packed columns under anaerobic conditions as compared to aerobic conditions. And higher ionic strengths were hypothesized to reduce the transport (Chen and Walker 2012). For **the first time**, the role of oxygen tension on the transport of *P. aeruginoas* in a packed bed column was assessed.

3. Evaluated the glycoprotein coated microspheres as surrogates for the filtration of viable *Cryptosporidium* under conditions broadly encountered during water

treatment processes by exploring the effects of flow rate, alum addition and humic acid (HA) adsorption on quartz sand filter.

Research questions: (1) How well will the modified microsphere represent viable *Cryptosporidium*? (2) What are the effects of increased flow rate, addition of alum, and adsorption of humic acid on collector surfaces on the filtration of the surrogates in sand?

We hypothesized that modifying microspheres with glycoprotein would provide fine surrogates for viable *Cyptosporidium* that could be used in filtration studies because the glycoprotein had a similar isoelectric point value as that of the *Cyptosporidium* (Pang et al. 2012). By doing the filtration studies, we hoped to achieve the best removal rate when operated at a relatively low flow rate and in the presence of alum with no adsorption of humic acid on the collector surfaces, i.e. under favorable conditions (mainly hydrodynamic and chemical) for deposition (Dai and Hozalski 2003, Kim et al. 2010). The glycoprotein modified microspheres were characterized and utilized for **the first time** to examine the removal through a sand bed under coupled factors mimicking the filtration in water treatment process.

1.3 Organization of thesis

This thesis includes six chapters, as follows:

Chapter 1 provides a brief introduction to the research topic. **Chapter 2** offers a description of the theoretical background and reviews the relevant literature on the transport and deposition of colloidal particles in porous media, and provides a

background introduction to the particles used in this work. **Chapter 3** concentrates on evaluating the transport and retention of an anaerobic dechlorinating mixed culture in anaerobic porous media under the impact of solution ionic strength**. Chapter 4** focuses on comparing the transport and deposition of *P. aeruginosa* under aerobic and anaerobic conditions. **Chapter 5** investigates the filtration of glycoprotein coated microspheres as surrogates for Cryptosporidium under conditions encountered during water treatment processes. **Chapter 6** summarizes the conclusions from this thesis, and provides recommendations for further investigation.

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Chapter 2. Theoretical Background and Literature Review

2.1 Theoretical background

2.1.1 Transport phenomena of microbial particles in porous media

The transport and retention of microbial particles in porous media have drawn increasing attention from researchers due to its critical impact in a variety of environmental contexts, such as bioremediation in contaminated soil and groundwater, pathogen transport in groundwater environments, and granular filtration processes for water and wastewater treatment (Costerton et al. 1995, Parsek and Fuqua 2004).

The transport processes of these microbial particles in the subsurface are often controlled by several mechanisms. Among these, physical mechanisms of interception, gravitational sedimentation, Brownian diffusion, straining, hydrodynamic retardation, and surface interaction forces, often affect the transport and attachment of microbial particles (Gollnitz et al. 1997, Tufenkji et al. 2002). Grazing of these microbial particles by microorganisms at higher trophic levels can also influence their transport processes (Gollnitz et al. 1997). Finally, the addition of chemicals can affect the surface chemical interactions, hydration, and hydrophobic interactions, which result in the attachment of biocolloidal particles to the grain surface (Yao et al. 1971). Worth noting, detachment of loosely attached microbial particles from sediment grains may occur during the transport process. However, this is not a dominant process because the detachment rate is much smaller than the deposition rate (Tufenkji et al. 2002).

Among the physical mechanisms described above, interception, gravitational sedimentation, and Brownian diffusion often influence the transfer of particles from the bulk solution to the collector surfaces (Crittenden et al. 2012). These three mechanisms

were used when Yao et al. (1971) developed the colloid filtration theory as described in Section 2.1.2. In interception, a suspended particle of virtually the same size and trajectory of the collector grain may come into contact with the collector surface when flowing past it (Yao et al. 1971). In gravitational sedimentation, particles denser than water are dragged by the combination of fluid and gravitational forces, and settle onto the collector grain (Yao et al. 1971). The transport of larger than 1 μm particles, such as a protozoa, are influenced mostly by sedimentation and (or) interception, as shown in Figure 2.1 (Yao et al. 1971). Higher removal efficiency for larger particles can be achieved under these conditions (Yao et al. 1971). In contrast, Brownian diffusion is the dominant mechanism for bringing microbial particles smaller than 1 μm, such as viruses, in contact with collector grains (Yao et al. 1971). Here particles in suspension are brought to the collector surface due to random bombardment by molecules in the suspending medium. Higher removal efficiency is achieved with smaller particles under these conditions (Yao et al. 1971). It should be noted, that microbial particles with sizes in the order of 1 μm, like bacteria, experience the least number of collisions with the collector grains, and therefore have the ability to travel the farthest distance in porous media (Yao et al. 1971). Other mechanisms controlling the transport processes will be discussed in Section 2.1.3.4.

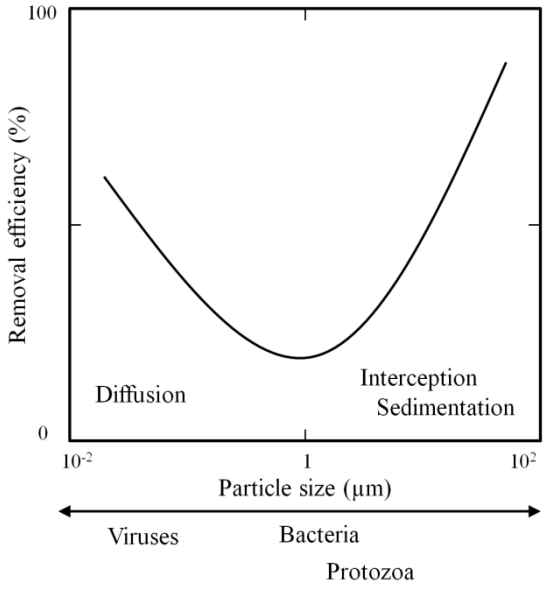

Figure 2.1 Schematic representation of removal efficiency to particle size distribution (adapted from Yao et al. 1971).

2.1.2 Classic colloid filtration theory (CFT)

2.1.2.1 What is CFT?

The colloid filtration theory (CFT) developed by Yao et al. (1971) is the most commonly used model for describing particle filtration. The removal of particles is controlled by the mass transfer of suspended particles from the bulk flow to the grain surface or particles previously attached and the attachment of particles to this solid surface as a result of colloid-surface interaction. Based on this approach, the performance of a packed bed for particle removal is expressed as a single collector efficiency η, and a collision (or attachment) efficiency α, as described by the equation (Yao et al. 1971):

$$\frac{dC}{dL} = -\frac{3}{2}\frac{(1-f)}{d}\alpha\eta C \qquad (2.1)$$

where C is the local particle concentration, f is the bed porosity, L is bed depth, d is grain diameter, α and η are represented as follow (Crittenden et al. 2012):

$$\eta = \frac{\text{particles contacting collector}}{\text{particles approaching collector}} \tag{2.2}$$

$$\alpha = \frac{\text{particles adhering to collector}}{\text{particles contacting collector}} \tag{2.3}$$

The parameter η represents the frequency at which the particles flowing toward the collector by diffusion, interception and sedimentation would strike the collector grain. And it depends on many factors such as filtration velocity, grain size, water temperature, and particle size and density (Yao et al. 1971). While α represents the probability that such collisions occurring between suspended particles and filter media would result in adhesion. α is equal to one in an ideal system where the suspended particles are completely destabilized (Yao et al. 1971).

Integration of Equation 2.1 yields the basis for Yao's filtration model, as follows (Yao et al. 1971):

$$\ln\frac{C}{C_0} = -\frac{3}{2}(1-f)\alpha\eta\left(\frac{L}{d}\right) \tag{2.4}$$

where C and C_0 are the effluent and influent concentrations for a packed bed.

As shown by Equation 2.4, in Yao's filtration model, the removal of suspended particles is presented by first-order kinetics with a spatially and temporally invariant colloid deposition rate coefficient. Therefore, the concentrations of suspended and retained particles are predicted to decrease exponentially with travel distance (Liu et al. 2007). Based on this assumption, the portion of particles recovered from the packed

column effluent is often used to estimate the physicochemical filtration rate, i.e. the overall deposition rate coefficient (k_d) as shown in the following equation (Tufenkji and Elimelech 2004a).

$$k_d = -\frac{U}{fL}\ln\left(\frac{C}{C_0}\right) \tag{2.5}$$

where U is the approach (superficial) velocity, L is the length of the column, and C/C_0 is the normalized breakthrough concentration relevant to "clean bed" conditions. The retained concentrations of particle S(X) in the column bed for a continuous injection of particles can be determined by the following equation (Liu et al. 2007, Tufenkji et al. 2003).

$$S(X) = \frac{t_0 f k_d C_0}{\rho_b}\exp\left(-\frac{k_d X f}{U}\right) \tag{2.6}$$

Here, X is column depth, ρ_b is the porous medium bulk density, and t_0 is the duration of continuous particle injection.

2.1.2.2 Deviations from CFT prediction

Yao's theory has been widely recognized for predicting particle filtration in packed bed columns. However, recent research suggests that the filtration of microbial particles may not be consistent with the filtration model, since experimental data from laboratory-scale column experiments has shown evidence of nonexponential decay in the deposition profiles. In the majority of the column experiments, deposition rates were found to decline with travel distance in a monotonic fashion (Bradford and Bettahar 2005, Bradford et al. 2005, Bradford et al. 2002, Kim and Tobiason 2004, Liu et al. 2007). Other patterns of nonexponential deposition, such as a power law distribution and

hyperexponential were also reported (Tong et al. 2005, Tufenkji et al. 2003). Table 2.1 summarizes several studies involving colloidal particles where a clear nonexponential deposition was observed.

Table 2.1 Review of several laboratory column studies with nonexponential deposition of colloidal particles (adapted from Bradford and Bettahar 2005, Tufenkji et al. 2003).

Colloidal Particle	**Sediment Grain**	**Column Length (cm)**	**Solution Chemistry**	**Observed Deposition Pattern**	**Ref.**
Carboxyl latex colloids	(a) Ottawa sand (b) glass bead	(a) 13 (b) 10	IS 1 mM, NaCl, , pH 6.98	monotonic	(Bradford et al. 2002)
Cryptosporidium parvum oocysts	Ottawa sand	13	IS 1 mM, NaCl, pH 6.98	monotonic	(Bradford and Bettahar 2005)
recombinant Norwalk virus	quartz sand	29	IS 10 mM, NaCl, pH 6.9,	power law (i.e. linear on a log-log plot, R^2 = 0.95, 0.99)	(Redman et al. 2001a)
motile, Gram-negative bacteria designated A1264	borosilicate glass	1, 2	MOPS[b] buffer, pH 7.7	power law (R^2 = 0.97)	(Albinger et al. 1994)
bacteria A1264 and nonmotile Gram-negative bacteria designated CD1	borosilicate glass	1	IS 10 mM, MOPS buffer, pH 7.0	power law (R^2 = 0.99)	(Baygents et al. 1998)
Pseudomonas fluorescens	Southern AZ soil	7	IS 4 mM, AGW[a]	power law pattern in	(Camesano and

P17 and *Burkholderia cepacia* G4				several experiments (R^2 = 0.83 - 0.99)	Logan 1998)
Pseudomonas fluorescens P17	quartz sand	1 - 10.5	(a) IS 3.6 mM, AGW[d], pH 8.0 (b) IS 60 mM, $CaCl_2$, pH 6.8	(a) power law (R^2 = 0.98) at low IS (b) exponential (R^2 = 0.90) at high IS	(Martin et al. 1996)
nonmotile Gram-negative bacteria designated W31	Oyster, VA sediment	15, 43, 45	AGW[c], pH 6.2	nonexponential in longer columns	(Bolster et al. 1999)
Comamonas DA001	quartz sand	20	IS 3.8, 20, and 50 mM, NaCl, pH 6.72	hyperexponential	(Tong et al. 2005)

[a] artificial groundwater (AGW) solution prepared as described by Camesano and Logan (1998).

[b] 3-[*N*-morpholino] propanesulfonic buffer.

[c] AGW consists of (per liter of DI water): 0.06g $MgSO_4 \cdot 7H_2O$, 0.036 g $NaHCO_3$, 0.036 g $CaCl_2$, 0.035 g $Ca(NO_3)_2$, 0.025 g $CaSO_4 \cdot 2H_2O$, and 0.02 g KNO_3.

[d] AGW consists of (per liter of DI water): 0.069 g $MgSO_4 \cdot 7H_2O$, 0.050 g $NaHCO_3$, 0.00145 g $CaCl_2 \cdot 2H_2O$, 0.064 g $Ca(NO_3)_2 \cdot 4H_2O$, and 0.002 g KF.

To explain the observed experimental deviation from CFT's theoretical prediction, various studies have been performed. The factors that may explain this deviation include heterogeneity in the microbial population (Baygents et al. 1998, Simoni et al. 1998) and on the collector surface (Silliman et al. 2001), straining in some cases (Bradford et al. 2002) and unfavorable conditions for deposition (Tufenkji and Elimelech 2004b). Some of the asymmetric distributions of the deposition rate coefficient reflecting the inherent heterogeneity of the microbial particles were found to give rise to nonexponential deposition patterns (Baygents et al. 1998). Specifically, Baygents et al. (1998) observed reduced affinity of bacteria for the glass beads with distance travelled through the columns due to intropopulational differences in the surface charge density of isolated ellipsoidal bacteria under conditions of the transport experiment. They believed that these variations in surface charge density could influence the colloidal interaction potentials, and the affinity of bacteria for negatively charged collectors (Baygents et al. 1998). Distributions in the surface properties among the microbial particles likely contributed to the deviation of deposition in the column. Individual microbial particles more prone to adhesion deposited near the column inlet and those less prone to adhesion travelled further then deposited, if at all (Tong et al. 2005). It should be noted that system conditions, such as porous media also affect the level of variations in deposition rate coefficient from CFT. When conducting transport experiments of *Pseudomonas fluorescens* strain M1 in a tank filled with heterogeneous sand media, Silliman et al. (2001) found great spatial variability in the attached bacteria with a particularly high number of bacteria cells retained in the fine-grained sand sediments. In addition, particle

deposition dynamics (blocking) and release of microbial particles from collector grains were considered as potential sources of deviations from CFT (Tufenkji et al. 2003).

2.1.3 Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloid stability

2.1.3.1 What is the DLVO theory?

The use of the DLVO theory to explain the effects of electrolyte concentration on initial bacterial adhesion was first reported by Marshall et al. when they interpreted the initial sorption of two marine bacteria on glass surface (Marshall et al. 1971). It has since been used by many as a qualitative approach, but in some cases as a quantitative way to calculate the adhesion free energy changes to explain microbial adhesion (Hermansson 1999). Microbial adhesion refers to the transfer of a cell from a free state in the bulk solution to a more or less firm attached state at an interface (Hermansson 1999). Briefly, the DLVO theory (Derjaguin and Landau 1941, Verwey 1948) is assumed to be additive and describes the combined interaction energies of van der Waals attraction and electrical double layer (EDL) repulsion between particles or particle-substratum (a flat surface) as a function of separation distance (h) as shown in Figure 2.2 (a). The total interaction, which is usually expressed as potential energy (V_T), is calculated as the sum of van der Waals attraction (V_A) and the EDL interaction (V_R) (Hermansson 1999):

$$V_T = V_A + V_R \tag{2.7}$$

V_R can be calculated using the following expression (Hogg et al. 1966):

$$V_R = \pi\varepsilon_0\varepsilon_r a_p \left\{2\psi_p\psi_c \ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + \left(\psi_p^2 + \psi_c^2\right)\ln[1-\exp(-2\kappa h)]\right\} \tag{2.8}$$

where ε_0 is the dielectric permittivity in a vacuum, ε_r is the relative dielectric permittivity of water, a_p is the colloidal particle radius, κ is the inverse Debye length, h is the separation distance between the colloidal particle and the collector surface, and ψ_p and ψ_c are the surface potentials of the colloidal particle and collector, respectively. In this expression, the surface potentials (ψ_p and ψ_c) are assumed to be constant (Redman et al. 2004).

V_A can be calculated based on the following equation (Elimelech and Omelia 1990, Gregory 1981):

$$V_A = -\frac{Aa_p}{6h}\left[1 + \frac{14h}{\lambda}\right]^{-1} \quad (2.9)$$

where A represents the Hamaker constant of the interacting media (colloidal particles – water – collector), and λ is the characteristic wavelength of the dielectric (usually assumed to be 100 nm).

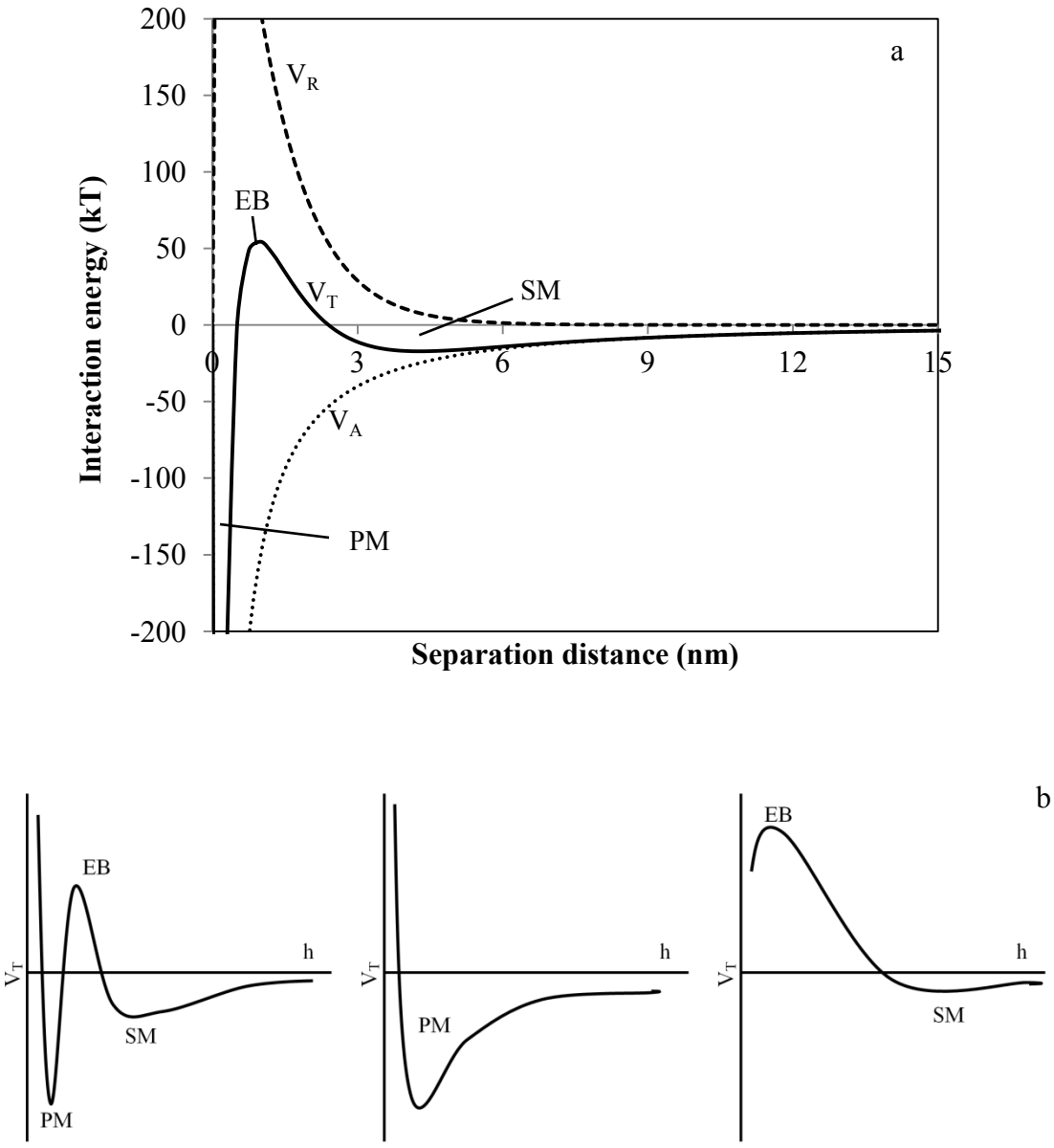

Figure 2.2 Schematic representations of total DLVO interaction energy profiles as a function of separation distance: (a) quantitative example for equal spherical particles of 1 μm diameter in 100 mM NaCl electrolyte, zeta potential (ζ) = -25 mV, A = 8.3×10^{-21} J; and (b) three patterns of the interaction energy profiles (EB: energy barrier; PM: primary minimum; SM: secondary minimum; h: separation distance; V_T: total potential energy; V_R: electrical double layer interaction; V_A: van der Waals attraction) (adapted from

Elimelech 1998).

2.1.3.2 Representations of the DLVO interaction energy profiles

Depending on the relative contributions of the two interactions, the energy profiles with separation distance can display one of three patterns as shown in Figure 2.2 (b) (Elimelech 1998):

(1) An infinitely deep minimum, the primary minimum (PM), at close separation distance, followed by a maximum, the energy barrier (EB), and another minimum, the secondary minimum (SM), at a larger distance;
(2) A primary minimum only;
(3) A primary minimum, and an energy barrier, but no significant secondary minimum.

Because of the different distance dependence of V_A (power law) and V_R (exponential) (Equations 2.8 and 2.9), V_A is usually greater than V_R at larger separation distances, which forms the secondary energy minimum as shown in Figure 2.2 (Elimelech 1998). The effects of secondary energy minima are considered to be more obvious on larger particles (greater than 1 μm in diameter), and at moderately high solution ionic strength, under which interacting particles are closer and facing greater attraction due to the reduced range of electrical repulsion (Elimelech 1998). Previous studies showed that when solution ionic strength increased from 1 mM to 31.6 mM, the depth of the secondary energy minima increased from 0.09 kT to 8.1 kT, resulting in more bacterial cell deposition (Redman et al. 2004). However, it should be noted that the deposition in the secondary minimum is reversible (Redman et al. 2004).

Additionally, the sole presence of a primary energy minimum is physically impossible in practice, because the short-range effects, such as those caused by ion hydration, can keep particles from coming into true physical contact (Elimelech 1998).

2.1.3.3 Factors affecting the interaction energies

Since the DLVO theory explores the effects of electrolyte concentrations on initial adhesion, the total interaction energies change with solution ionic strengths and zeta potentials. Zeta potential refers to the charge at the shear plane of particles, and is typically used as a relative measure of particle surface charge. Empirically, when the absolute value of a particle's zeta potential is below 20 mV, the particles in suspension are prone to aggregate (Crittenden et al. 2012). When the zeta potential and electrolyte ionic strength is at such that the repulsion outweighs the attraction, particles approaching the substratum encounters an energy barrier, which tends to inhibit the contact between the particle and substratum (Elimelech 1998). In the presence of an energy barrier, colliding particles must overcome this barrier in order to come into true contact. In Brownian diffusion, particles rarely have energy of more than a few of kT (unit of the interaction energy); therefore an energy barrier of 20 kT or more usually represents a very stable system, where only a minimum fraction of particles have sufficient energy to overcome the barrier (Elimelech 1998). As the zeta potential is reduced and/or ionic strength is increased, the energy barrier gets lower making contact easier. Eventually, the energy barrier disappears, leading to favorable conditions for adhesion, i.e. attractive interaction in primary minimum, under which condition, in principle, particles can adhere each time when they collide (i.e. $\alpha = 1$). Such adhesion is most likely irreversible (Elimelech 1998).

In addition to solution ionic strength and zeta potential, the interaction profiles are also affected by the following physicochemical parameters (Elimelech 1998, Hermansson 1999).

(1) *Particle size and surface structures/molecules*. Adhesion is facilitated by the reduction of the contacting region (Weiss and Harlos 1972). Decreasing the radius of the contact region generally reduces both V_R and V_A, and consequently the total interaction energy, or conversely as discussed in the previous section (Weiss and Harlos 1972). Polymers on the surface of particles trapped in the secondary minimum well can also contribute to adhesion through bridging. Surface structures, such as fimbriae, lipopolysaccharides (LPS), capsule material and flagella of bacteria are not accounted for in the DLVO theory (Hermansson 1999). However, their presence can alter the overall properties of bacterial cells, such as cell surface hydrophobicity, and surface charge. In such cases, the measured overall cell properties do not fully represent the adhesion ability of the cells (Hermansson 1999).

(2) *Substratum roughness*. In DLVO prediction, the surface of the collector was assumed to be perfectly smooth, which does not exist in practice. When two particles come into contact, the roughness may also affect the effective contacting area and restrict the minimum separation distance attainable (Elimelech 1998). If the rough layer is thick in comparison to the separation distance, the unretarded interaction can be substantially reduced due to attenuation of van der Waals interaction (Hermansson 1999).

(3) *The Hamaker constant (A)*. The Hamaker constant represents a material property that describes the strength of the interaction between a surface and the medium and the interaction between two interacting bodies in the medium, and determines the van der Waals interaction (Norde and Lyklema 1989). In aqueous media, A is usually positive for the substratum-media-colloidal particles, and varies between different substrata, media and colloidal particles (Norde and Lyklema 1989).

2.1.3.4 Non-DLVO interactions

Recently, DLVO theory was found unable to adequately describe biotic and abiotic particle adhesion. Several non-DLVO forces, such as steric repulsion, polymer bridging, hydrophobic interaction and hydration were found to contribute to the colloidal interactions (Elimelech 1998, Hermansson 1999). Polymers on a particle surface can either enhance the colloidal stability through the effect of steric repulsion, or promote aggregation of particles and/or attachment to macroscopic surfaces through polymer bridging, depending on the amount and chain-size of the surface polymers (Elimelech 1998). The presence of an adsorbed layer of solvent, surfactant, and macromolecules can sometimes influence the stability of colloidal suspension, through repulsive hydration interaction, and attractive hydrophobic interaction (Elimelech 1998).

2.2 Literature review

2.2.1 Factors affecting the transport and retention of colloidal particles

The transport and retention of particles in a natural environment is a complex process and can be affected by many factors. Therefore, studies evaluating the impacts of these

factors on the fate and transport of particles in porous media have been intensively explored using laboratory scale columns. These factors and their effects are discussed below.

(1) *Solution ionic strength and composition*. Solution ionic strength and composition are the most readily controlled conditions for transport experiments. Research has shown that electrolyte with higher ionic strength and/or composed of divalent ions rather than monovalent ions enhanced the aggregation or the deposition of suspended particles due to reduced absolute values of zeta potentials (Chen and Walker 2012, Lerner et al. 2012). A decreased zeta potential is usually caused by the compression of the electrical double layer, indicating a less stable colloidal suspension (Chen and Walker 2007, 2012, Saleh et al. 2008, Zhao et al. 2014).

(2) *Porous media saturation*. Chen and Walker (2012) compared deposition of two model fecal indicators *E. faecalis* and *E. coli* in saturated and unsaturated quartz sand bed and observed increased retention of the two bacterial species in unsaturated rather than saturated porous media. They further showed a preferred affinity of the *E. faecalis* at the air/water interface for attachment rather than the sand surface (Chen and Walker 2012). This accumulation at the air/water interface is likely controlled by a combined effect of the DLVO and hydrophobic interactions between approaching cells and air/water interface (Bradford and Torkzaban 2008, Schäfer et al. 1998). Other possible mechanisms such as film straining and air/water interfacial capture were also proposed to contribute to the increased retention in unsaturated porous media (Zhuang et al. 2005).

(3) *Colloidal particle size and shape*. As discussed in Section 2.1.1, the removal of a spherical particle is proportional to its size when it is larger than 1 μm and counter-proportional when it is smaller than 1 μm. However, in practice, perfect spheres are not always present, especially for bacteria, which are sometimes rod-shaped for example. When interpreting the transport of bacteria, normally it is assumed they are spheres. However, it has been shown that particles with higher aspect ratios in terms of shape are believed to exhibit preferential retention during the transport process since they minimize the interaction energy when approaching collector surfaces (Seymour et al. 2013, Wang et al. 2008, Weiss et al. 1995). Salerno et al. (2006) observed that rod-shaped particles with aspect ratios of 2:1 and 4:1 displayed higher retention than spherical particles with an aspect ratio of 1:1. Liu et al. (2010) also reported that rod-shaped particles displayed higher retention than spherical particles.

(4) *Flow velocity*. Higher flow velocity was generally reported to reduce retention and facilitate the transport of colloidal particles with an exception as discussed in point (5) (Camesano and Logan 1998, Kim et al. 2010). Hydrodynamically favorable conditions for retention generated in low velocity regions in porous media was believed to play a role in enhancing the motility of flow-through particles (Torkzaban et al. 2007, Torkzaban et al. 2008).

(5) *Motility of microbial cells*. When brought near collector surfaces, motile cells can swim along the surfaces at various orientations by either tracing out circles around the surfaces, or moving in parallel or perpendicular to the surfaces (Frymier et al. 1995, Vigeant and Ford 1997). Previous studies have shown that cell motility can

either increase or inhibit cell attachment (Jenneman et al. 1985, Korber et al. 1994). Camesano and Logan (1998) discovered that swimming cells were able to avoid adhering to a solid surface leading to reduced cell adhesion at lower flow velocity (0.56 m/day), which was opposite to the prediction of increased cell retention at decreased bulk fluid rate based on the colloid filtration theory (Camesano and Logan 1998) . At higher fluid velocities, cell motility corresponded to a clear reduction in cell adhesion due to increased hydrodynamic force. Therefore, the variation of deposition between motile and nonmotile cells was most obvious at low fluid velocities (Camesano and Logan 1998, McCaulou et al. 1995)

(6) *Natural organic matter (NOM)*. NOM usually refers to those naturally occurring molecules and macromolecules (i.e. anionic, organic) that are ubiquitous in water supplies. Its presence was broadly found to facilitate the transport of bacteria (Yang et al. 2012a, Zhao et al. 2014), nanoparticles (Wang et al. 2012, Xie et al. 2008), and protozoa such as *Cryptosporidium* (Abudalo et al. 2010). However, increased removal was also reported in rare occasion when *Cryptosporidium parvum* oocysts travelled through organic matter-coated silica surfaces under the combined effects of surface charge heterogeneity and presence of positively-charged area on the organic matter-coated surface (Liu et al. 2009). In general, the adsorption of negatively charge NOM on to a sand surface can either reversely alter the positively charged collector surfaces (Abudalo et al. 2010) or increase the negativity of similarly charged collectors (Dai and Hozalski 2003) and compete with approaching particles for effective site of deposition.

(7) *Extracellular polymeric substances (EPS)*. The EPS matrix generally consists of a mixture of macromolecules such as proteins, polysaccharides, nucleic acids, lipids, and other polymeric compounds. It usually functions as an initial adhesive, however the extent of this function was unclear because of the various compositions among different organisms (Hwang et al. 2012, Liu et al. 2007). Therefore, EPS can either promote (Liu et al. 2007) or hinder (Hwang et al. 2012) cell adhesion depending specifically on the components of the EPS and experimental systems.

(8) *Grain size and roughness*. In general, coarse sized collectors were reported to filter more particles than fine sized collectors (Bradford et al. 2007). In Yao's theory, the collector was assumed to be perfectly smooth spheres, which is hardly achievable in practice. The presence of uneven surfaces of the collector grains can provide valleys with lower interaction energy wells, which are favorable for attachment (Elimelech 1998, Hoek et al. 2003).

(9) *Bacterial cell type*. When comparing the transport behaviors of the Gram-positive *E. faecalis* and Gram-negative *E. coli* in quartz sand bed, Chen and Walker (2012) observed much more reduced motility of the *E. faecalis* to increased solution ionic strength and decreased porous media saturation level. The distinct surface characteristics of the Gram-positive *E. faecalis* (i.e. much more negatively charged zeta potential and higher cell surface acidity) distinguished it from the Gram-negative *E. coli* in response to increased ionic strength and presence of air/water interface. Other surface properties including EPS content (protein and polysaccharides) and surface hydrophobicity that also

differed between Gram-positive and Gram-negative were also found to be involved in cell transport (Yang et al. 2012b, Zhao et al. 2014).

(10) *Colloidal particle concentration*. Although the clean bed theory predicts that cell retention in porous media is independent of cell concentration (Yao et al. 1971), high cell concentrations are known to affect the overall retention of cells either positively by providing more favorable surfaces for adhesion (ripening) or negatively by providing less favorable surfaces (blocking) (Camesano and Logan 1998, Camesano et al. 1999).

2.2.2 Introduction to the particles used in this dissertation

This section provided information about the microorganisms and the glycoprotein modified microspheres utilized in this dissertation.

2.2.2.1 The dechlorination culture – KB-1®

The KB-1® culture is a strictly anaerobic enrichment culture that is capable of complete degradation of tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE) and vinyl chloride (VC) to non-toxic ethene. It was mainly used for in situ dechlorination of chlorinated ethenes contaminated sites by injecting the enriched culture into the contaminated plume (http://www.siremlab.com/products/kb-1).

The culture was initially constructed with soil and groundwater samples obtained from a TCE-contaminated site in Southern Ontario, Canada, which contained high concentrations of TCE and daughter products including high levels of ethene in the groundwater. The coexistence of TCE and its daughter products suggested the in situ

presence of active organisms capable of complete dechlorination of TCE. Further incubation and enrichment with selective electron acceptors (e.g. TCE or VC) and electron donor (methanol) in the laboratory produced this stable culture exhibiting promising dechlorination ability (Duhamel et al. 2002).

The microbial composition of the enrichment culture was carefully explored using combined techniques of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and cloning to identify the phylotype in the culture, and quantitative PCR (qPCR) estimate their relative abundances based on the 16S rRNA sequence (Duhamel and Edwards 2006, Duhamel et al. 2004), as shown in Table 2.2.

Table 2.2 Operational taxonomic unit (OTU) detected in the KB-1® clone libraries (adapted from Duhamel and Edwards 2006)

Sequence name	**Closest GenBank match (as of 1st February, 2006)**	**Accession no.**	**% identity[a]**	**Number of clones**
Bacterial OTUs				
Bacteroidetes KB-1 (AY780552)	Uncultured bacterium clone 060B03_B_SD_P93 from a municipal anaerobic sludge digester	CR933316	99	2/37
	(closest isolate) *Bacteroides uniformis* strain S13	AB247146	81	
Dehalococcoides KB-1/VC (AY146779) and KB-1/PCE (AY146780)	*Dehalococcoides* strain CBDB1	AF230641	100	15/37[b]
	Dehalococcoides strain FL2	AF357918	100	
	Dehalococcoides strain BAV1	AF165308	100	
'Desulfo' KB-1 (AY780555)	Uncultured bacterium clone SJA-173 from a trichlorobenzene-degrading culture	AJ009503	99	1/37
	(closest isolates) *Desulfotomaculum Auripigmentum*	AJ493051	94	
	Desulfosporosinus sp. S8	AF076247	94	
Geobacter KB-1	*Geobacter lovleyi* strain SZ	AY914177	99	11/37

(AY780563)				
Spirochaetes KB-1 (AY780558)	Spirochaetes bacterium SA-8	AY695839	99	4/37
Sulfurospirillum KB-1 (AY780560)	Uncultured bacterium clone DS155 from mangrove bacterioplankton	DQ234237	99	1/37
	(closest isolate) *Sulfurospirillum multivorans*	X82931	98	
Syntrophobacter KB-1 (AY780561)	*Syntrophobacter sulfatireducens* strain TB8106	AY651787	96	1/37
Syntrophus KB-1 (AY780562)	Uncultured bacterium clone R4b9 from granular sludge	AF482443	99	2/37
	(closest isolates) *Syntrophus* sp. LYP	AF126282	91	
	Syntrophus gentianae	X85132	93	
Archaeal OTUs				
Methanosarcina KB-1 (AY780570)	Uncultured archaeon clone 221 from landfill leachate	AJ831161	99	12/20
	(closest isolate) *Methanosarcina barkeri* strain Sar	AF028692	99	
Methanomethylovorans KB-1 1	*Methanomethylovorans hollandica* strain	AY260433	100	1/20

(AY780564)	ZB			
Methanomethylovorans KB-1 2 (AY780565)	Uncultured archaeon clone SHB-143 from a 1,2-dichloropropanedechlorinating culture	AJ312013	99	4/20
	(closest isolate) *M. hollandica* strain ZB	AY260433	98	
Methanomicrobiales KB-1 1 (AY780566)	Uncultured archaeon clone CBd-364F from acidic peatlands	DQ301905	97	1/20
	(closest isolate) *Methanoculleus palmolei*	Y16382	91	
Methanomicrobiales KB-1 2 (AY780567)	Uncultured archaeon clone 244 from landfill leachate	AJ831168	99	1/20
	(closest isolate) Methanogenic archaeon NOBI-1	AB162774	94	
Methanosaeta KB-1 (AY780568)	Uncultured archaeon clone PL-39A4 from a biodegraded oil reservoir	AY570685	99	1/20
	(closest isolate) *Methanosaeta Concilii*	X51423	99	
[a] in the TCE-methanol culture [b] sum of Dehalococcoides clones. Because the clones were not sequenced in the region that differentiates between the two phylotypes.				

As shown, *Dehalococcoides* and *Geobacter* were the most abundant phylotypes in the enrichment culture. They are the TCE dechlorinators in the mixed culture. In terms of TCE dechlorination, *Geobacter* and several strains of *Dehalococcides* were reported to quickly degrade TCE to DCE, however further reduction was inhibited due to the inability to derive energy from DCE and VC (Duhamel and Edwards 2007). Only limited strains of *Dehalococcoides* were found to further degrade DCE and VC to nontoxic ethene (He et al. 2003b, Sung et al. 2006). Complete degradation of TCE to nontoxic ethene can be achieved most efficiently when *Geobacter* and *Dehalococcoides* are both present. Other nondechlorinating microorganisms are also believed to contribute to dechlorination as trace nutrient and electron donor providers (Duhamel and Edwards 2006). The dechlorination process cannot effectively proceed without the cooperation among these microorganisms. Based on this, *Dehalococcoides*, *Geobacter* and *Methanomethylovorans* were selected as the model microorganisms to track in this study.

Therefore, successful in situ biodegradation of chlorinated ethenes contaminated sites depends on equal or similar motility of the microorganisms in KB-1® to arrive at the contaminated plume together.

2.2.2.2 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a facultative Gram-negative bacterium that is noted for its environmental metabolic versatility, ability to cause disease in particular susceptible individuals, and its resistance to antibiotics. Being able to grow using a large variety of carbon sources under either aerobic or anaerobic conditions, *P. aeruginosa* can adapt to and colonize in various environmental niches such as soil and marine habitats, plants, and

animals (Palmer et al. 2007, Williams et al. 1978, Yoon et al. 2002). In humans, *P. aeruginosa* are predominant in chronic lung infections of cystic fibrosis patients (de Kievit and Iglewski 2000). *P. aeruginosa* can produce a number of toxic proteins which can cause extensive tissue damage, and interfere with the human immune system. These proteins include potent toxins that enter and kill host cells at or near the site of colonization and degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs (Pier et al. 1996).

In addition, though more widely recognized as an opportunistic pathogen in humans and animals, *P. aeruginosa*'s degradative abilities are apparent in soil (Alonso et al. 1999). Isolates of *P. aeruginosa* have shown an ability to degrade various petroleum products such as gasoline, kerosene, diesel oil, and crude oil, and pollutants such as n-alkanes and polycyclic aromatic hydrocarbons (PAH) despite their origins (clinic or environment) (Alonso et al. 1999, Karamalidis et al. 2010).

This versatility allows *P.aeruginosa* to exist and migrate in a wide range of environments. Therefore, understanding the transport and deposition of *P. aeruginosa* is important with respect to increasing knowledge of pathogenic transport, and in some cases, biodegradation of hydrocarbons.

When travelling in the subsurface, various dissolved oxygen (DO) concentrations are always encountered due to different geologic conditions (Castro and Tufenkji 2008). Different oxygen concentrations have been shown to affect the growth of *P. aeruginosa* and induce physiological change (Sabra et al. 2003). An anaerobic environment was reported to promote the production of the viscous exopolysaccharide alginate, and

introduce alterations in the outer membrane of *P. aeruginosa*, e.g., loss of O-specific antigens (previously known as B-band polysaccharides), which eventually influenced the adhesion of *P. aeruginosa* on various surfaces (Schobert and Jahn 2010, Singh et al. 2000).

Therefore, to evaluate the fate and transport of pathogenic *P. aeruginosa* in porous media, the role of oxygen tension requires equal attention as other factors, such as solution chemistry, and flow rate, etc.

2.2.2.3 Cryptosporidium and its surrogate

Cryptosporidium parvum is a disease-causing microbial contaminant frequently found in surface waters. It is usually released into the environment via feces of infected host organisms, posing great challenges in health risk management for drinking water supplies. Ingestion of a small number of viable oocysts can lead to cryptosporidiosis, a severe gastrointestinal disease that can prove fatal for immune-compromised individuals (Dai and Hozalski 2003). Outbreaks of cryptosporidiosis disease are often caused by the penetration of *C. parvum* oocysts through drinking water treatment systems (Gitis 2008). The *C. parvum* oocysts are unusually resistant to traditional disinfectants such as free chlorine and chloramines due to their thick cell walls (Finch et al. 1993). As a result, water utilities are showing increased interest in oocyst removal in porous media such as riverbank filtration, deep-bed (granular) filtration, and slow sand filtration to control drinking water quality (Gottinger et al. 2013, Huck et al. 2002, Tufenkji et al. 2004).

In consideration of *Cryptosporidium*'s impact on experimental personnel health, a growing number of studies, have been conducted using microspheres of similar size

instead of viable *Cryptosporidium* (Amburgey 2011, Amburgey et al. 2005, Chung 2012, Dai and Hozalski 2003, Emelko and Huck 2004, Harvey et al. 2009, Muhammad et al. 2008). However, the use of these types of microspheres can potentially neglect the impact of *Cryptosporidium* surface properties, such as zeta potential and surface structure, therefore rendering the microspheres useless in predicting the filtration removal efficiency satisfactorily. Even when the size and shape of microspheres are the same, the micro-heterogeneity on the surface of viable *Cryptosporidium* can also alter the adhesion behaviors (Pang et al. 2012).

Very recently, efforts have been made to explore a more suitable surrogate that can satisfy the need for closely mimicking viable *Cryptosporidium,* while eliminating personnel health hazards. Pang et al. modified the surfaces of oocyst-sized carboxylated polystyrene microspheres with biotin (free and containing amine, NH_2) and glycoprotein and compared their filtration in porous media (sand) with viable *Cryptosporidium* and unmodified microspheres, and found that the glycoprotein modified microspheres with similar zeta potential and size resembled the viable *Cryptosporidium* the best, indicating the significant role of surface protein, which is lacking on unmodified microspheres, in particle attachment to solid surfaces (Pang et al. 2012).

Therefore, the use of the glycoprotein modified microspheres can be a more cost-effective alternative for viable *Cryptosporidium* and unmodified microspheres for assessing *Cryptosporidium* filtration in porous media. These modified microspheres can be used for evaluating sand filter performance in water and wastewater treatment processes with sufficient validation in various environmental conditions.

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Chapter 3. Retention and Transport of an Anaerobic Trichloroethene Dechlorinating Microbial Culture in Anaerobic Porous Media*

*A version of this chapter has been published.

Zhang, H., Ulrich, A.C., and Liu, Y. (2015). Colloids and surfaces B: Biointerfaces, 130, 110-118.

3.1 Introduction

Chlorinated ethenes such as trichloroethene (TCE) and tetrachloroethene (PCE) are toxic environmental contaminants that are introduced into soil and groundwater systems through their extensive usage in industry (Lemming et al. 2010, Mundle et al. 2012). Remediation strategies mainly focus on biological and chemical transformations that replace chlorine atoms with hydrogen atoms, producing the more toxic incomplete degradation products dichloroethene (DCE) and vinyl chloride (VC), and eventually producing the nontoxic complete degradation product ethene (Duhamel et al. 2004, Friis et al. 2007, Mundle et al. 2012). Strains of the anaerobic *Dehalococcoides* species are currently the only bacteria that can completely degrade chlorinated ethenes to ethene (Duhamel and Edwards 2006, Haggblom and Bossert 2003, He et al. 2003a, He et al. 2003b, Loffler and Edwards 2006). Bioremediation of TCE using the *Dehalococcoides* bacteria has been shown to be a cost-effective way to decontaminate polluted sites (Duhamel and Edwards 2006, Friis et al. 2007, Lemming et al. 2010, Mundle et al. 2012).

The population of indigenous *Dehalococcoides* species in contaminated sites is generally low or absent. To successfully apply bioremediation strategies, bioaugmentation is required to introduce TCE degradation microorganisms to the contaminated subsurface (Camesano and Logan 1998). A major problem with the application of bioaugmentation is the limited transfer distance of bacteria injected into contaminated sites due to their strong adhesion to solid surfaces. Bacteria tend to remain localized at the injection well head, causing serious biofouling of injection wells and insufficient dispersion of degradative organisms. Consequently, the successful transport of degrading bacteria away from injection sites and into contaminated zones is the

determining element of in situ bioremediation (DeFlaun and Condee 1997). Reduced adhesion and enhanced transport of the bacterial strains can minimize bioclogging at the injection well head (Gross and Logan 1995, Redman et al. 2004). The distance TCE degradation microorganisms must travel to perform TCE degradation is an important factor in successful remediation of contaminated waters.

Previous studies on the retention and transport of bacteria showed that grain size and shape (Bradford et al. 2007, Syngouna and Chrysikopoulos 2011), saturation of porous media (Chen and Walker 2012, Schafer et al. 1998), chemical treatments (Gross and Logan 1995), solution ionic strength and composition (Chen and Walker 2012, Kim et al. 2009, Wang et al. 2011), natural organic matter (Foppen et al. 2008, Yang et al. 2012a), bacteria growth phase (Walker et al. 2005), bacterial cell type and concentration (Haznedaroglu et al. 2009, Yang et al. 2012b), cell motility (Camesano and Logan 1998, De Kerchove and Elimelech 2008), cell surface lipopolysaccharides (LPS) (Walker et al. 2004), extracellular polymeric substances (EPS) (Liu et al. 2007), and the presence of biofilm (Liu and Li 2008, Liu et al. 2008) played important roles in controlling bacterial transport behaviors. Research on the transport and adhesion behaviors of dechlorinators is limited (Camesano and Logan 1998, Chen et al. 2010b, Chen and Walker 2007, DeFlaun and Condee 1997, DeFlaun et al. 1999, Nelson et al. 1986, Salerno et al. 2007). Most studies have focused on aerobic and pure cultured conditions, and no study has evaluated the retention and transport of commonly used bioaugmentation cultures in anaerobic environments, which are representative of groundwater conditions; also many contaminated subsurface environments are under oxygen depletion conditions (He et al. 2003a).

Previous studies showed that the presence of oxygen significantly impacted bacterial adhesion. van Schie and Fletcher (van Schie and Fletcher 1999) studied the adhesion to coverslips of a hypothetical PCE- and TCE-dechlorinating consortium comprised of four strictly anaerobic pure cultures (*Desulfomonile tiedjei*, *Syntrophomonas wolfei*, *Syntrophobacter wolinii*, and *Desulfovibrio* sp. strain G11) under anaerobic and aerobic conditions by merging the coverslips in cell suspensions. They found that exposure to air reduced the adhesion ability of *S. wolfei* and *Desulfovibrio* sp. strain G11 but not the adhesion ability of *Desulfomonile tiedjei* and *S. wolinii*, thus it is possible that the influence of dissolved oxygen on bacterial retention is organism dependent (Castro and Tufenkji 2008). Their work also suggested that individual species exhibited different adhesion characteristics and abilities. Subsequent work from this group (Cutter et al. 2003) confirmed that the attachment of one bacterial species could considerably affect the adhesion of other bacteria species, either positively or negatively, and that the dechlorinator (*D. tiedjei*) had the strongest attachment when solid surfaces were exposed to the premixed community, demonstrating that interspecies interactions could affect bacterial adhesion properties. Their work provided insight into the community biofilm formation of dechlorinating bacteria in an anaerobic condition but the retention and transport of complex anaerobic microbial communities in porous media was not evaluated.

In the present study the retention and transport of the TCE-bioaugmentation culture KB-1®, which has been shown to be effective in TCE remediation (Peale et al. 2008, Scheutz et al. 2010), was investigated. The KB-1® culture includes more than fourteen defined species, of which eight are bacterial and six are archaeal (Duhamel and Edwards

2006, 2007, Duhamel et al. 2004). Of the three major functional microorganisms—*Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*—in KB-1®, *Geobacter* and several strains of *Dehalococcides* could degrade TCE to DCE, but were unable to derive energy from DCE and VC. Only limited strains of *Dehalococcoides* were found be able to further degrade DCE and VC to nontoxic ethene (He et al. 2003b, Sung et al. 2006). Without the presence of *Dehalococcoides* strains that could degrade DCE and VC, it is possible that complete TCE degradation would stop beyond DCE, leading to the accumulation of more toxic daughter products, DCE and VC (Duhamel et al. 2002). Therefore, complete biodegradation of TCE to nontoxic ethene requires the presence of both species. As the most frequently detected putative methanogen in methanol-amended cultures, *Methanomethylovorans* converts methanol to methane (Duhamel and Edwards 2006). Other microorganisms detected in the culture were *Spirochaetes* (identified to taxonomic class), *Sulfurospirillum*, *Syntrophobacter*, *Syntrophus*, and Methanomicrobiales (identified to taxonomic class). The abundant presence of these nondechlorinators are believed to contribute to dechlorination by generating electron donors and trace nutrients to dechlorinators (Duhamel and Edwards 2006).

Microbial cell retention of the three major functional microorganisms *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans* in KB-1® culture was evaluated in porous media in a well controlled anaerobic condition. Cell quantification was accomplished using a quantitative polymerase chain reaction (qPCR). Cell surface electrokinetic potentials were characterized to estimate the DLVO interaction forces between microorganisms and the collector surfaces. To our knowledge this is the first

study to investigate the retention and transport of a mixed TCE-bioaugmentation culture in porous media under strictly anaerobic conditions.

3.2 Materials and methods

3.2.1 Anaerobic microbial culture maintenance and preparation

A strictly anaerobic dechlorinating enrichment culture KB-1® (SiREM, Guelph, Ontario) was used in this study. The detailed microbial composition and characteristics of the culture are described elsewhere (Duhamel and Edwards 2006, 2007, Duhamel et al. 2004). The three major functional microorganisms, *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*, were chosen as the targeting microorganisms.

KB-1® was cultured in a sterile defined anaerobic mineral medium (Edwards and Grbicgalic 1994) statically in an anaerobic chamber (Coy Laboratory Products Inc., MI) and fed with TCE (150 μM aqueous concentration) and methanol (1.5 mM) every two weeks as described previously (Duhamel et al. 2002). Minor modifications were made when preparing the mineral medium by reducing the KH_2PO_4 concentration to 20.96 g/L and increasing the K_2HPO_4 concentration to 42.85 g/L. A redox indicator, resazurin, was included in the preparation of defined mineral medium. Under anaerobic conditions, resazurin remains colorless in the media solution. The anaerobic chamber was regularly flushed with a gas mixture containing 5% H_2, 5% CO_2, and 90% N_2 (Praxair Inc., CA) to maintain a strictly anaerobic condition.

In each experiment, approximately 40 mL of KB-1® culture during exponential phase was transferred into 50 mL sterile polystyrene tubes in the anaerobic chamber and

harvested by centrifugation at 3000 g and 4 °C for 10 min. The supernatant was decanted and the pellets were resuspended in appropriate anaerobic electrolyte (1, 10, and 100 mM NaCl). The pH of the NaCl solutions ranged from 6.3 to 6.6. The NaCl solution was prepared by dissolving an appropriate amount of NaCl in ultrapure water (Milli-Q water, Millipore Corp., MA). The NaCl solution was autoclaved and purged with ultrapure N_2 (Praxair Inc., CA) and stored in an anaerobic chamber for at least 1 week prior to use to remove all traces of residual oxygen (van Schie and Fletcher 1999). Due to the high sensitivity of the cells to oxygen, recentrifugation and resuspension was performed once to minimize possible cell damage and loss. The cell suspension was mixed using a stir bar throughout the experiment to maintain uniform cell distribution. To maintain anaerobic conditions, culture bottles and centrifuge tubes were opened only inside the anaerobic chamber. To prevent oxygen intake during centrifugation, centrifuge tubes were sealed with vinyl tape (Coy Laboratory Products Inc., MI) in the chamber. Plastic centrifuge tubes were stored inside the chamber for at least one week before use to ensure sufficient desorption of oxygen. To ensure the anaerobic condition of samples before, during, and after centrifugation, a redox indicator, resazurin, was added in the defined mineral medium. Only samples remained colorless after the centrifugation were used for following transport experiments. Concentrations of the targeting microorganisms in the KB-1® cell suspensions were determined to be $10^6 – 10^7$ cells/mL for *Dehalococcoides*, $10^3 – 10^4$ cells/mL for *Geobacter*, and $10^4 – 10^5$ cells/mL for *Methanomethylovorans* using a qPCR.

3.2.2 Electrokinetic potential measurements and DLVO interaction energy calculations

The electrokinetic potentials of the three targeting microbial species were determined by a Malvern Zetasizer Nano-ZS Analyzer (Malvern Instruments Ltd., UK). Pure cultured *Dehalococcoides* strain BAV1 (ATCC BAA-2100), *Geobacter lovleyi* strain SZ (ATCC BAA-1151), and *Methanomethylovorans hollandica* strain DMS 1(DSMZ 15978) were prepared as model microorganisms, and were obtained from ATCC (American Type Culture Collection, Rockville, MD) and DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig). The microorganisms were grown in a defined anaerobic mineral medium and fed with representative carbon sources as described previously (He et al. 2003b, Lomans et al. 1999, Sung et al. 2006). The microorganisms for zeta potential measurements were prepared under anaerobic condition. Approximately 30 mL of actively growing pure cultures were collected by centrifugation at 3000 g and 4 °C for 10 min. The freshly harvested cells were resuspended in 1, 10, and 100 mM anaerobic NaCl solutions to yield a final cell concentration of 10^7 cell/mL, and divided into several sterile 2 mL microcentrifuge tubes. The microcentrifuge tubes were taken out of the anaerobic chamber only when measuring zeta potentials. To guarantee the anaerobic conditions of the cell suspension, each tube was used for one measurement only. All measurements were performed at least three times using freshly prepared cell suspensions. The zeta potential of crushed glass beads was determined in the three ionic strengths at 22 ± 1 °C. The results were averaged over at least three measurements.

To better understand the mechanism controlling the observed microorganisms transport and deposition behaviors in column experiments, the DLVO theory was used to

estimate the total interaction energy as a microbial cell approaching a glass bead for each condition. The total interaction energy, including the sum of attractive van der Waals, and the repulsive electrostatic double layer interactions, was determined by modeling the microbial cell-glass bead system as a sphere-plate interaction (Redman et al. 2004, Tufenkji and Elimelech 2004b).

The repulsive electrostatic double layer interaction energies were calculated using the expression berived by Hogg et al.(1966):

$$\Phi_{EDL} = \pi\varepsilon_0\varepsilon_r a_p \left\{2\psi_p\psi_c ln\left[\frac{1+exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + \left(\psi_p^2 + \psi_c^2\right) ln[1 - \exp(-2\kappa h)]\right\} \quad (3.1)$$

where ε_0 is the dielectric permittivity in a vacuum, ε_r is the relative dielectric permittivity of water, a_p is the microbial radius, κ is the inverse Debye length, h is the separation distance between the microorganism and the collector surface, and ψ_p and ψ_c are the surface potentials of the microbial cell and bead collector, respectively. The retarded van der Waals attractive interaction energy was calculated based on (Elimelech and Omelia 1990, Gregory 1981):

$$\Phi_{VDW} = -\frac{Aa_p}{6h}\left[1 + \frac{14h}{\lambda}\right]^{-1} \quad (3.2)$$

where A is the Hamaker constant of the interacting media (microorganism – water – glass bead), and λ is the characteristic wavelength of the dielectric (assumed to be 100 nm). A value of 4.16×10^{-21} J was used for the Hamaker constant implying attractive van der Waals interaction as a microbial cell approaching a glass bead-water interface (Chen and Walker 2012, Israelachvili 2011, Lerner et al. 2012).

3.2.3 Porous media preparation and transport experiments in packed-bed columns

The packing material was spherical glass beads (MO-SCI Specialty Products, LLC., MO) with an average diameter of 550 μm and a specific gravity of 2.45 g/cm^3. Prior to column assemblage, glass beads were thoroughly cleaned following a method reported previously (Li et al. 2006), oven-dried at 80 °C, and autoclaved.

Cell filtration was performed in acrylic columns with an internal diameter of 3.175 cm and a length of 14 cm. Columns were uniformly dry-packed to a height of 14 cm with vibration in the anaerobic chamber. The column porosity was estimated to be 0.4 using a standard gravimetric method. Once packed, the column was stored in an anaerobic chamber for at least one week (van Schie and Fletcher 1999) to remove all traces of oxygen before performing column experiments.

A peristaltic pump (Cole Parmer, IL) was used to pump the solutions in an up flow mode. Prior to each experiment, the filter was equilibrated by pumping at least 6 pore volumes of cell free NaCl solution (1, 10, or 100 mM) through the column at a constant velocity of 0.0042 cm/s. Approximately 4 pore volumes of cell suspension were immediately injected into the column after switching the influent from the NaCl solution to a cell suspension. Following cell injection, columns were eluted with 4 pore volumes of background NaCl solution. The column effluent was collected in 50 mL sterile polystyrene tubes. Following each transport experiment, the column media were equally extruded and dissected into five segments of 2.8 cm length. Approximately 42 g of glass beads were obtained from each segment. Column effluent and segment samples were immediately used for DNA extraction. All experiments were performed in triplicate at

room temperature (22 ± 1 °C) in a well-controlled anaerobic condition. The solution pH of the influent and effluent were measured and remained in the range of 6.5 ± 0.1 throughout the experimental process.

To quantitatively compare the overall deposition of the three species of anaerobic organisms at different ionic strengths, the deposition rate coefficient k_d was determined using the steady state breakthrough cell concentrations according to the following equation (Walker et al. 2004):

$$k_d = -\frac{U}{fL} ln\left(\frac{C}{C_0}\right) \tag{3.3}$$

where f is the bed porosity, U is the approach (superficial) velocity, and L is the length of the column. C/C_0 is the normalized breakthrough cell concentration related to "clean bed" conditions; C/C_0 was obtained by averaging the breakthrough concentrations between 3 and 4 pore volumes from each breakthrough curve. Based on classic colloid filtration theory, the theoretical particle deposition pattern in the packed column can be determined using the following equation (Tufenkji et al. 2003):

$$S(X) = \frac{t_0 f k_d C_0}{\rho_b} exp\left(-\frac{k_d X f}{U}\right) \tag{3.4}$$

where S(X) is the particle deposition pattern, i.e., the number of deposited cells per mass of the granular collector, X is the column depth, ρ_b is the porous media bulk density, and t_0 is the duration of the continuous particle injection.

3.2.4 DNA extraction and cell enumeration

A PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc.) was used to extract DNA from the effluent and the segments, with the following modifications to the manufacturer's instructions. For the effluents and initial cell suspension, an aliquot of 300 µL was transferred into the PowerBead Tubes provided. For the segments, duplicate glass beads samples with approximately 0.3 grams were extracted following the manufacturer's protocol and were gathered during the separation step using the spin filter.

A qPCR was used to enumerate the cells in the samples. *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans* were targeted in this study. Primer pairs (Duhamel and Edwards 2006, Duhamel et al. 2004) (Table 3.1) were used in a CFX 96 real-time PCR system with a C1000 Thermal Cycler (Bio-Rad Laboratories, Inc.). Each 20 µL reaction contained 10 µL of SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Inc.), 6 µL of sterile water, 10 pmol of each primer, and 2 µL of DNA template. The thermocycling program was as follows: initial denaturation for 3 min at 95 °C; two-step cycles of 95 °C for 10 s, and 58 °C annealing for 30 s for a total of 40 cycles; a final melting curve from 65–95 °C was analyzed by measuring fluorescence every 0.5 °C. Calibration was performed with serial dilutions of a known quantity of identical 16S rRNA gene, which contained a targeting sequence generated using the PCR. Triplicate reactions of each standard dilution were run alongside triplicate reactions of all samples analyzed. The reaction conditions for the PCR were as follows: initial denaturation for 5 min at 95 °C; 35 cycles of 95 °C for 30 s, 58 °C for 1 min, and 72 °C for 1 min; and a final 10 min extension at 72 °C. Each 25 uL of PCR mixture contained 5× PCR buffer, 37.5 nmol $MgCl_2$, 5 nmol deoxynucleoside triphosphate, 25 pmol each primer, 1.25 U GoTaq®

DNA polymerase (Promega Corporation), and 1 uL DNA template. Melting curves showed reproducible single peaks without smaller peaks that would indicate primer dimer formation or nonspecific amplification.

Table 3.1 Targeting microorganims and primer sequences

Microorganism	**Primer**	**Sequence**
Dehalococcoides	1f	5'-GGA TGA ACG CTA GCG GCG-3'
	259r	5'-CAG ACC AGC TAC CGA TCG AA-3'
Geobacter	Geo 73f	5'-CTT GCT CTT TCA TTT AGT GG-3'
	Geo 485r	5'-AAG AAA ACC GGG TAT TAA CC-3'
Methanomethylovorans	Mvorans 166f	5'-AAA GCT TTT GTG CCT AAG GA-3'
	Mvorans 413r	5'-ATG GAC AGC CAA CAT AGG AT-3'

3.2.5 Statistical analysis

Electrokinetic potentials and breakthrough concentrations were analyzed with a single factor analysis of variance (ANOVA) and reported as p-values. ANOVA was performed using Microsoft Excel software; a p-value smaller than 0.05 suggests that the difference is statistically significant.

3.3 Results and discussion

3.3.1 Electrokinetic potentials of microbial cells

The zeta potentials of the *Dehalococcoides*, *Geobacter*, *Methanomethylovorans* cells, and glass beads are shown in Figure 3.1. The results suggest that the three microorganisms and the glass beads were negatively charged over the range of ionic strengths (1, 10, 100 mM) and pH (6.3–6.6) tested. The zeta potentials of the three

microorganisms became less negative with increasing ionic strength due to compression of the electrostatic double layer at high ionic strength. *Methanomethylovorans* cells had the lowest zeta potential and *Dehalococcoides* had the highest zeta potential among the three microbial strains ($p < 0.05$) at the tested ionic strengths, except at 100 mM. The zeta potentials of the glass beads remained relatively constant at 1 and 10 mM ionic strength, then became less negative at 100 mM. The measured zeta potentials were used to calculate DLVO interaction energy profiles for the microorganism-glass bead system to elucidate the microbial cell adhesion mechanism.

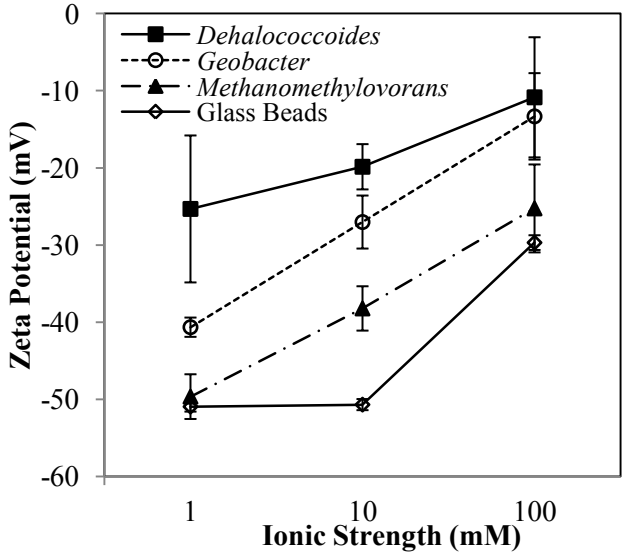

Figure 3.1 Surface zeta potential of three microbial species in KB-1® culture (*Dehalococcoides*, *Geobacter*, *Methanomethylovorans*), and glass beads. Ionic strengths: 1 mM, 10 mM, 100 mM NaCl. Error bars represent standard deviations of three measurements.

3.3.2 Column transport experiments

Microbial breakthrough curves were constructed by plotting normalized cell concentrations (C/C_0) in the column outflow against the number of pore volumes passed through the packed bed (Figure 3.2). Injected bacteria were detected in the column effluent within one pore volume upon switching from the cell free NaCl solution to the cell suspension. Similar breakthrough patterns were observed for all three species of bacteria over the range of ionic strengths tested. The injected cells broke through the packed bed after approximately 2 pore volumes, and the breakthrough concentrations appeared to be constant for all three species. After 4 pore volumes, the columns were flushed with cell free solutions of identical chemical composition; microorganism concentrations in the effluent decreased continuously, eventually reaching zero, except for *Geobacter* at 1 mM ionic strength. During flushing, a certain degree of tailing was observed during the washout, indicating cell detachment.

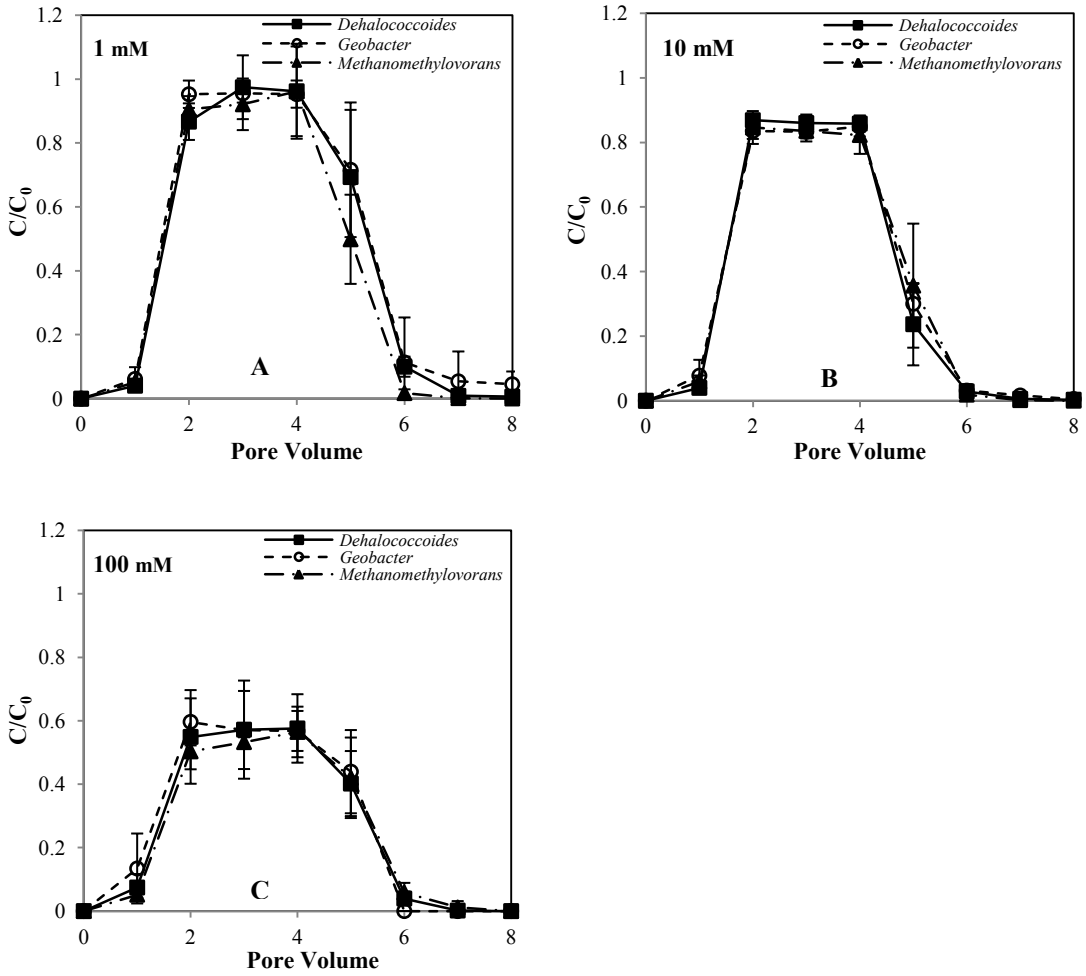

Figure 3.2 Breakthrough curves of three microbial species in KB-1® culture (*Dehalococcoides*, *Geobacter*, *Methanomethylovorans*) under three solution ionic strengths: 1 (A), 10 (B), and 100 mM (C) NaCl. Cells injection started at pore volume 0 and ended at pore volume 4. Experimental conditions were as follows: approach velocity = 0.0042 cm/s, porosity = 0.4, and pH = 6.3 – 6.6. Error bars represent standard deviations of three measurements.

The breakthrough cell concentration decreased significantly with an increase in ionic strength ($p < 0.05$), indicating increased cell retention. The increased cell removal observed was in qualitative agreement with colloidal stability predictions of DLVO

theory, which state that the diffuse double layers are compressed with increasing ionic strength, causing a reduction in repulsive electrostatic double layer forces and an increase in particle deposition rate.

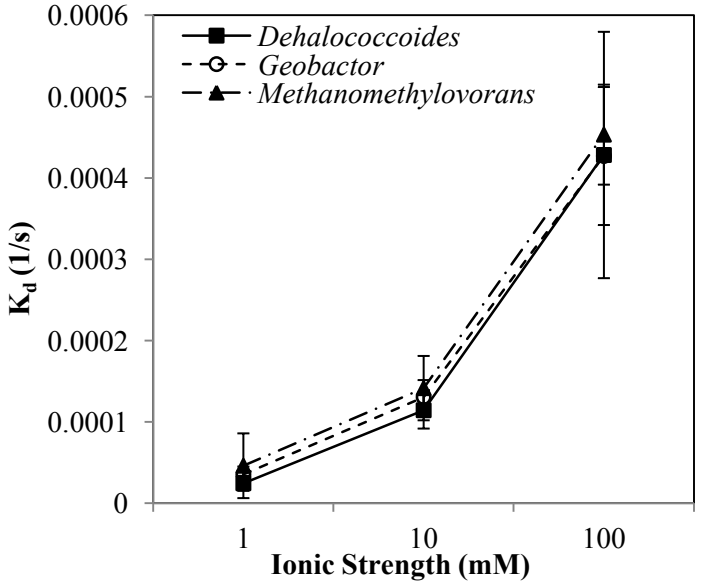

Figure 3.3 Microbial deposition rate coefficient (k_d) determined from the breakthrough curves (Figure 3.2) using equation 3.3. Ionic strengths: 1 mM, 10 mM, 100 mM NaCl. Error bars represent standard deviations of three measurements.

Generally, an increase in ionic strength leads to a higher deposition rate coefficient. As shown in Figure 3.3, the deposition rate increased approximately 20 times over the range of examined ionic strengths tested for all three microorganisms. Specifically, from 1 to 10 and 100 mM, the k_d increased from 2.43E-05 to 1.14E-04 and 4.28E-04 s^{-1} for *Dehalococcoides*, from 3.52E-05, to 1.30E-04 and 4.27E-04 s^{-1} for *Geobacter*, and from 4.60E-05 to 1.41E-04 and 4.53E-04 s^{-1} for *Methanomethylovorans*. Trends in the deposition rates of the three species were consistent with respect to their zeta potentials

(Figure 3.1) as predicted by DLVO theory. However, it was noted that under the same solution chemistry conditions, the deposition rate coefficients were similar among the three bacterial species, although the measured zeta potentials were distinct. That is, the least negatively charged microbial species (*Dehalococcoides*) exhibited deposition behavior similar to the most negatively charged microbial species (*Methanomethylovorans*). One possible reason is that previously attached particles may have provided favorable sites for approaching particles to deposit. This may be because the measured electrokinetic potentials of microbial cells were less negative than those of the glass beads. Therefore, particle-particle interactions may become more favorable than particle-collector surface interactions. It is also suspected that the power of theoretical DLVO theory to predict microbial deposition behavior in this experimental system was limited.

3.3.3 Profiles of retained microbial cells

Figures 3.4A, B, and C were obtained by plotting the number of microbial cells recovered from the glass beads as a function of the distance travelled and the number of microbial cells actually recovered with the number of microbial cells expected to be recovered according to colloid filtration theory (CFT). The magnitudes of the retained cell profiles for all three microbial species under three examined conditions varied contrarily to the breakthrough plateaus, based on the mass balance consideration. That is, an increase in ionic strength induced greater cell retention in the porous media.

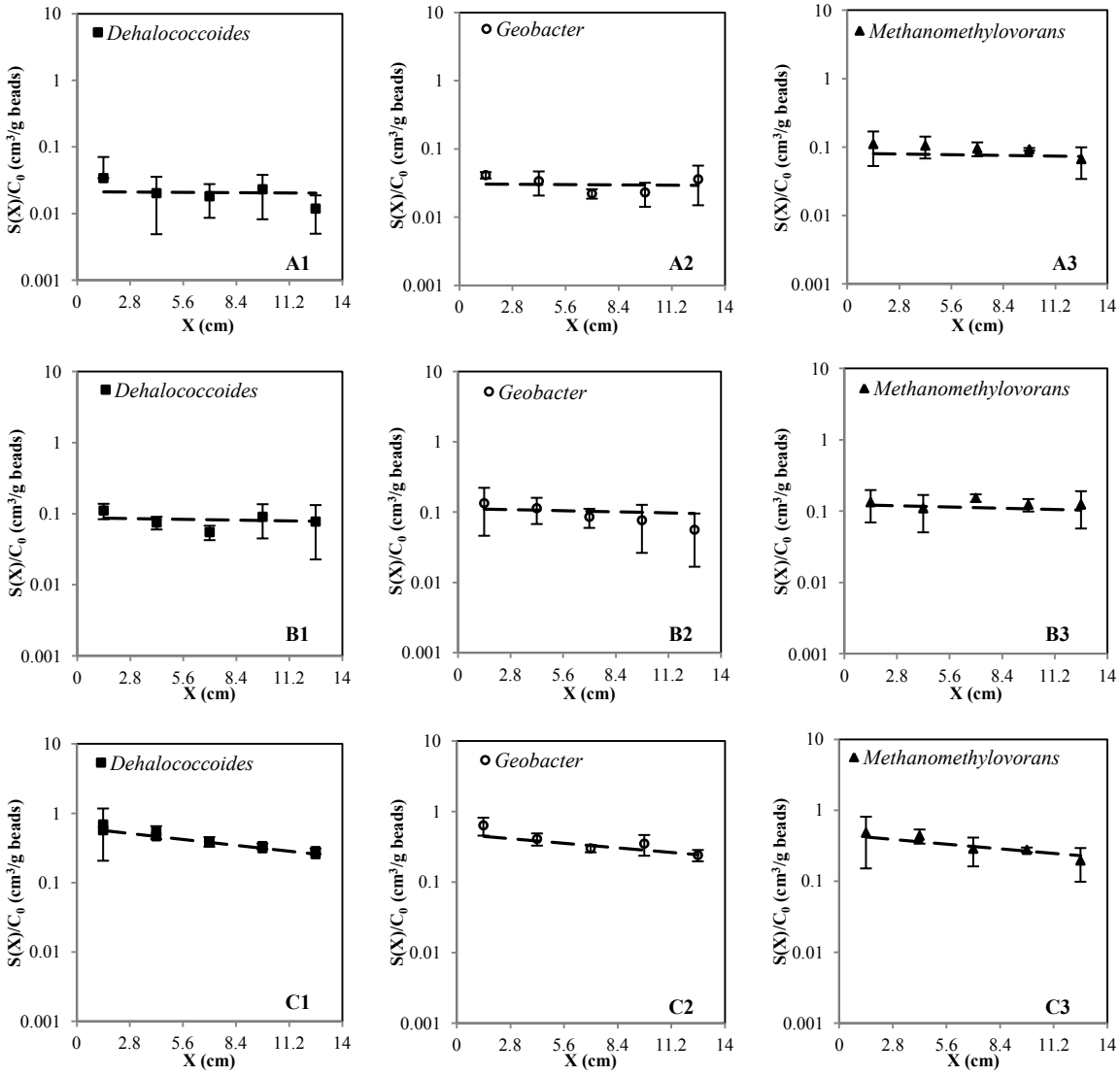

Figure 3.4 Measured retained cell concentration profiles in single 14 cm column (symbols in A1-A3, B1-B3, C1-C3). The microbial species are: *Dehalococcoides* (A1, B1, and C1); *Geobacter* (A2, B2, and C2); and *Methanomethylovorans* (A3, B3, and C3). The ionic strengths for the carrying solutions are as follows: 1 mM NaCl (A1-A3); 10 mM NaCl (B1-B3); and 100 mM NaCl (C1-C3). Dashed lines represent predictions based on the classic colloid filtration theory (CFT) using deposition rate coefficient (k_d). Data

were presented in a semilog format. Error bars represent standard deviations of three measurements.

Log-linear retained profiles were observed for *Methanomethylovorans*, *Dehalococcoides*, and *Geobacter* under the three ionic strengths examined, suggested that the microbial removal in the porous media decreased exponentially with travel distance, indicating spatially constant deposition rate coefficients. A reasonable mass balance was generally achieved in the column experiments (77–118%) (Table 3.2).

Table 3.2 Cell recovery percentage from column experiments

Ionic Strength	**Microorganism**					
	Dehalococcoides		*Geobacter*		*Methanomethylovorans*	
	Effluent[a]	Total[b]	Effluent[a]	Total[b]	Effluent[a]	Total[b]
1 mM	91.9%	95.0%	96.0%	98.4%	83.9%	84.6%
10 mM	73.0%	79.6%	74.2%	82.6%	74.0%	77.3%
100 mM	54.7%	118.1%	57.2%	118.5%	53.0%	113.6%

[a]The percentage of injected cells that were recovered by integration of the breakthrough curves in Figure 3.2 during the transport stage. [b]The combined percentage of cells retained in the columns and cells that broke through the columns based on the cell mass balance.

3.3.4 Modeling using DLVO theory-interaction energy profiles

Under all ionic strengths analyzed, microbial cells and glass beads had a negative zeta potential. Repulsive electrostatic interactions should inhibit cell attachment. However, a clear trend of increasing deposition rate coefficient was observed with an increase in

ionic strength. To better understand the mechanism controlling the observed microorganism transport and deposition behaviors in column experiments, the DLVO interaction energy profiles were determined for cells interacting with glass beads upon close approach. The average equivalent spherical radius of a spheroid having the same average volume as a disc-shaped *Dehalococcoides* bacterium was assumed to be 0.27 μm (He et al. 2003b). The average equivalent spherical radius of a rod-shaped *Geobacter* bacterium was determined to be 0.33 μm (Sung et al. 2006). *Methanomethylovorans* cells were considered to be spheres with an average spherical radius of 0.63 μm (Lomans et al. 1999). Additionally, the zeta potentials of the microorganisms and glass beads were used in place of the respective surface potentials (Tufenkji and Elimelech 2004b). DLVO interaction energy versus separation distance for the microorganism-glass bead system in the three conditions used in the deposition experiments is presented in Figure 3.5. The calculations revealed the presence of a significant repulsive energy barrier to deposition at all ionic strengths for all three bacterial species. The repulsive energy barriers exhibited marked decreases with increases in ionic strength; specifically, the energy barrier decreased from 337 kT at 1 mM to 9.92 kT at 100 mM, from 883 kT at 1 mM to 29.5 kT at 100 mM, and from 2140 kT at 1 mM to 270 kT at 100 mM for *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*, respectively, as listed in Table 3.3. Therefore, DLVO interaction energy calculations predicted that the microbial cells would be unlikely to deposit onto the glass bead surfaces (i.e., overcome the repulsive energy barrier) under these conditions. However, cell deposition was observed in the presence of a significant repulsive energy barrier even at the lowest ionic strength examined (e.g., $C_0/C \approx 0.95$ for all three species at 1 mM).

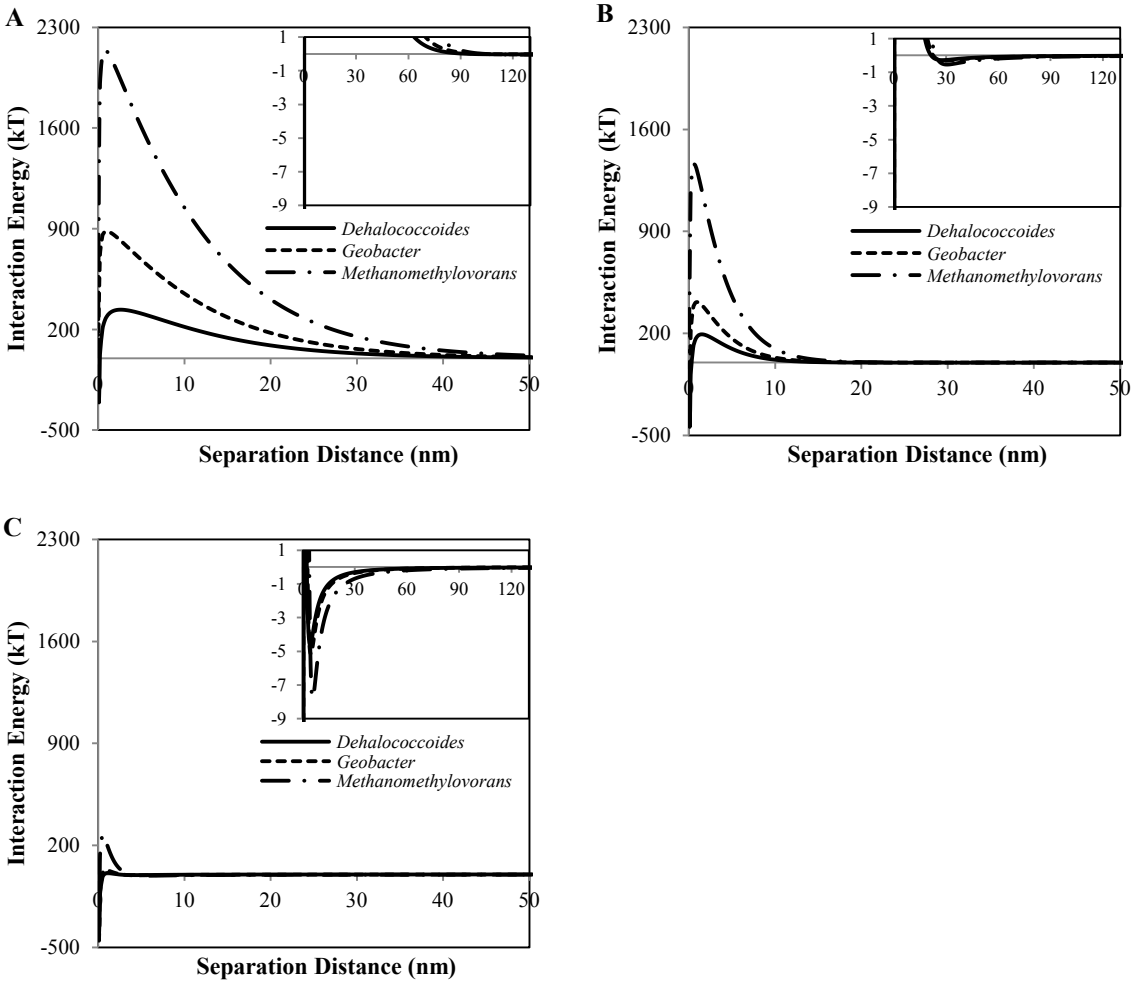

Figure 3.5 Calculated DLVO interaction energy plotted as a function of separation distance for different solution ionic strengths: 1 mM (A), 10 mM (B), and 100 mM (C); and (insets) the expanded drawing to highlight the secondary energy minimum. Experimentally measured zeta potentials (Figure 3.1) and a Hamaker constant of 4.16×10^{-21} J were used to calculate interaction energies.

Replots of the DLVO interaction energy profiles on a different scale (insets in Figure 3.5) showed the presence of a secondary energy minimum at a greater separation distance than that of the repulsive energy barrier. A secondary energy minimum exists as a consequence of different dependencies of the van der Waals and electrostatic double

layer on the separation distance (power-law and exponential, respectively). Microorganisms approaching a bead collector would experience an attractive force before encountering a repulsive energy barrier. Cells would remain associated with the bead collector within the secondary energy minimum unless they had sufficient energy to escape the well (Redman et al. 2004). As shown in Figure 3.5, the magnitude of the secondary energy minimum increased with increasing ionic strength for all three species over the range of ionic strengths tested. In particular, the depth of the secondary energy minimum increased from 0.0187 kT at 1 mM to 4.61 kT at 100 mM, from 0.0208 kT at 1 mM to 5.15 kT at 100 mM, and from 0.0381 kT at 1 mM to 7.77 kT at 100 mM with respect to corresponding separation distances of 70–4.25 nm, 122.5–4.75 nm, and 126–5.75 nm for *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*, respectively (Table 3.2).

Table 3.3 Calculated DLVO interaction parameters

	Energy Barrier (kT)	**Secondary Minimum Depth (kT)**	**Secondary Minimum Separation (nm)**
Dehalococcoides			
1 mM NaCl	337	0.0187	70
10 mM NaCl	194	0.275	27.5
100 mM NaCl	9.92	4.61	4.25
Geobacter			
1 mM NaCl	883	0.0208	122.5
10 mM NaCl	414	0.311	29
100 mM NaCl	29.5	5.15	4.75
Methanomethylovorans			
1 mM NaCl	2140	0.0381	126
10 mM NaCl	1360	0.544	30.5
100 mM NaCl	270	7.77	5.75

Compared to the microbial deposition rate coefficient (Figure 3.3), the increases in calculated secondary energy minimum depths with increasing ionic strength were in parallel to the k_d, which also depends on ionic strength. So it is possible that the microbial deposition was mostly in the secondary energy minimum wells several to hundreds of nanometers from the collector surface instead of in the primary energy minima (Redman et al. 2004). In summary, the DLVO energy profiles suggested that increasing the ionic strength substantially lowered the repulsive energy barriers that prevented microbial cells from attaching to glass bead surfaces, and also deepened the secondary energy minimum wells that might retain cells in the column. Consequently, cell deposition was expected to increase with increasing ionic strength, as shown previously (Figures 3.2, 3.3 and 3.4). Therefore, the transport and retention behavior of each bacterial species—

Dehalococcoides, *Geobacter*, and *Methanomethylovorans*—was in qualitative agreement with the DLVO interaction energy profiles presented in Figure 3.5.

3.3.5 Deviations from DLVO predictions

Even though the transport and retention behavior of each of the individual microbial species (i.e., *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*) was in qualitative agreement with the DLVO prediction, limitations of the theoretical DLVO predictions in the experimental system are worth noting. Although each individual species exhibited a distinct DLVO interaction energy profile consisting of a repulsive energy barrier and a secondary energy minimum, the transport and retention behaviors of all species were virtually the same. It is possible that the population heterogeneity in the KB-1® culture might have contributed to the deviant transport and retention behaviors observed among the different species (Tong and Johnson 2006, 2007, Tufenkji and Elimelech 2005a). To evaluate factors that could cause deviation between experimental deposition behavior and behavior predicted by DLVO theory, several aspects of population heterogeneity in the KB-1® culture are considered.

(1) Cell size and shape

Previous studies (Tong and Johnson 2006, Yao et al. 1971) suggest that particles with about 1.0 μm diameter exhibit minimal particle deposition efficiency. According to Yao (Yao et al. 1971), sedimentation and interception are the predominant removal mechanisms of particles larger than 1 μm, for which removal efficiency increases with increasing particle size; particles smaller than 1 μm are effectively removed by diffusion due to their Brownian movement, and their deposition efficiency decreases with an

increase in particle size (Yao et al. 1971). In our study, the equivalent spherical diameters of *Dehalococcoides* were estimated to be approximately 0.54 μm. As the cell diameters of *Geobacter* were 0.62–0.70 μm, respectively, it is likely that both strains undergo diffusion, and the smaller sized *Dehalococcoides* would have higher attachment efficiency than the larger *Geobacter*. *Methanomethtlovorans*, with the largest cell size (diameter = 1–1.5 μm), would be more likely removed by sedimentation and interception.

Bacterial cell shape is reported to play a role in the filterability of bacterial cells, and cells with higher aspect ratios are believed to exhibit preferential retention during the transport process (Wang et al. 2008, Weiss et al. 1995). Salerno et al. (2006) observed that rod-shaped particles with aspect ratios of 2:1 and 4:1 displayed higher retention than spherical particles with an aspect ratio of 1:1. Liu et al. (2010) also reported that rod-shaped particles displayed higher retention than spherical particles. Rod-shaped particles would adopt a preferred orientation (end-on position) to minimize the interaction energy when approaching collector surfaces (Seymour et al. 2013). It is possible that the rod-shaped *Geobacter* cells oriented themselves to an end-on position when approaching to the glass bead surfaces, rendering the collector surfaces additional roughness, which may enhance subsequent attachment (Chen et al. 2010a). As described by He et al. (2003b), the suspended disc-shaped cells of *Dehalococcoides* would tumble end over end in solutions (He et al. 2003b). It is suspected that the disc-shaped *Dehalococcoides* cells would adopt a similar end-on position as *Geobacter* cells when approaching to surfaces. However, further experiments using single strains are needed to examine the suspension. Despite the coccoid shapes of *Methanomethylovorans* cells, their minor difference from

the other two species in retention behavior might have contributed to the available favorable sites provided by previously attached cells.

(2) Cell concentration

Although the clean bed theory predicts that cell retention in porous media is independent of cell concentration, high cell concentrations are known to affect the overall retention of cells either positively by providing more favorable surfaces for adhesion (ripening) or negatively by providing less favorable surfaces (blocking) (Camesano and Logan 1998, Camesano et al. 1999). Blocking was shown in a previous study where cell concentrations of 10^8 cell/mL increased the breakthrough concentrations, however, no obvious decrease in retention was observed at concentrations of 10^7 cell/mL and lower, indicating that blocking is not predominant when cell concentrations are below the tested 10^8 cell/mL level (Haznedaroglu et al. 2009).

In our study, initial injected concentrations of *Dehalococcoides* were around 10^6–10^7 cells/mL, approximately 1000 times higher than that of *Geobacter*, and 100 times higher than that of *Methanomethylovorans*. It is possible that previously attached *Dehalococcides* cells provided a more favorable site of adhesion, and promoted further cell attachment, resulting in multilayer coverage. Ripening is potentially involved in cell adhesion and could have provided favorable adhesion sites.

(3) Cell motility

Bacterial motility is known to affect adhesion behavior. When brought near collector surfaces, motile cells can swim along the surfaces at various orientations by either tracing

out circles around the surfaces, or moving in parallel or perpendicular to the surfaces (Frymier et al. 1995, Vigeant and Ford 1997). Previous studies have shown that cell motility can either increase or inhibit cell attachment (Jenneman et al. 1985, Korber et al. 1994). Camesano and Logan (1998) discovered that as fluid velocities decreased from 120 to 0.56 m/day, unlike nonmotile cells, swimming cells were able to avoid adhering to a solid surface leading to reduced cell adhesion. At higher fluid velocities, cell motility corresponded to a clear reduction in cell adhesion. They concluded that the variation between motile and nonmotile cells at lower fluid velocities could potentially cause a difference in the degree of cell retention from the colloid filtration theory prediction, which predicts that a decrease in bulk fluid rate results in increased cell retention (Camesano and Logan 1998, McCaulou et al. 1995). Under the experimental flow rate (3.63 m/day), the motile *Geobacter* cells might have exhibited less attachment to the collector surfaces than the nonmotile *Dehalococcides* and *Methanomethylovorans* cells.

(4) Microbial interactions

Cutter et al. (2003) indicated that one bacterial species adhering to a solid surface could either positively or negatively affect the adhesion of other bacteria. Macromolecules such as polymers, proteins, surfactants, and metabolites secreted by bacteria were shown to either increase or decrease the attachment of other bacteria (Pringle et al. 1983, Sutherland 1983, Tosteson et al. 1985). Previously attached bacteria could also impact subsequent bacterial attachment by producing polymers and surfactants on the collector surface (Pringle and Fletcher 1986); such surface alterations could influence the subsequent adhesion of other microorganisms. In the present study it is possible that the adhesions of *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans*

were to some extent affected competitively or cooperatively by each other and by other cells in the KB-1® culture.

The above discussion regarding different aspects of population heterogeneity is heavily dependent on the three microorganisms targeted. However, other bacterial and archaeal species present in the culture are believed to have contributed to the observed transport and deposition behaviors of the three species examined in present study. In addition, surface properties of *Dehalococcoiddes*, *Geobacter*, and *Methanomethylovornas* were characterized solely based on the chosen model microorganisms and could not fully represent the overall cell surface characterizations in the mixed culture, and hence might not reflect predicted DLVO interactions. A change in model microorganisms could potentially change the calculated DLVO interaction energy profiles.

3.4 Conclusions

Transport and retention trends of *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans* in KB-1® culture were investigated with a focus on the influence of solution chemistry. Both experimental results and theoretical calculations were employed to predict interactions and transport behaviors of the three species in representative groundwater conditions. Cell surface characterizations were investigated and classic DLVO theory interaction energy profiles were estimated. The influence of mixed species interactions on the deposition behaviors of targeted bacteria was discussed.

Electrokinetic potential analysis revealed that *Methanomethylovorans* was the most negatively charged species followed by *Geobacter*. Column experiment results showed that all three species travelled similar distances, and were almost equally retained. Size,

shape, motility, and initial concentration of the microorganisms were found to impact transport and deposition behaviors by balancing the effects caused by differences in electrokinetic potentials; the result of transfer and deposition rate coefficients for *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans* was similar. A similarity in cell transport behavior is greatly preferred for the TCE-bioargumentation process because the coexistence of two major TCE dechlorinators - *Dehalococcoides* and *Geobacter* - is crucial to complete dechlorination of TCE contaminated sites, with the presence of other nondechlorinators. In our study, the three functional species travelled the same distance, which is desirable. Future studies could track *Dehalococcoides*, *Geobacter*, and *Methanomethylovorans* on a longer trajectory, and perhaps investigate the retention and transport of other species in the KB-1® culture in porous media.

The results of the study suggest that bioaugmentation of KB-1® would be most successful in low ionic strength water. Other factors, such as flow velocity, cell loading, solution composition, grain size and shape, and saturation of porous media (not examined in the present study) could also impact the retention and transport of the culture and could be optimized with further testing. This study is the first to document the comparative retention and transport behaviors of *Dehalococcoides*, *Geobacter*, and *Methanemethylovorans* in the mixed TCE - bioaugmentation culture KB-1® in a range of anaerobic groundwater conditions.

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Chapter 4. Comparison of the Transport and Deposition of *Pseudomonas aeruginosa* under Aerobic and Anaerobic Conditions*

*A version of this chapter has been submitted for journal publication.

4.1 Introduction

Bacterial transport and retention in granular porous media affects environmental applications such as in-situ bioremediation, water and wastewater filtration, and pathogen transport in subsurface (Parsek and Fuqua 2004, Steffan et al. 1999, Tufenkji et al. 2002). To evaluate the fate and transport of organisms in groundwater and other subsurfaces, numerous studies have been conducted to determine mechanisms governing microbial migration and retention, particularly those toxigenic and degradative bacterial strains (Camesano and Logan 1998, Haznedaroglu et al. 2009, Kim et al. 2009, Li et al. 2006, Liu and Li 2008, Liu et al. 2007).

P. aeruginosa are facultative, functioning aerobically and anaerobically, and are capable of growing using a large variety of carbon sources (Palmer et al. 2007, Williams et al. 1978, Yoon et al. 2002). Metabolically versatile, *P. aeruginosa* can adapt to and colonize in various environmental niches such as soil and marine habitats, plants, and animals. In humans, *P. aeruginosa* are predominant in the chronic lung infections of cystic fibrosis patients (de Kievit and Iglewski 2000). Though more widely recognized as an opportunistic pathogen in humans and animals, *P. aeruginosa*'s degradative abilities are apparent in soil (Alonso et al. 1999). Isolates of *P. aeruginosa* had shown efficient ability to degrade various petroleum products such as gasoline, kerosene, diesel oil, and crude oil, and pollutants such as n-alkanes and polycyclic aromatic hydrocarbons (PAH) despite their origins (clinic or environment) (Alonso et al. 1999, Karamalidis et al. 2010).

Although factors that affect the fate and transport of *P. aeruginosa* under aerobic conditions are well documented, including solution ionic strength (Martin et al. 1992),

hydrophobicity (Marcus et al. 2012), presence of divalent cations (Simoni et al. 2000), extracellular polymeric substances (EPS) (Liu et al. 2007), cell motility (de Kerchove and Elimelech 2007, 2008), little information is available on their transport under anaerobic conditions.

Bacteria travelling in the subsurface may face various dissolved oxygen (DO) concentrations. Variation in oxygen concentration has been shown to affect the growth of *P. aeruginosa* and induce physiological change (Sabra et al. 2003). An anaerobic environment was found to promote the production of the viscous exopolysaccharide alginate, and introduce alterations in the outer membrane of *P. aeruginosa*, e.g., loss of O-specific antigens (previously known as B-band polysaccharides) (Schobert and Jahn 2010, Singh et al. 2000).

Studies on the impact of oxygen levels on *E. coli* deposition in porous media (Castro and Tufenkji 2008, Landini and Zehnder 2002, McCaulou et al. 1995, Yang et al. 2006) are scarce. Landini and Zehnder (2002) found that anoxia reduces *E. coli* adhesion to a sand column, and proposed that the reduced adhesion was a result of increased transcription of the *fliC* gene, encoding the major flagellar subunit, and increased production of lipopolysaccharides (LPS). Yang et al. (2006) observed that environmental *E. coli* isolates showed the least transport and most adhesion to various granular materials when growing under aerobic condition compared to intestinal growth. Castro and Tufenkji (2008) performed laboratory scale filtration experiments in electrolyte solution with low or high DO using *E. coli* and *Yersinia enterocolitica* grown in either aerobic or anaerobic condition. It was observed that the effect of DO on bacterial transport and

retention depended on microbial growth conditions and microorganism types. Studies on different organisms are needed to determine whether these general principles apply.

To our knowledge, the effects of oxygen tension on the transport of *P. aeruginosa* PAO1 in granular porous media have not been previously reported. This study investigates the effects of oxygen on the growth and transport of *P. aeruginosa* PAO1. Experiments were carried out in oxygen-depleted and oxygen-rich electrolytes to examine bacterial transport and deposition behaviors. The results obtained from packed column experiments were further supported by the measurements of electrokinetic potentials and characterization of cell surfaces.

4.2 Materials and methods

4.2.1 Bacterial cultivation and cell preparation

Gram negative, rod-shaped *Pseudomonas aeruginosa*, wild-type PAO1, was stored at -80 °C until utilized in the study. For each aerobic experiment, *P. aeruginosa* PAO1 was streaked onto a Luria-Bertani (LB) agar plate and incubated at 37 °C overnight. A single colony was transferred into 15 mL of LB broth and grown in a shaker incubator (New Brunswick Scientific Co., NJ) at 200 rpm and 37 °C for 18 h. Early stationary-phase bacterial cells were harvested by centrifugation at 3000 g and 4 °C for 10 min using 50 mL sterile polystyrene tubes. After the supernatant was decanted, the pellets were resuspended in appropriate electrolytes (1, 10, and 100 mM NaCl). The centrifugation and resuspension procedure was repeated twice to remove possible traces of growth media. A final cell density of approximately 10^6 colony-forming units (CFU)/mL was obtained. Cell suspensions were kept on ice before the filtration experiment to minimize

bacterial growth. The pH of the electrolytes ranged from 6.3-6.6. Cell suspensions were mixed using a stir bar throughout the experiment to maintain uniform cell distribution.

For each anaerobic experiment, a single colony was transferred into 15 mL of anaerobic LB broth amended with 5 mM $NaNO_3$ and cells were grown in an anaerobic chamber (Coy laboratory Products Inc., MI) at room temperature for 38-40 h to reach early stationary growth phase. For anaerobic experiments, cell suspensions of 10^6 CFU/mL using anaerobic 0.9% NaCl were prepared in the manner described for aerobic experiments. To maintain the anaerobic condition of the cell suspension, the 50 mL tubes were taken out of the anaerobic chamber only for centrifugation and were opened only in the anaerobic chamber. To minimize oxygen intake during centrifugation, centrifuge tubes were sealed with vinyl tape (Coy laboratory Products Inc., MI) before being taken out of the anaerobic chamber. Anaerobic LB broth and 0.9% NaCl solution were prepared by purging with ultrahigh purity N_2 (Praxair Inc., CA) for at least 30 min per liter of liquid immediately after autoclaving. All liquids and plastic tubes used for anaerobic transport experiments were stored in the anaerobic chamber for at least one week prior to use (van Schie and Fletcher 1999). To control the anaerobic condition, the anaerobic chamber was routinely flushed with a gas mixture containing 5% H_2, 5% CO_2, and 90% N_2 (Praxair Inc., CA).

4.2.2 Electrokinetic potential measurements

A Malvern Zetasizer Nano-ZS Analyzer (Malvern Instruments Ltd., UK) was used to measure the zeta potentials of both aerobic and anaerobic PAO1 cells. Cell suspensions were prepared from aerobic and anaerobic cultures as described in section 4.2.1.

Approximately 10 mL of stationary phase PAO1 cells were collected by centrifugation at 3000 g and 4 °C for 10 min. The rinsed stock cell suspensions were diluted in 1, 10, and 100 mM NaCl (aerobic or anaerobic) to a final cell density of 10^7 CFU/mL. The anaerobic bacteria cell suspension was divided into several sterile 2 mL microcentrifuge tubes, and kept in the anaerobic chamber until zeta potentials were measured. To maintain the anaerobic condition of the cell suspension, each microcentrifuge tube was used for only one measurement. Anaerobic NaCl solutions (1 mM, 10 mM, and 100 mM) were prepared by purging with ultrapure N_2 (Praxair Inc., CA) for at least 30 min per liter immediately after autoclaving. All measurements were performed at least three times using freshly prepared cell suspensions. The zeta potential of crushed glass beads was determined under the three ionic strengths at 22 ± 1 °C. The results were averaged over at least three measurements. The measured zeta potentials were used to calculate the Derjaguin-Landau-Verwey-Overbeek (DLVO) interaction energy.

4.2.3 Bacterial cell size

To measure the dimensions of PAO1 in aerobic and anaerobic conditions, scanning electron microscopy (SEM) images were taken using a scanning auger microprobe spectrometer (JAMP9500F, Oxford Instruments). Freshly harvested stationary bacteria were suspended in 10 mM NaCl, and fixed with 2.5% glutaraldehyde for 30 min. Bacterial suspensions were then washed three times with 10 mM NaCl and fixed again using 1% OsO_4 for 30 min. Bacteria were then dehydrated in a series of 50%, 70%, 90%, and 100% ethanol, followed by critical point drying at 31 °C for 30 min. The samples were sputter-coated with gold (Edwards, Model S150B, UK) and examined with SEM. Individual cell lengths and widths were determined. Measured values were used to

calculate the effective cell radius and corresponding bacterial cell surface area. The average lengths were found to be 1.95 ± 0.15 μm and 1.55 ± 0.05 μm for aerobic and anaerobic PAO1, respectively, while the widths (0.55 ± 0.05 μm) were similar. The resulting equivalent spherical radii were 0.48 μm and 0.44 μm for aerobic and anaerobic PAO1, respectively.

4.2.4 EPS composition analysis

Components of extracellular polymeric substances (EPS) were extracted from the bacterial surfaces with a heating method. Briefly, freshly harvested bacterial cells in early stationary phase were washed twice using 0.9% NaCl before proceeding to EPS extraction. After being washed, the bacterial cell pellet was resuspended in ultrapure water and heated at 80 °C for 10 min (Zhang et al. 1999). The heated bacterial suspension was centrifuged at 15,000 g, 4 °C for 15 min. Supernatants were filtered (0.22 μm pore) and filtrate was collected as EPS extracts and stored at -20 °C (Hwang et al. 2012).

Colorimetric methods were employed to determine the composition of the EPS extracts using a UV-Visible spectrophotometer (Varian Inc., CA). Protein and polysaccharides concentrations were estimated by the bicinchoninic acid assay (Frolund et al. 1995, Lowry et al. 1951) and the phenol-sulfuric acid assay (Dubois et al. 1956), respectively. Bovine serum albumin (BSA) (Fisher Scientific Inc., USA), and glucose (Fisher Scientific Inc., USA) were used as standards for measuring protein and polysaccharides, respectively. Spectrophotometer readings of absorbance at 595 nm and 480 nm were taken to determine protein and polysaccharides concentrations, respectively (Hwang et al. 2012).

4.2.5 XPS analysis of bacterial cells

Cryo-X-ray photoelectron spectroscopy (Cryo-XPS) (Axis 165, Kratos Analytical, Japan) was used to determine the surface composition of both aerobic and anaerobic PAO1 cells. Bacteria aerobically grown to early stationary phase were harvested and washed twice with 0.9% NaCl. After the final washing, cell pellets were dissolved in 1 mL 0.9% NaCl, yielding a final cell density of 10^9 CFU/mL. Bacteria grown in the anaerobic condition were prepared in the same manner using anaerobic 0.9% NaCl in a well controlled anaerobic chamber. To maintain an anaerobic condition in bacterial cell suspensions during centrifugation, centrifuge tubes were sealed with vinyl tape and opened only in the anaerobic chamber. Cell suspensions were quickly cooled and frozen in liquid nitrogen, and placed in the instrument for XPS analysis. CasaXPS instrument software was used for data processing. The sum of atomic concentrations of O, C, and N for each sample was scaled to 100%.

The concentrations of the three major bacterial surface constituents-proteins (PR), polysaccharides (PS), and hydrocarbon-like products (HC) were determined using the following equations (Shapiro and Dworkin 1997).

$$O/C = 0.325(C_{PR}/C) + 0.833(C_{PS}/C) \quad (4.1)$$

$$N/C = 0.279(C_{PR}/C) \quad (4.2)$$

$$1 = (C_{PR}/C) + (C_{PS}/C) + (C_{HC}/C) \quad (4.3)$$

where O/C and N/C are the atomic concentration ratios of oxygen/carbon and nitrogen/carbon, respectively, in the sample surface. C_{PS}, C_{PR}, and C_{HC} are the atomic

concentrations of carbon present in polysaccharides, proteins, and hydrocarbon-like products, respectively.

4.2.6 Granular porous medium preparation

The filtration system consisted of acrylic columns with an internal diameter of 3.175 cm, and a length of 14 cm. The packing material was spherical glass beads (MO-SCI Specialty Products, LLC., MO) with an average diameter of 550 μm and a specific gravity of 2.45 g/cm^3. Columns were uniformly dry-packed to the height of 14 cm with vibration. The column porosity was estimated to be 0.4 using the standard gravimetric method. Prior to column assemblage, glass beads were thoroughly cleaned following a method reported previously (Li et al. 2006). Specifically, the glass beads were first washed with ultrapure water (Milli-Q water, Millipore Corp., MA), then ultrasonicated in 0.01 M NaOH for 15 min followed by repeating ultrasonication in ultrapure water until rinsed solution become transparent, then were ultrasonicated in 1 M HNO_3 for 20 min, with final rinse with ultrapure water. The cleaned glass beads were then autoclaved and oven-dried at 80 °C. For the anaerobic experiments, the columns were packed in the anaerobic chamber, and stored in the anaerobic chamber for at least 1 week after assemblage to remove all traces of oxygen before performing column experiments.

4.2.7 Transport experiments in packed-bed columns

A peristaltic pump (Cole Parmer, IL) was used to pump the solutions in an up-flow direction. Prior to each experiment, the columns were equilibrated by injecting at least 6 pore volumes of background NaCl solution. After reaching equilibrium, approximately 4 pore volumes of cell suspension were injected into the column, followed by 5 pore

volumes of background NaCl solution. The approach velocity was 0.0063 cm/s. Column effluent was collected in 50 mL sterile polystyrene tubes. To determine the distribution of PAO1 cells in the column, column media was extruded and dissected into five equal segments of 2.8 cm length following each transport experiment. Approximately 42 g of glass beads were obtained from each segment. Column effluent was immediately placed on ice and cells were enumerated by plating appropriate dilutions. The segment samples were added into representative background NaCl solution, sonicated for 10 min, then cells were enumerated by plating appropriate dilutions. Ten minutes of sonication was shown to produce less than 0.1% cell lysis as described previously (Liu and Li 2008). Anaerobic experiments were performed in an anaerobic chamber using anaerobic NaCl solution. Tubes containing segment samples from the anaerobic experiments were sealed with vinyl tape before they were removed from the chamber for sonication. All experiments were performed at least in triplicate at room temperature (22 ± 1 °C).

To quantitatively compare the overall deposition of aerobic and anaerobic PAO1 cells at different ionic strengths, the deposition rate coefficient k_d was determined using the steady state breakthrough cell concentrations using equation (4.4) (Walker et al. 2004):

$$k_d = -\frac{U}{fL} ln\left(\frac{C}{C_0}\right) \tag{4.4}$$

where f is the media porosity, U is the approach (superficial) velocity, and L is the length of the column. C/C_0 is the normalized breakthrough cell concentration related to "clean bed" conditions; C/C_0 was obtained by averaging the breakthrough concentrations between 3 and 4 pore volumes from each breakthrough curve. Based on classic colloid

filtration theory, the theoretical particle deposition pattern S(X) in the packed column can be determined using equation (4.5) (Tufenkji et al. 2003):

$$S(X) = \frac{t_0 f k_d C_0}{\rho_b} exp\left(-\frac{k_d X f}{U}\right) \quad (4.5)$$

where S(X) is the particle deposition pattern, i.e., the number of deposited cells per mass of the granular collector, X is the column depth, ρ_b is the porous media bulk density, and t_0 is the duration of the continuous particle injection.

4.2.8 Bacterial enumeration procedures

Viable bacterial cell counts were obtained using the drop plate method (Ellery and Schleyer 1984). A series of 10-fold dilutions was performed and 10 μL of each dilution was plated on LB agar plates (aerobic) or LB agar plates amended with 5 mM $NaNO_3$ (anaerobic) in triplicate. In aerobic experiments, plates were incubated at 37 °C for 20 h before counting. In anaerobic experiments, cells were plated and stored in the anaerobic chamber at room temperature for 2 days (early stationary phase, data not shown) before counting. The lower limit of this detection method is 10^2 CFU/mL. To guarantee the sterile condition, anaerobic LB agar plates were prepared in the sterile biosafety hood and stored in the anaerobic chamber for at least one week before use (Borriello et al. 2004).

4.2.9 Statistical analysis

Electrokinetic potentials and breakthrough concentrations were analyzed with a single factor analysis of variance (ANOVA) and reported as *p*-values. ANOVA was performed using Microsoft Excel software; a p-value smaller than 0.05 suggests that the difference was statistically significant.

4.3 Results and discussion

4.3.1 Electrokinetic potential of bacterial cells

The zeta potentials of *P. aeruginosa* PAO1 are presented in Figure 4.1. The results indicated that under both aerobic and anaerobic conditions, PAO1 bacteria were negatively charged over the range of ionic strengths (1, 10, and 100 mM) and pH conditions (6.3-6.6) tested. Under both conditions, the absolute magnitude of the cell zeta potentials decreased with an increase in NaCl concentration, as expected from compression of the electrostatic double layer. Differences in the bacterial zeta potential between aerobic and anaerobic conditions were not significant ($p > 0.05$). The zeta potentials of the glass beads indicated that the glass beads were more negatively charged than the PAO1 cells under all ionic strengths tested.

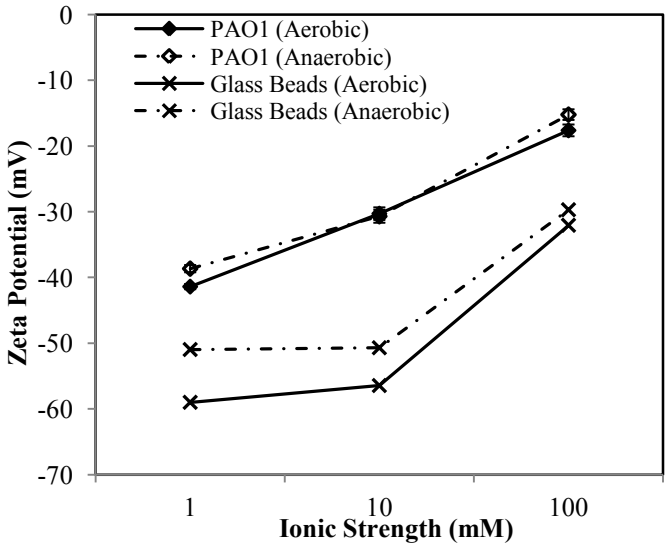

Figure 4.1 Surface zeta potentials of *P. aeruginosa* PAO1, and glass beads as a function of solution chemistry. Ionic strengths: 1 mM, 10 mM, 100 mM NaCl solution. Error bars represent standard deviations of three measurements.

4.3.2 Column transport experiments

Figure 4.2 presents the bacterial breakthrough curves showing the normalized effluent concentration, C/C_0, during the injection and elution of *P. aeruginosa* cells under aerobic (A) and anaerobic (B) conditions. Under both conditions, the injected bacteria were detected in the column effluent within one pore volume. Effluent concentrations of PAO1 cells increased gradually with the injection of cell suspension. Relatively constant breakthrough plateaus were reached after three pore volumes' injection and remained relatively steady until switching to cell-free solutions after injecting four pore volumes at all three ionic strengths. The magnitude of the steady-state breakthrough plateau was highest at 1 mM ionic strength, and decreased with an increase in salt concentration. At 10 and 100 mM ionic strength, PAO1 anaerobic breakthrough concentrations were significantly higher than aerobic breakthrough concentrations ($p < 0.05$), indicating that fewer PAO1 cells were retained in the columns under the anaerobic condition. However, at the lowest ionic strength (1 mM), anaerobic and aerobic breakthrough concentrations were the same ($C/C_0 \approx 0.9$) ($p > 0.05$). The fact that the change in magnitude of the breakthrough concentrations of anaerobic PAO1 cells among different ionic strength was less than that of aerobic PAO1 cells suggested that aerobic PAO1 cells were more sensitive than anaerobic PAO1 cells to solution ionic strength changes.

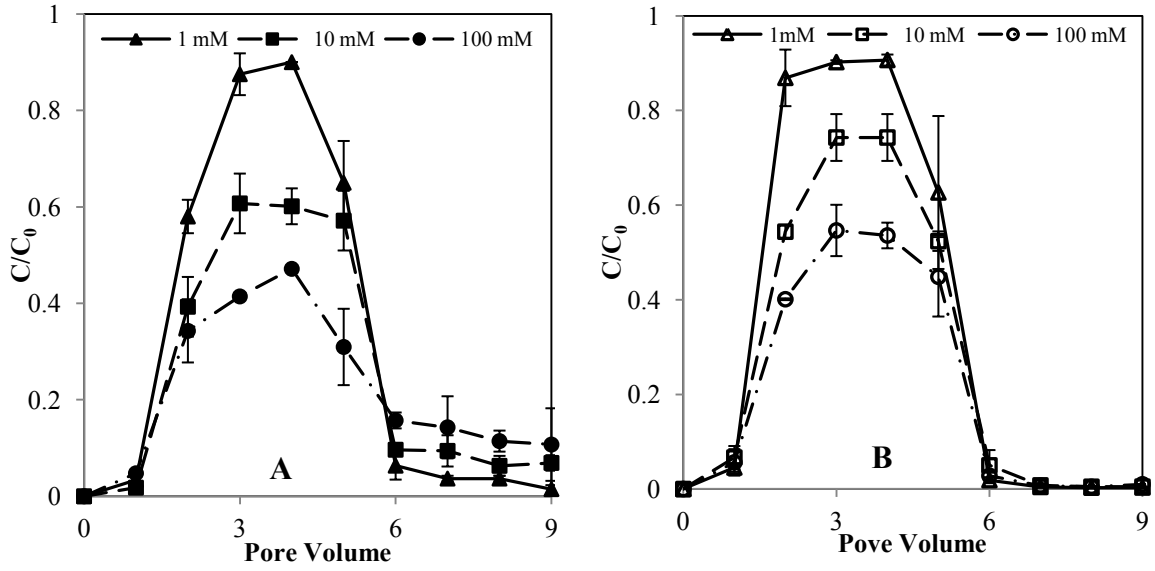

Figure 4.2 Breakthrough curves of *P. aeruginosa* PAO1 under three solution ionic strengths: 1 mM, 10 mM, and 100 mM NaCl, under aerobic (A) and anaerobic (B) conditions. Error bars represent standard deviations of three measurements.

During elution, PAO1 tailings were observed in the aerobic columns over the three ionic strengths. The magnitude of PAO1 tailings increased with an increase in ionic strength, with the most significant tailings observed at 100 mM ionic strength. However, PAO1 tailings in anaerobic columns were not obvious. It is likely that tailings were caused by the detachment of previously attached bacteria (Zhang et al. 2001). The solution pH of the influent and effluent were measured and remained in the range of 6.3-6.6 throughout the experimental process.

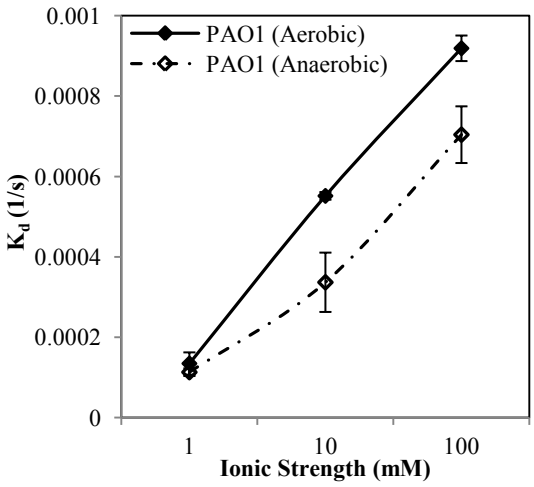

Figure 4.3 Comparison of deposition rate coefficient (k_d) under aerobic and anaerobic conditions determined from the breakthrough under various solution chemistry. Error bars represent standard deviations of three measurements.

As shown in Figure 4.3, the deposition rate coefficients increased at higher ionic strengths under both aerobic and anaerobic conditions. This is in qualitative agreement with the prediction of DLVO theory, which posits that increasing the ionic strength reduces the electrostatic double layer repulsion between negatively charged cells and glass beads. PAO1 cells in aerobic and anaerobic conditions showed different deposition rate coefficients when ionic strengths were 10 mM and 100 mM ($p < 0.05$), with the aerobic PAO1 having higher deposition rates. However, deposition rate coefficients of aerobic and anaerobic PAO1 cells at 1 mM were not statistically different ($p > 0.05$). The deposition rate coefficients results were consistent with the breakthrough concentrations analysis.

4.3.3 Profiles of retained bacterial cells

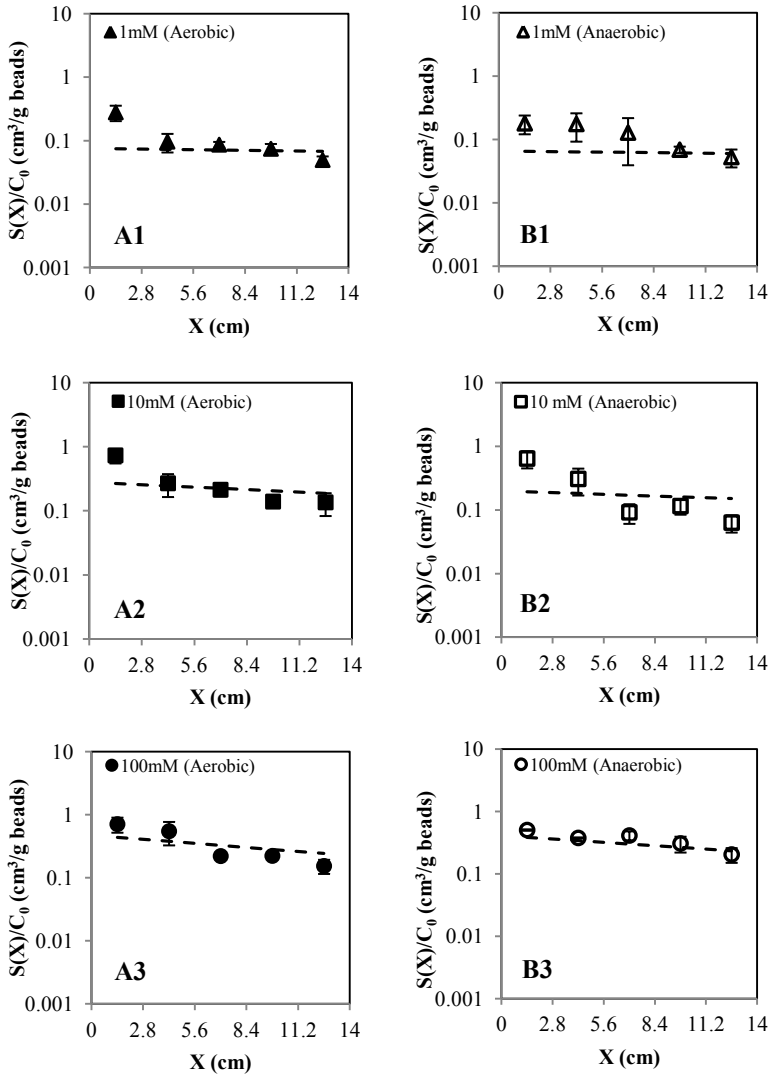

Figure 4.4. Measured retained PAO1 cell concentration profiles in single 14 cm column under aerobic (A1-A3), and anaerobic (B1-B3) conditions. The ionic strengths for the carrying solutions are as follows: 1 mM NaCl (A1, B1); 10 mM NaCl (A2, B2); and 100 mM NaCl (A3, B3). Dashed lines represent predictions based on the classic colloid filtration theory (CFT) using deposition rate coefficient (k_d). Data were presented in a semilog format. Error bars represent standard deviations of three measurements.

Figure 4.4 compares the retained bacterial profiles obtained by plotting the number of bacteria recovered from glass beads as a function of travelled distance and those to be recovered from the classic colloid filtration theory. The magnitudes of retained profiles for anaerobic and aerobic PAO1 cells under three examined ionic strengths varied contrarily with the breakthrough plateaus, in agreement with the mass balance consideration, which indicated that the increase of ionic strength introduced greater cell retention in the porous media. Further inspection of Figure 4.4 indicated that the retained bacterial concentrations decreased hyperexponentially, indicating that the deposition rate coefficients decreased with increasing travel distance in both aerobic and anaerobic conditions. Since a spatially constant deposition rate coefficient could yield a log-linear retained bacterial profile, the hyperexponential profiles were demonstrated by comparison to log-linear profiles developed using the deposition rate coefficients calculated from equation 4.4. However, the discrepancy was less distinct at 100 mM for both aerobic and anaerobic experiments. This observation was consistent with what Li et al. (Li et al. 2004) had reported previously. According to Li et al. (2004), the observed colloidal deposition profiles were much steeper than the expected profiles based on a constant deposition rate coefficient in porous media with travel distance were greatest at highly unfavorable conditions (low ionic strength). This indicated that the deposition rate coefficient decreased with travel distance, as shown in our deposition profiles at lower ionic strength. They further concluded that when solution ionic strength increased, this deviation in the deposition profile from log-linear decreases with distance was reduced and eventually eliminated under favorable conditions as the deposition profiles fell into the expected log-linear profiles based on a constant rate. Furthermore, the

hyperexponential decreases were also possibly driven by the biocolloid population heterogeneity (Tong and Johnson 2007) and steric repulsion due to surface polymers (Tong et al. 2005). Tong and Johnson (2007) demonstrated that colloid surface heterogeneity, such as variations in electrophoretic mobilities among individuals within a population, may lead to a distribution of deposition rate coefficients among the colloid population. They observed that the fast-depositing (sticky) fraction of the colloid population with less electrophoretic mobilities was removed in the up-gradient column and further concluded that this hyperexponential deposition in the inlet did not happen preferentially due to deposition in the secondary energy minimum. Therefore, it was possible that the stickier PAO1 cells were mostly retained in the up-gradient column where less deposition happened in the down-gradient column, leading to a hyperexponential deposition profile. Additionally, a reasonable mass balance (76–97%) was achieved in the column experiments (Table 4.1).

Table 4.1 Cell recovery percentage from column experiments

	Ionic strength (mM)		
	1	10	100
Aerobic	84.8±1.0%	79.8±4.2%	74.8±1.9%
Anaerobic	93.6±6.2	97.4±6.9%	92.5±2.1%

Consider the PAO1 retention profiles and the breakthrough curves, it can be concluded that PAO1 showed higher deposition concentrations on glass bead surfaces in the aerobic condition than in the anaerobic condition. Our results were consistent with those of Sabra et al. (2003) who studied bacterial adhesion on glass tubes. They showed that PAO1

statically grown at higher oxygen concentrations had an increased capacity to adhere to hydrophilic glass tube surfaces, and concluded that the increased adhesion was mainly caused by the oxygen-dependent alterations in cell-surface components (B-band lipopolysaccharides). B-band lipopolysaccharides, which are considered necessary for initial bacterial attachment to hydrophilic surfaces, are high molecular weight serotype-specific lipopolysaccharides produced by *P. aeruginosa*; they consist of two uronic acid deviates and one *N*-acetylfucosamine residue, and are barely expressed in cells grown in a dissolved oxygen tension lower than 3% of air saturation (Makin and Beveridge 1996, Sabra et al. 2003). Under the experimental anaerobic condition, PAO1 likely diminished the expression of B-band LPS, which consequently led to reduced adhesion.

4.3.4 Extracted EPS compositions

The composition of the extracted aerobic PAO1 EPS was significantly different from that of the anaerobic EPS. Both protein and polysaccharides contents were much higher in PAO1 cultivated anaerobically (14.96 ± 0.27 μg/10^9 cells and 53.46 ± 4.05 μg/10^9 cells, respectively) than in PAO1 cultivated aerobically (12.48 ± 0.08 μg/10^9 cells and 43.28 ± 2.10 μg/10^9 cells, respectively) ($p < 0.05$). In general, polysaccharides concentrations were higher than protein concentrations. Although it was reported previously that polysaccharides and proteins enhanced cell deposition (Gomez-Suarez et al. 2002), it is not likely applicable in our experiments for the following reason, discussed below.

The impact of EPS on cell adhesion depends on the specific components of the EPS and the experimental system (Liu et al. 2007). The EPS matrix generally consists of a mixture of macromolecules such as proteins, polysaccharides, nucleic acids, lipids, and

other polymeric compounds. Even though the EPS functions as an initial adhesive, the extent of this function was still unclear due to the various compositions among organisms (Hwang et al. 2012, Liu et al. 2007). Liu et al. (2007) discovered that the presence of EPS significantly increased the PAO1 adhesion in comparison to an EPS deficient PAO1 strain over a wide range of ionic strength (1.65–165 mM phosphate buffered saline). However, Hwang et al. (2012) examined the adhesion of *P. aeruginosa* and *B. cepacia* on glass slides coated with EPS extracted from the two bacteria, and observed a general trend of hindered adhesion of *B. cepacia* onto glass slides coated with both types of EPS throughout the ionic strengths tested (1, 10, 100 mM KCl); but a reduced adhesion of *P. aeruginosa* on the coated glass slides was only observed at high ionic strength (100 mM KCl). In our attempt to discover the EPS composition in aerobic and anaerobic PAO1, bovine serum albumin and glucose were selected as the standards for protein and polysaccharides, respectively, and only concentrations of protein and polysaccharides were measured. Even though the colorimetric methods are well established and widely used (Haznedaroglu et al. 2009, Hwang et al. 2012, Liu et al. 2007), limitations exist (Dubois et al. 1956, Lowry et al. 1951). The protein and polysaccharides content might not be a comprehensive representation of the complex EPS matrix. It is possible that other macromolecules in the EPS matrix also interacted with the glass bead surfaces, leading to a reduced deposition of anaerobic PAO1 in the column porous media.

4.3.5 Bacterial surface elements

Table 4.2 shows the elemental composition of the bacterial surface that resulted from integrating C1s, O1s, and N1s peaks from the XPS scan of the spectrum and the ratios of N/C and O/C (spectra not shown). The N/C and O/C ratios were 0.14 and 0.37,

respectively under anaerobic conditions, while under aerobic conditions, the N/C and O/C ratios were 0.10 and 0.36, respectively. The variations may be caused by the fact that the anaerobic PAO1 were grown in a 5% H_2, 5% CO_2, 90% N_2 atmosphere instead of in ambient air (~ 20% O_2, 80% N_2). Denyer et al. (1990) observed similar results when growing *Staphylococcus epidermidis*. The N/C and O/C ratios (0.190 and 0.428) of *Staphylococcus epidermidis* strains grown in 5% CO_2/95% air atmosphere were higher than N/C and O/C ratios (0.161 and 0.359) of *Staphylococcus epidermidis* grown in air, and they noticed reduced bacterial colonization on polystyrene surfaces in the presence of higher N/C and O/C ratios (Denyer et al. 1990, van der Mei et al. 2000). This was consistent with our results, as fewer PAO1 cells were retained in the column under the anaerobic condition (Figure 2). Additionally, the N/C ratio is also related to the amount of protein presented on the cell surface (Hwang et al. 2012). The higher N/C ratio in the anaerobic PAO1 cells coincided with the higher protein concentration in anaerobic PAO1 EPS extract.

Table 4.2 The percentage elemental composition (atomic concentration %), ratio of N/C and O/C, proportion of carbon associated with three classes of molecular constituents (proteins, polysaccharides, and hydrocarbon-like products), and the weight fractions of each constituent for the bacterial cell surface

						Proportion			**Weight fraction**		
	%C	%N	%O	N/C	O/C	C_{PR}/C	C_{PS}/C	C_{HC}/C	C_{PR}	C_{PS}	C_{HC}
Aerobic	68.41	7.07	24.52	0.10	0.36	0.37	0.29	0.34	0.40	0.37	0.23
Anaerobic	66.10	9.45	24.45	0.14	0.37	0.51	0.23	0.26	0.54	0.29	0.17
Note: The sum of atomic concentrations of O, C, and N for each sample was scaled to 100%.											

Table 4.2 also shows the proportion of carbon associated with the three classes of molecular constituents (proteins, polysaccharides, and hydrocarbon-like products) on bacterial surfaces. Hydrocarbon-like products include phospholipids, glycolipids, and other lipid compounds. Phospholipids and glycolipids are the major components of membrane lipids and extracellular lipids, respectively (Haferburg et al. 1986). Solving equations (4.1-4.3) gave the proportions of carbon in each of the molecular constituents. Dividing this proportion by the carbon concentration in the constituent gave the weight fraction of the constituent in the total concentration of carbon in the sample. In this case, the carbon concentrations were 43.5, 37.0, and 71.4 mmol/g for proteins, polysaccharides, and hydrocarbon-like products, respectively (Shapiro and Dworkin 1997). As shown in Table 4.2, the anaerobic PAO1 cells had a higher protein weight fraction, and lower weight fractions of polysaccharides and hydrocarbon-like products than aerobic PAO1 cells. The predominant constituent of PAO1 under both conditions was protein, followed by polysaccharides, and hydrocarbon-like products. These results complement the EPS analysis. The EPS analysis measured the protein and polysaccharides of the extractable bacterial surface polymer, whereas the XPS analysis provides information on the overall cell surface characteristics. In addition to the EPS, other cell surface components such as LPS are analyzed by the XPS measurement. Therefore, the XPS results provide a more detailed analysis of the overall cell surface properties.

4.3.6 Interpretation using DLVO theory

Theoretical interaction energy profiles predicted using the DLVO theory for the bacterium-glass bead system are presented in Figure 4.5 at the different solution conditions tested in the transport experiments under both aerobic and anaerobic

conditions. The calculations revealed the presence of a significant repulsive energy barrier for deposition at all ionic strengths, ranging from 1440 kT at 1 mM to 755 and 109 kT at 10 and 100 mM, respectively, in the aerobic condition, and 1110 kT at 1 mM to 692 and 60.4 kT at 10 and 100 mM, respectively, in the anaerobic condition. The theoretical predictions that PAO1 experienced the highest energy barriers to attachment at 1 mM ionic strength in aerobic and anaerobic conditions coincide with the experimental results that much higher breakthrough concentrations ($C/C_0 \approx 0.90$) were observed at 1 mM ionic strength than at 10 mM and 100 mM ionic strength, where relatively smaller energy barriers were predicted. Therefore, at 1 mM ionic strength, PAO1 travelled the longest distance and the pathogen had the most negative zeta potential.

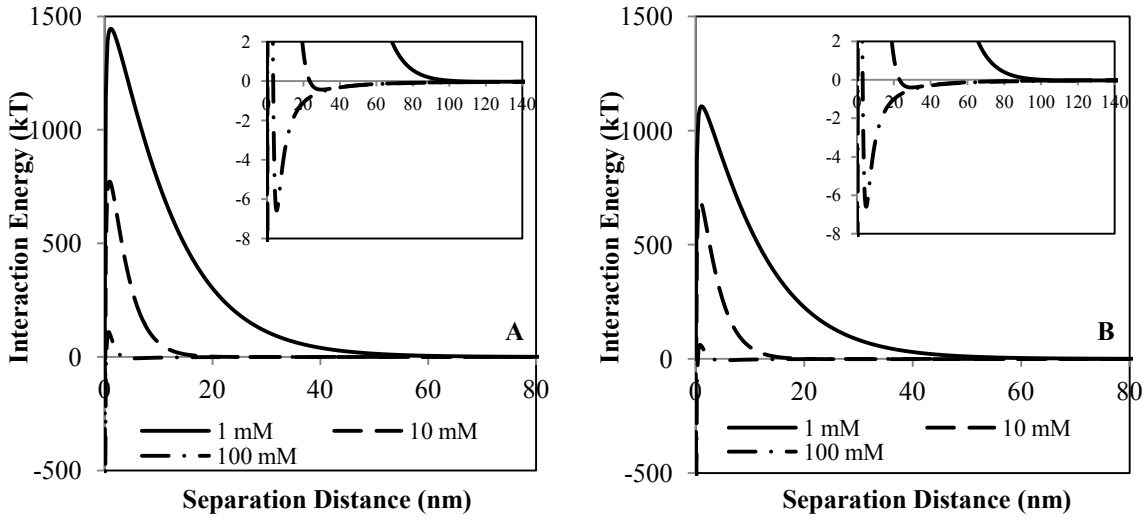

Figure 4.5 Calculated DLVO interaction energy plotted as a function of separation distance for different solution ionic strengths: 1 mM, 10 mM, and 100 mM under aerobic (A) and anaerobic (B) conditions; and (insets) the expanded drawing to highlight the secondary energy minimum. Experimentally measured zeta potentials (Figure 4.1) and a Hamaker constant of 4.16×10^{-21} J were used to calculate interaction energies.

A replot of the DLVO interaction energy profiles on a different scale presented a secondary energy well at a greater separation distance from the collector grain surface than that of the repulsive energy barrier. The depths of the secondary minimum for the PAO1 bacterium-glass bead interaction increased from 0.0294 kT at 1 mM to 0.43 kT and 6.55 kT at 10 mM and 100 mM (aerobic), respectively, and 0.0283 kT at 1 mM to 0.407 kT and 6.58 kT at 10 mM and 100 mM (anaerobic), respectively. The location of the secondary energy wells got closer to the collector surface with respect to a corresponding separation distance of 128.5-5.25 nm, and 125.5-5 nm in aerobic and anaerobic conditions, respectively. It was noticed that despite different growth and experiment conditions, the PAO1 cells had similar profiles of secondary energy wells under aerobic and anaerobic conditions. The increased secondary energy minimum might be responsible for the increased retention at higher ionic strength in the column porous media, because more PAO1 cells could be trapped in the deeper secondary energy wells when approaching the collector surfaces at higher ionic strength (100 mM). When comparing the experimental deposition profiles, it was clear that in the aerobic condition, PAO1 had a significantly higher deposited concentration in the porous media at 10 and 100 mM than in the anaerobic condition. The higher energy barrier and slightly higher/or similar secondary minimum in the aerobic condition however suggested that it would have less deposition concentration in the porous media than that in the anaerobic condition, which do not agree with the experimental results. The above discussion suggested that DLVO forces might not be the only interaction that play a role in the attachment of bacterial to collector beads at relatively high ionic strength (10 and 100 mM). Particularly, at 10 and 100 mM, the electrical double layer repulsion has been

significantly compressed (Chen and Walker 2012). Possible contributions to the cell attachment include the surface heterogeneity as recently reported by Pazmino et al. (2014). In addition, the attachment would be due to some specific interaction between cell surface polymers such as EPS and LPS, and glass beads. The surface composition characterization suggested that the cells in the aerobic condition was slightly less hydrophilic (with less N/C and O/C), suggesting that it might have weaker steric interactions between the hydrophilic surface polymers and the beads than those in the anaerobic condition (Liu 2013, Lu et al. 2011, Pradier et al. 2005).

4.4 Conclusions

We have studied the deposition behavior of *P. aeruginosa* PAO1 in granular porous media and analyzed PAO1 cell surface properties under aerobic and anaerobic conditions. To our knowledge, the effects of oxygen tension during growth and transport of *P. aeruginosa* PAO1 in granular porous media have not been previously reported. PAO1 showed distinctly different transport and deposition behaviors when grown and travelling in respective anaerobic and aerobic conditions. Specifically, at higher solution ionic strengths (10 and 100 mM), aerobic PAO1 showed significantly higher retention in the porous media and travelled less distance than anaerobic PAO1. However, at low solution ionic strength (1 mM), differences in retention and travel distance were not observed.

Significant variation in cell surface electrokinetic potentials was not observed in PAO1 between anaerobic and aerobic conditions. DLVO theory predicted higher energy barrier and slightly higher/or similar secondary minimum in the aerobic condition than in the anaerobic condition, suggested a less deposition in the porous media in aerobic condition, which did not agree with the experimental results. Analysis of cell surface

composition using XPS revealed variations in constituents, as presented in the form of N/C and O/C ratios of the bacterial cell surface when cells were grown at different DO levels. Cell surface variations in the N/C and O/C ratios were believed have contributed to the different levels of deposition, as higher disposition was observed in anaerobic condition with higher N/C and O/C ratios. It was believed that DLVO forces might not be the only interaction that played a role in the attachment of bacteria to collector beads, interactions between cell surface polymers and glass bead surfaces were most likely involved. The increase in PAO1 deposition with increasing ionic strength under both aerobic and anaerobic conditions is thought to be due to compression of the electrostatic double layer.

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Chapter 5. Filtration of *Cryptosporidium* Oocysts Surrogates in Porous Media: Effects of Flow Rate, Alum, and Humic Acid*

*A version of this chapter has been submitted for journal publication.

5.1 Introduction

Cryptosporidium parvum is one of the disease-causing microbial contaminants frequently found in surface waters, posing great health risk management challenges for drinking water supplies. Outbreaks of cryptosporidiosis disease, a severe gastrointestinal illness are often caused by the penetration of *C. parvum* oocysts through drinking water treatment systems and are potentially fatal for people with compromised immune systems (Gitis 2008). They are usually released into the environment via feces of infected host organisms, and have unusual resistance to traditional disinfectants such as free chlorine and chloramines due to their thick cell walls (Finch et al. 1993). The typical disinfectant dosages and contact times used in practice are not as effective at inactivating *C. parvum* oocysts (Dai and Hozalski 2003). Ozone is found to be a much more effective disinfectant, but the formation of disinfection by-products bromate compounds is also a great concern for bromide contaminated water. The widespread use of ultraviolet light irradiation (UV), however, is limited due to the high operation price. Although the UV irradiation is quite effective (Clancy et al. 2000).

The high resistance of *Cryptosporidium* to disinfection has led to a growing emphasis on oocyst removal by filtration (Dai and Hozalski 2003). The filtration is considered as the most critical barrier for *Cryptosporidium* removal in drinking water treatment plant. Some of the reported filtration systems include granular filtration, riverbank filtration, and slow sand filtration (Gottinger et al. 2013, Huck et al. 2002, Tufenkji et al. 2004). However, utilization of *Cryptosporidium* has its own limitations. Firstly, the cultivation and maintenance is not easy and the biohazard disposal requires very careful attention (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds48e-eng.php). Secondly, viable

Cryptosporidium often poses high health concerns on research personnel and has restricted access in some areas, which makes research using *Cryptosporidium* difficult. For example, according to Public Health Agency of Canada, *Cryptosporidium parvum* is classified as a Risk Group 2 pathogen and should be handled in facilities no lower than Containment Level 2 (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds48e-eng.php). Lastly, the surface characteristic of inactivated oocysts is often less well defined and may not be representative of live oocysts (Pang et al. 2012).

Therefore, surrogates with well defined properties to mimic live *Cryptosporidium* have been explored. The commonly used surrogates are microspheres (Dai and Hozalski 2003, Harvey et al. 2008, Tufenkji and Elimelech 2005a). Dai and Hozalski (2003) compared the filtration of 5 μm carboxylated latex microspheres and oocysts through 550 μm spherical glass beads packed polycarbonate plastic column and obtained similar removal efficiencies. They suggested that microspheres could be used as a conservative surrogate of *C. parvum* oocysts in filters containing hydrophilic negatively charged filter media. However, microspheres can potentially either over- or under-predict the transport of *C. parvum* oocysts in aquifer due to the various surface properties of oocysts and diverse characteristics of aquifers from site to site (Harvey et al. 2010). For example, Tufenkji and Elimelech observed underestimated removal of microspheres in silica beads (Tufenkji and Elimelech 2005a), but overestimated removal of the oocyst-sized carboxylated polystyrene microspheres was observed by Harvey et al. in columns packed with intact limestone cores (Harvey et al. 2008).

Since surface charge has the greatest effect on particle transport and retention (Harvey et al. 1989), Pang et al. (2009) proposed that mimicking the surface charges of

microorganisms by modifying microspheres using protein that had similar surface charge to *Cryptosporidium* could potentially provide better defined surrogates and more accurate results (Pang et al. 2009). Later they showed that glycoprotein-coated microspheres best resembled the filtration of oocysts in sand media in 1 mM NaCl solution in terms of surface charge (Pang et al. 2012). However, further validation of these surrogates in various environmental conditions is needed, and mechanisms involved in the fate and transport process need to be explored. In addition, since these surrogates were prepared only to mimic the surface charges of viable *Cryptosporidium*, limitations of these surrogates such as surface hydrophobicity, still exist.

Besides surface charge, many factors can affect the particle removal in porous media, such as grain size and shape (Bradford et al. 2007, Syngouna and Chrysikopoulos 2011), flow velocity (Syngouna and Chrysikopoulos 2011), chemical treatment (Gross and Logan 1995), natural organic matter (NOM) such as humic acid (HA) (Yang et al. 2012a), particle size (Tufenkji and Elimelech 2005a). In water treatment, the varied flow rate is perhaps one of the most often encountered situations during filtration process. Higher flow velocity is shown to reduce the retardant and increase the transport of colloidal particles in porous media through increased hydrodynamic force (Kim et al. 2010), therefore leads to reduced removal of *Cryptosporidium* in the filter bed. Serving as the most commonly used coagulant in water treatment, alum can effectively remove suspended particles (58.1%) for the water purification, and increase the size of colloidal particles to be settled by decreasing the stability of suspended particles and promoting the formation of flocs (Karanis and Kimura 2002), which additionally increases the removal in porous media (Yao et al. 1971).

Further, the raw water quality does not remain unchanged. In particular, natural organic matter such as humic acid is ubiquitous in water supplies. When *Cryptosporidium* enters the natural water systems, its surface properties can be altered by the adsorption of negatively charged HA, and these changes could reduce the removal of *Cryptosporidium* in filters (Hsu et al. 2001, Yang et al. 2012a). Additionally, HA in water can absorb onto solid surfaces and hereby change the solid surface properties. It was found that HA ubiquitous in water interacted with the divalent ion (Ca^{2+}) in a more complicated fashion than when either of HA or Ca^{2+} is present and could either enhance or reduce particle stability depending on the surface properties of particles and the relative concentrations of divalent ions and humic acid (Chen and Elimelech 2007, Dong and Lo 2013, Liu et al. 2011). Therefore, it is hypothesized that the HA presenting in water would also interact with alum and influence the removal of *Cryptosporidium*. However, experimental validation for this hypothesis is required to examine if, how and to what extent HA could affect the removal efficiency.

Therefore, it is important to examine the effects of flow rate, alum addition and HA adsorption on collector surfaces on the fate and transport of *Cryptosporidium* in porous media and explore the mechanisms involved in the deposition progress. The objective of this study was to evaluate the filtration of the *Cryptosporidium* surrogates and verify the applicability of the surrogates under effects commonly encountered in water treatment, and explore the mechanisms involved in the transport and deposition process. The surface properties of the surrogates were tested, and filtration experiments were performed in different solution and hydrodynamic conditions broadly representing water treatment conditions. The microsphere breakthrough curves and deposition profiles were

constructed. DLVO interaction energy was calculated to interpreting the deposition behavior.

5.2 Materials and methods

5.2.1 Microspheres and conjugation of biomolecules with microspheres

The carboxylated polystyrene microspheres from Polysciences, Inc. (Warrington, PA) were used in this study. To mimic the size and shape of *C. parvum* oocysts, microspheres with an average diameter of 4.5 μm (diameter coefficient variation (CV) = 7%) were chosen. Based on the manufacturer's description, the microspheres had yellow green (excitation 441 nm, emission 486 nm) fluorescence. Per milliliter of the 2.5% aqueous suspension had approximately 4.99×10^8 particles. Additional 10 μm microspheres (CV = 10%, 4.55×10^7 particles/mL 2.5 % aqueous suspension) were also obtained to examine the effect of increases particle size on the filtration efficiency. The microsphere suspension was stored in dark at 4 °C.

To mimic the surface charge of the oocysts, an alpha-1-acid glycoprotein (AGP) (Sigma-Aldrich, Oakville, ON) with isoelectric point (IP) value (indicating surface charge) similar to that of oocysts was selected and stored at 4 °C for conjugation. According to the manufacturer product information, the AGP has a molecular weight of 33,000 to 40,800 g/mol, an IP of 2.7 in phosphate buffer, and a water solubility of 10 mg/mL. Details of the sequence of the polypeptide portion of AGP have been described elsewhere (Pang et al. 2012, Schmid et al. 1973).

5.2.2 Humic acid solution preparation

The influence of humic acid on the transport behavior of modified microspheres was also examined in this study. The Suwannee River Humic Acid standard II purchased from International Humic Substances Society was selected as model natural organic matter in this study. HA solution was prepared following the method described previously (Yang et al. 2012a). The total organic carbon (TOC) content of the HA stock solution was 47.2 mg/L as determined by a TOC analyzer (Mandel, ON, Canada).

5.2.3 Determination of zeta potentials and particle size

The zeta potentials of the unmodified and modified microspheres (4.5 and 10 μm) with or without 5 mg/L alum addition were determined using a Brookhaven Zetaplus (Brookhaven Instruments Co., Holtsville, NY) at 25 °C. Prior to the zeta potential measurement, each sample was sonicated in a cold water bath for 1 min. The zeta potentials (ζ) were measured in a background electrolyte of 1 mM KCl at the experimental pH values. To measure the zeta potentials of sands coated with or without HA, the sand samples were oven-dried, crushed to fine powders using a bench-scale grinder, and measured in the same background electrolyte. The obtained zeta potential values were used in place of the surface potentials of microspheres to determine the interaction energies between sand and the 4.5 μm microspheres using the DLVO theory as described previously (Zhang et al. 2015). A value of 6.5×10^{-21} J was used as the Hamaker constant for the quartz-microsphere-water system (Kuznar and Elimelech 2006). The average diameter ($n \geq 30$) of the modified microspheres was obtained from the microscope observation.

5.2.4 Porous media

The sands with an effective size of 0.35 mm, and a uniformity coefficient of 1.5 were used for microsphere deposition experiments in porous media. The sand particles are often used by water treatment utilities as rapid sand filter media (Crittenden et al. 2012). The procedure used for cleaning the sands was described previously (Xu et al. 2008). Briefly, the sands were cleaned with boiling nitric acid (70%) for 24 h, followed by alternating rinse with 0.1 M NaOH solutions and deionized water. The cleaned sands were oven-dried at 80 °C and then used in the column experiments. The acid-base washing procedure was adopted to remove surface impurities, such as iron hydroxide, organic coatings, and very fine particles that could promote favorable deposition of the colloids.

5.2.5 Experimental conditions

The filtration system consisted of acrylic columns with an internal diameter of 3.175 cm, and a length of 14 cm. The porosity of the packed columns was approximately 0.46. Columns were dry-packed with mild vibration to ensure uniformity. Pre-equilibration was performed by introducing at least 6 pore volumes of microsphere-free 1 mM KCl solution into the columns in an up-flow mode using a peristaltic pump (Cole Parmer, IL). For experiments examining the effects of HA, pre-equilibration was done by firstly introducing 3 pore volumes of 1 mg/L HA in 1 mM KCl solution, and then rinsing with 2 pore volumes of microsphere-free 1 mM KCl solution (Yang et al. 2012a). The remaining HA in column was found to be approximately 3×10^{-4} mg/g sand.

Following pre-equilibration, 4 pore volumes of modified microspheres (4.5 μm or 10 μm) with or without 5 mg/L alum in solutions were injected into the column, followed by elution with 2 pore volumes of microsphere-free 1 mM KCl solution. The transport experiments were conducted in 1 mM KCl solutions at pH = 6.0 ± 0.1 without pre-adjustment of the saline solution. Experimental conditions tested in this study are listed in Table 5.1. It should be noted that for experiments that considered the effects of alum, a reduced pH of 5.7 ± 0.1 was observed due to the addition of alum. Three pore water velocities, 4.6 m/day (2.5 mL/min), 10.9 m/day (6 mL/min), and 21.8 m/day (12 mL/min), were selected to broadly represent fluid velocities in various environments, such as coarse aquifer sediments, forced-gradient conditions, and engineered water filtration units (Yang et al. 2012a).

Table 5.1 Porous media experimental conditions (1 mM KCl) and mass recovery

Modified microspheres	Flow rate (m/day)	Alum in solution	HA coated sand	Recovery (%)
4.5 μm	4.6	No	No	82.0
		Yes	No	90.4
	10.9	No	No	86.4
		Yes	No	86.0
		No	Yes	95.0
		Yes	Yes	98.0
	21.8	No	No	99.7
		Yes	No	97.8
10 μm	10.9	No	No	87.6
		Yes	No	76.9
		No	Yes	93.0
		Yes	Yes	99.4

5.2.6 Sample collection and analysis

Samples from the column effluent were collected in 50 mL polystyrene tubes for every half pore volume (PV). Following the experiments, the column media were equally extruded and dissected into five segments of 2.8 cm length. Approximately 39 g of sands were obtained from each segment. Retained microspheres were recovered by placing sands from each segment into specified volumes of 1 mM KCl solution and sonicating for 10 min, followed by vigorous vortexing for 1 min. Aqueous effluent samples and supernatant samples from the recovered retained microspheres were analyzed using a flow cytometry (BD FACScan, Beckton Dickinson & Co., FranklinLakes, NJ) at a flow rate of 1 μL/s and were counted for 1.5 min. Conversion of counts on the flow cytometer

to microspheres concentration was achieved by calibrating using a BD Trucount™ Absolute Counting Tube with known bead count in each tube.

5.3 Results and discussion

5.3.1 Electrokinetic properties of microspheres and DLVO interaction energy calculations

Table 5.2 represented the measured zeta potentials of modified and unmodified microspheres (4.5 and 10 μm) in 1 mM KCl. The zeta potentials of the unmodified microspheres were negative for both sizes, and the absolute values were close, indicating similar surface properties. After surface coating with AGP, the zeta potential of the 4.5 μm microspheres became less negative, and the absolute value decreased from the original 33.9 mV to 19.9 mV (pH = 6.0). Our measured zeta potential of the 4.5 μm modified microspheres was close to what was reported previously by Pang et al. (2012) where they obtained a value of approximately -25 mV of glycoprotein coated microspheres at the pH of 6. To better interpret the surface electrokinetic potential of the modified microspheres, zeta potentials of viable and irradiated *Cryptosporidium* in the same background solution were also measured as shown in Table 5.2. The irradiated *Cryptosporidium* (-28.7 mV) was more negatively charged than the viable ones (-14.2 mV), indicating altered surface properties after irradiation. Upon introducing 5 mg/L alum, the zeta potentials of the modified 4.5 μm microspheres in 1 mM KCl became positive (34.4 mV) due to charge neutralization. Similar results were also reported by Bustamante et al. (2001), they observed a reversal of zeta potential to positive values for oocysts with increased alum dosage. The free aluminum and aluminum hydroxide species

in solution were able to form complexes with or to neutralize the negative charges on the particle and collector surfaces (Dai and Hozalski 2003).

Table 5.2 Zeta potentials of microspheres in 1 mM KCl and sands

Zeta potential (mV)								
Microspheres (μm)			Sand				*Cryptosporidium*	
	4.5	10	Clean	HA[b]	Alum[c]	Alum[c]+HA[b]	Viable	Irradiated
Unmodified	-33.9	-30.7	-35.3	-46.4	26.2	20.6	-14.2	-28.7
Modified	-19.9	-17.5						
Modified+ alum[a]	34.4	9.88						
[a] modified microsphere supplied with 5 mg/L alum in solution [b] sand pre-coated with 1 mg/L HA [c] sand in solution containing 5 mg/L alum								

The zeta potentials of sand and sand pre-soaped in HA in our study were -35.3 and -46.4 mV, respectively, possibly due to the adsorption of humic acid onto the negatively charged sand surfaces (Dai and Hozalski 2003). Dai and Hozalski (2003) observed a consistent more negative charge of oocyst and microspheres in the present of NOM, and pointed out that NOM could adsorbed onto negatively charged surfaces regardless of electrostatic repulsion inducing more negative surface. It appeared that HA adsorption onto negatively charged surfaces was likely attributed to hydrophobic interactions (Fein et al. 1999) and polyvalent cations generating complexes with negatively charged groups on both the sand surface and HA (Busch and Stumm 1968). However, after introducing alum into columns, the same reversal of the zeta potential of sand and HA coated sand to

positive values of 26.2 mV, and 20.6 mV, respectively, were again observed likely due to reasons discussed before.

Table 5.3 DLVO interaction energy calculations

			Depth of secondary energy minimum[a] (kT)	Separation distance to secondary energy well (nm)	Energy barrier (kT)
Sand	Modified	i	0.332	106	1730
	Modified + alum	ii	NA[b]	NA	NB[c]
Sand + HA	Modified	iii	0.314	109	1930
	Modified + alum	iv	NA	NA	NB

[a] values calculated within the separation distance of 150 nm

[b] not applicable

[c] no energy barrier

The DLVO interaction energy between the approaching 4.5 μm modified microspheres and sand surfaces were calculated using the measured zeta potential values in four different experimental systems: (i) modified microspheres approaching clean sand; (ii) modified microspheres with alum approaching clean sand; (iii) modified microspheres approaching sand coated with HA; and (iv) modified microspheres with alum approaching sand coated with HA, and the results were presented in Table 5.3. It was noticed that continuously injecting solution containing alum could change the zeta potentials of sand (from -35.3 to 26.2 mV) and HA-coated sand (-46.4 to 20.6 mV) (Table 5.2). Since the zeta potentials of collectors kept changing during the period of experiments, it was difficult to determine the average values that can better represent the zeta potentials during the experiments. Therefore, initial zeta potentials of sand (-35.3

mV) and HA-coated sand (-46.4 mV) were used for the calculation of DLVO interaction energies.

As shown in Table 5.3, an insurmountable energy barrier to attachment was observed when alum is not present in the system (i.e., systems i and iii). System iii showed a higher level of energy barrier (1930 kT) when HA was present than system i (1730 kT) when no HA was present. Closer inspection indicated the present of shallow secondary energy minimum wells for both systems (-0.332 and -0.314 kT for system i and iii at separation distance of 106 and 109 nm, respectively). No energy barrier was observed when the modified microspheres were approaching both clean and HA pre-coated sands when alum was added in the feeding solution (i.e., systems ii and iv), indicating favorable condition for adhesion. But it was possible that with the injection of solution containing alum, the interaction energies would change to less favorable due to the reduced negativity to collector surfaces.

5.3.2 Observed transport and retention behaviors of modified microspheres

5.3.2.1 Influence of flow rate

The influence of different flow rate on the transport and deposition of modified microspheres in clean sand bed column was examined using the modified 4.5 μm microspheres, as the size and zeta potential better mimic the viable *Cryptosporidium*. The breakthrough curves in clean sand as a function of flow rate and the surrogate deposition profiles are shown in Figure 5.1. The relative effluent concentrations (C/C_0) were plotted as a function of ½ pore volume (PV). The normalized concentration of microspheres retained in the clean sand ($S(X)/C_0$ mL/g sand; where $S(X)$ and C_0 were the

number of retained microspheres per gram of sand and the initial concentration of injected microspheres, respectively) were plotted as a function of equal distance from the column inlet. Bradford et al. (2003) reported that Yao's classic colloid filtration theory (CFT) can no longer adequately describe the measured deposition profiles when the ratio of particle diameter to collector grain diameter is greater than around 0.005, and under such conditions, colloid transport in heterogeneous systems was mainly controlled by straining (Bradford et al. 2004). In our study, the ratio is averaged at 0.014 for the modified 4.5 μm microspheres. Therefore, straining likely occurred under all test conditions because the heterogeneity of the sands used in the experiments. However, attachment and the flow bypassing of the finer-textured sands are also important during the transport processes (Bradford et al. 2004). Theoretical prediction based the CFT was not included for all the column experiments. Error bars were obtained from replicate test results.

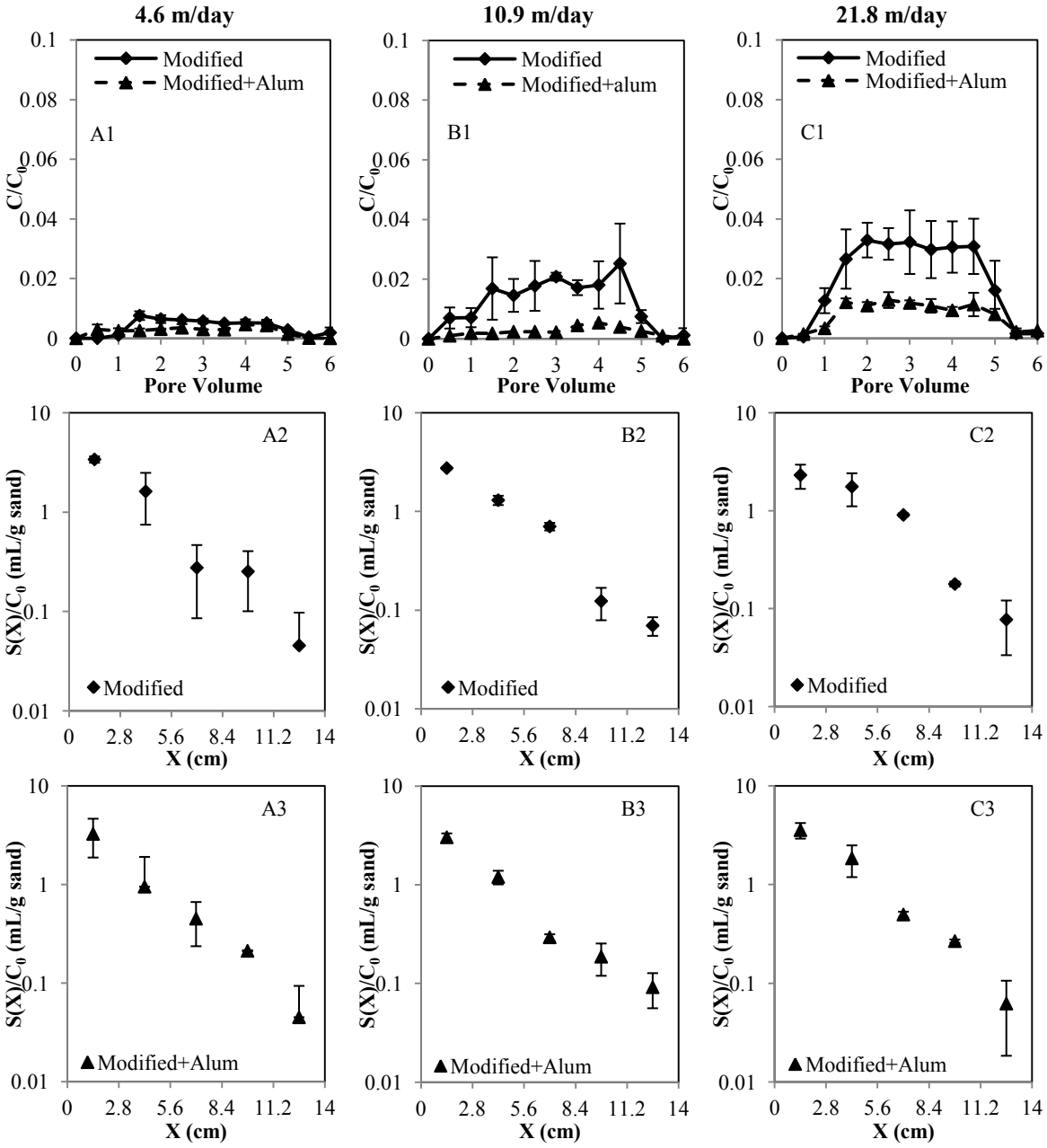


Figure 5.1 Breakthrough curves (A1, B1, C1) and deposition profiles in column bed of (i) modified microspheres in 1 mM KCl and (ii) modified microspheres with alum in 1 mM KCl in the sand column at three pore water velocities: 4.6 m/day (A2, A3); 10.9 m/day (B2, B3); and 21.8 m/day (C2, C3). Error bars were obtained from replicated test results.

As the water velocity increased, the magnitudes of the drag and lift forces that act on particles near collector surfaces would increase accordingly (Li et al. 2005), and the volume of low velocity regions was expected to decrease (Bradford et al. 2007). Enhanced transport of microspheres is usually expected at higher flow rate. The increased hydrodynamic forces may inhibit the retention of particles in the smallest regions of the soil pore space, which is usually described as straining, and induce the shape change of the deposition profile (Bradford et al. 2007). Research has demonstrated that straining of oocysts primarily occurred at the sand surface and at textural interfaces when water flows from a coarser to a finer medium (Bradford and Bettahar 2005). Figure 5.1(A1, B1 and C1) showed that increasing flow rate led to greater transport of the modified microspheres in the sand bed regardless of the addition of alum. Similar trend of flow rate influence was observed by Kim et al. (2010), where they observed greater transport of *C. parvum* oocyst at increased velocity in quartz and Ottawa sands (Kim et al. 2010). Longer travel distance was observed when microspheres were moving without the addition of alum. The addition of alum greatly hindered the motility of microspheres at all levels of flow rate, as agreed by the DLVO interaction energies where favorable condition for adhesion was predicted. The effect of alum was further discussed in Section 5.3.2.2.

The deposition profiles showed less microsphere retention with higher flow rate, consistent with information presented in the breakthrough curves. The shape of the profiles did not seem to have changed greatly, especially for conditions with alum addition. Based on this observation, increase of flow rates seemed have little effect on the shape of the deposition profiles of retained microspheres at the tested range of flow rate. Review on the literature revealed that high velocity (6.48 m/day) could generate fluid

drag forces sufficient enough to inhibit particle retention in pore spaces that readily occurred at lower velocity, eventually produce a less hyperexponential deposition (Bradford et al. 2007). For conditions where no alum was added, the deposited microspheres were found to decline hyperexponentially at 10.9 and 21.8 m/day, while slower than the hyperexponential decrease at 4.6 m/day. In addition, good recovery rates were obtained from the experiments (Table 5.1).

Despite the predicted unfavorable conditions for deposition by the DLVO theory for system i, the majority of microspheres were still retained in the packed sand bed with only rather small amount successfully travelled through the column. As shown in Table 5.3, when approaching to the sand surfaces, the modified microspheres faced a significant energy barrier inhibiting attachment (1730 kT for system i). However, a shallow secondary energy minimum (0.332 kT) existed between the microspheres and sand before encountering the energy barrier. Since larger particles (greater than 1 μm in diameter) are more influenced by the secondary minimum well (Elimelech 1998), it was possible that besides straining, secondary energy minimum well retained a portion of the microspheres. DLVO predictions were calculated based on the assumption that the overall zeta potential represented the surface potential, and the collector had a smooth surface. However, in this study, the surface roughness and heterogeneity of the sand collector cannot be neglected. Hoek et al. (2003) reported that the presence of rough surface produced lower energy barrier for attachment and created valleys with lower interaction energy wells favoring colloidal particle deposition (Hoek et al. 2003). It was possible that in this experiment the pore structure and pore spaces formed by grain-grain contact provided preferred location for particles that were weakly associated with the solid surface under secondary energy

minimum to retain due to reduced hydrodynamic force, pore size limitation, and enhanced DLVO interaction (Hoek and Agarwal 2006). Nanoscale chemical heterogeneity might enhance adhesion between two surfaces as well (Kozlova and Santore 2006).

5.3.2.2 Influence of alum

The effect of alum addition on the filtration of modified microspheres was also examined because alum is a commonly used coagulant in drinking water treatment. The influence of alum on the transport and deposition of modified microspheres in clean sand bed column was examined using 4.5 μm microspheres. Results were shown in Figure 5.1 as well. From the breakthrough curves in Figure 5.1 (A1, B1, C1), it was noticed that the addition of alum greatly reduced the breakthrough concentrations of microspheres comparing to the condition when alum was absent, except at low flow velocity (4.6 m/day). Specifically, at 4.6 m/day, the difference in breakthrough curves between conditions with and without alum was quite minimum; at 10.9 m/day, breakthrough concentration of microspheres without alum started to increase while the one with alum addition remained relatively steady; at the highest 21.8 m/day, microspheres were able to travel the farthest distance under both conditions, and the difference of the breakthrough concentrations between two conditions was most obvious (i.e. $C/C_{0(microspheres)}$ - $C/C_{0(microspheres + alum)}$ = 0.07, 0.017 and 0.003 for 21.8, 10.9, and 4.6 m/day, respectively). It was reported previously that low velocity regions in porous media are hydrodynamically favorable for retention (Torkzaban et al. 2007, Torkzaban et al. 2008). At the low flow rate condition, the microspheres weakly associated with the collector surface could be transported along the grain surface by hydrodynamic shear to regions

that are more favorable for deposition and remain attached (Bradford et al. 2007). As mentioned in Section 5.3.2.1, the addition of alum introduced very minimal change of the shape of the deposition profile. Deposited particle concentration declined monotonically at a rather constant rate (i.e. relatively constant slope). It should be noted that within the experimental duration (< 50 min), no aggregation of the modified 4.5 μm microspheres was observed based on a preliminary size distribution determination (details provided in section 5.3.2.4). Therefore, the effect of size change on the transport of microspheres was not observed under this condition.

Favorable interaction for deposition was obtained under this condition (system ii in Table 5.3). This favorable condition for adhesion well explained the observed less transport and more deposition of microspheres in system ii as compared to the unfavorable condition in system i. Another possible reason for the observed higher deposition was the reduced pH (from 6.0±0.1 to 5.7±0.1) due to the addition of alum. Reducing pH from 8 to 6 was found to reduce the electrophoretic motility and eventually led to greater aggregation and deposition of the negatively charged zero valent iron nano-particles (Kim et al. 2012). The increased acidity in this study might also increased the deposition of the negatively charged microspheres, however the effect could be minimal since the change of pH was not significant. However, very low amount of microspheres still transported through the column bed and were detected in the column effluent as shown by the breakthrough curves. One thing need to mention was that the zeta potential of sand surface used for DLVO calculation was the initial value before alum was supplied, however, with the continuous addition of alum, the collector surfaces might become less negative (as shown in Table 5.2), resulting to a less favorable condition for deposition as

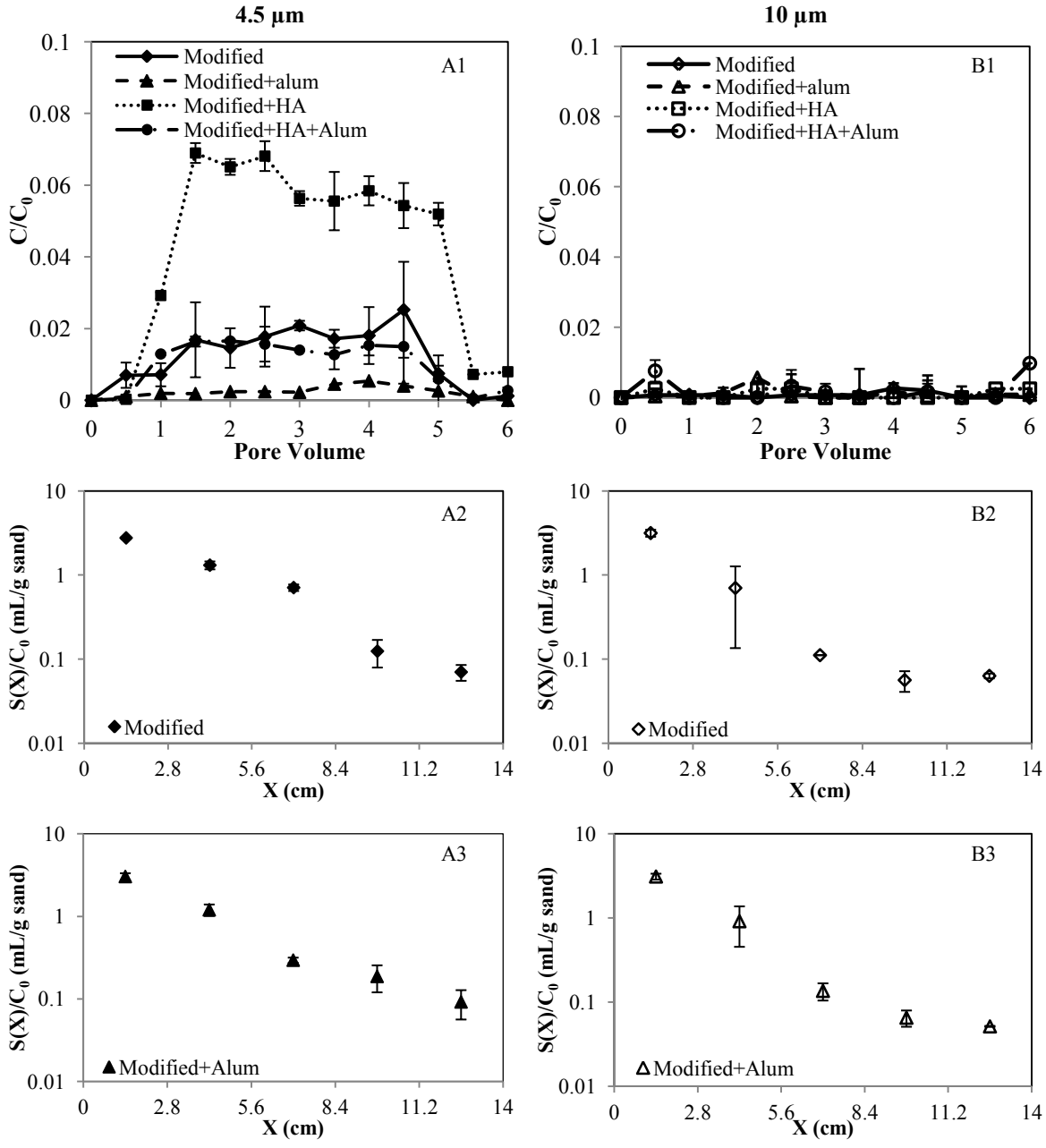
compared to the beginning of the experiments. Less deposition could possible happen towards the end of the experiment period, leading to the low breakthrough concentrations. In addition to that, the steric repulsion between the sand and microsphere surfaces (Kuznar and Elimelech 2006) and blocking (Camesano et al. 1999) were also possible reasons for the observed breakthroughs under the DLVO favorable condition. Microspheres weakly attached to the collectors under the force of steric repulsion may get detached from collector surface and travel along with the flow under hydrodynamic drag force (Kim et al. 2010).

5.3.2.3 Influence of HA

The dissolved HA in solution was reported to hinder the aggregation of colloidal particles and enhance transport in porous media through several mechanisms, including inducing changes in the surface properties of colloidal particles, altering the surface charge of the collector, increasing repulsive electrostatic force between particle and collector, and competing with the particles for deposition site (Dai and Hozalski 2003, Dong and Lo 2013, Liu et al. 2011, Yang et al. 2012a). In our study, to examine the effect of HA on the filtration efficiency of modified microspheres, 3 pore volumes of HA solution (1 mg/L) were injected into the column bed prior to microsphere injection. The zeta potential of sand pre-coated with HA was more negative (-46.4 mV) than that of the clean sand (-35.3 mV). The 4.5 μm modified microspheres and a flow rate of 10.9 m/day were chosen, results were shown in Figure 5.2.

From Figure 5.2 (A1), it was noticed that in the presence of HA solely (system iii), modified microspheres were able to travel the farthest in comparison to the other test

condition (i.e. system i). Previous studies have demonstrated that the site-blocking caused by pre-equilibrating the porous media with HA could inhibit the colloid deposition mostly likely through steric repulsion and perhaps increased electrical repulsion (Foppen et al. 2008, Foppen et al. 2006). Same results were reported by Yang et al. (2012a), as they observed that regardless of solution chemistry, cell types, motility, and EPS, HA enhanced bacterial cell transport. According to them, a site competition by a portion of HA and the repelling deposition (potential steric repulsion) caused by suspended HA in solution seemed to contribute to the reduced cell deposition (Yang et al. 2012a). What should be noticed though, was that in their observation, zeta potential of sand in the presence of HA was similar to that of clean sand. But a more negatively charged zeta potential of sand with HA was obtained in our study, indicating change in the surface charge of the sand. Therefore, it seemed that surface charge alteration resulting in an increased electrostatic repulsion was also a mechanism enhancing the transport of microspheres.


4.5 µm
10 µm
A1
B1
Modified
Modified+alum
Modified+HA
Modified+HA+Alum
C/C0
Pore Volume
A2
B2
A3
B3
S(X)/C0 (mL/g sand)
X (cm)
Modified
Modified+Alum

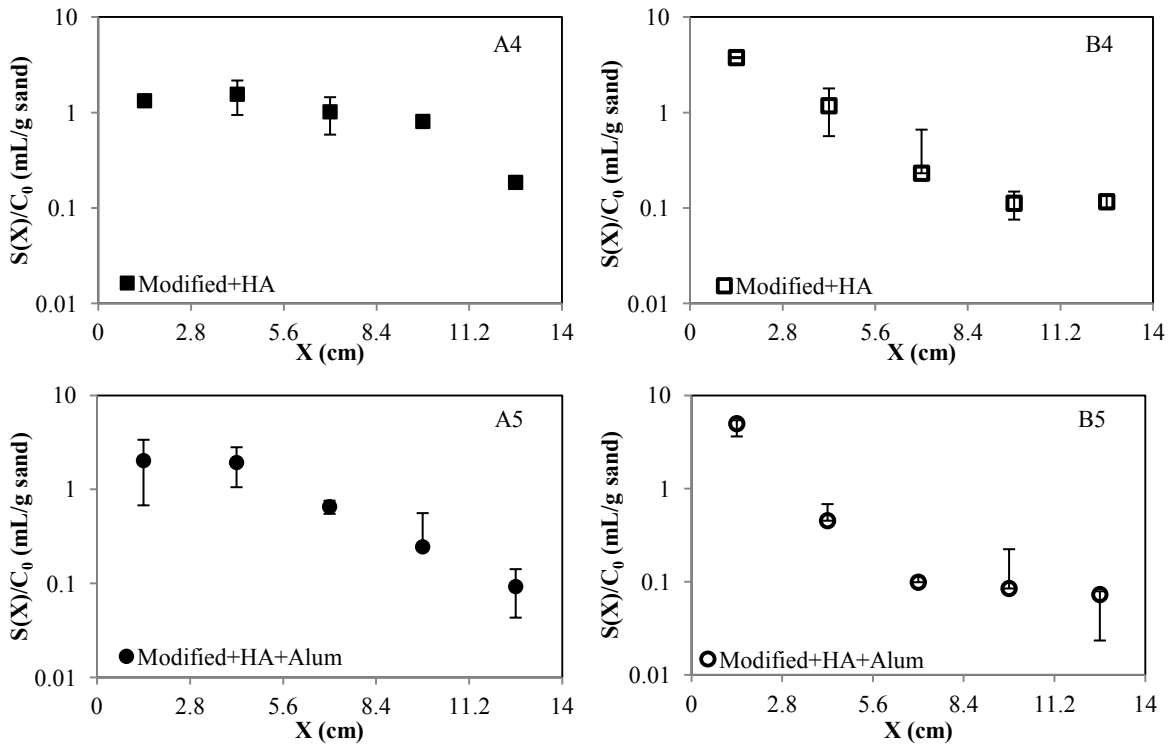


Figure 5.2 Comparison of breakthrough curves (A1, B1) and deposition profiles in column bed of modified microspheres of 4.5 µm (A2, A3, A4, A5) and 10 µm (B2, B3, B4, B5) at (i) modified microspheres in 1 mM KCl only; (ii) modified microspheres with alum in 1 mM KCl; (iii) modified microshperes in 1 mM KCl in the columm pre-equilibrated with HA; and (iv) modified microspheres with alum in 1 mM KCl in the sand column pre-equilibrated with HA. Pore water velocity = 10.9 m/day. Error bars were obtained from replicated test results.

Interestingly when alum was present in solution, the microspheres were not able to travel the similar distance in HA-coated porous media, as the breakthrough plateau of microspheres in the presence of alum and HA (system iv) was much lower than that when only HA (system iii) was present, and it almost overlapped with the breakthrough curves when only microspheres (system i) were present. Specifically, the breakthrough

concentrations of microspheres were in the order of (from high to low): systems iii > i > iv > ii. Compared to conditions where only HA (system iii) was present, the addition of alum also reduced the breakthrough concentrations of microspheres in the presence of HA (system iv). Dai and Hozalski (2003) observed similar results where increased removal of oocyst and unmodified microspheres was obtained in the presence of NOM when alum was supplied as coagulant for particle destabilization as compared to conditions when no alum was added. In their study, the highest collision efficiency was obtained when alum was supported as coagulant in the presence of NOM, possibly due to particle flocculation prior to entering filter bed under such condition. Yao et al. (1971) suggested that flocculation within the pores of filter bed could also increase the filtration efficiency of particles larger than 1 μm. It was possible that flocculation promoted the deposition of microspheres when alum was added in the condition where sands were coated with HA. Another noteworthy observation was the reduced pH (from 6.0 ± 0.1 to 5.7 ± 0.1) when alum was supplied as coagulant, as discussed in Section 5.3.2.2. In addition, studies showed that in the presence of HA, when divalent ions (such as Ca^{2+}) was supplied, nanoparticles had greater aggregation due to bridging interaction of humic acid and Ca^{2+} (Chen and Elimelech 2007, Dong and Lo 2013, Liu et al. 2011). It seemed that instead of steric repulsion, humic acid likely aggregated through intermolecular briding with alum complexation (Dai and Hozalski 2003) to form humic acid aggregates, which then bridged the microspheres and aggregated together, forming bigger size of aggregates that could be more easily removed by filtration (Chen and Elimelech 2007). Deposition profiles of the microspheres in the HA-coated porous media with or without alum addition showed similar pattern. The deposited concentrations first increased

slightly then gradually decreased, mostly likely due to the interactions between the microspheres and HA adsorbed on the sand surface, indicating that the deposition rate changed non-monotonically through travel distance. Giving that the sand was pre-equilibrated, HA adsorbed on the sand surface likely contributed to this pattern of deposition as discussed by Redman et al. (2001a, 2001b), where they attributed the increase-then-decrease deposition pattern with distance across the column to the adsorption of organic matter. According to them (Redman et al. 2001a, 2001b), the adsorbed organic matter near the column inlet could decrease colloid attachment through increased electrostatic repulsion between the colloids and the collector surfaces, leading to the down-gradient movement of maximum concentrations of retained microspheres in the column bed.

By looking at the DLVO interaction energy profiles, a bigger energy barrier (1930 kT) and a shallower secondary energy minimum (0.314 kT) was observed when microspheres and HA were together (system iii), indicating more unfavorable condition for deposition in comparison to system i. Evidence was the higher breakthrough concentration of system iii as compared to system i as shown in Figure 5.2 (A1). The DLVO interaction profiles of system iv showed favorable conditions for deposition as well, which also explained the higher deposition of microspheres in system iv as compared to system iii. Compared to system ii, which was also favorable for deposition, microspheres in system iv had higher breakthrough concentrations. This may be attributed to blocking of previously attached microspheres (Camesano et al. 1999) and electrostatic repulsion between the micropsheres and sands (Redman et al. 2001a, Redman et al. 2001b).

5.3.2.4 Influence of particle size

With the consideration of potential size increase due to aggregation of oocyst-sized microspheres, additional experiments using modified 10 μm particles were perform at flow velocity of 10.9 m/day under conditions with or without alum/HA (Table 5.1). Preliminary size distribution results with modified 4.5 μm microspheres showed that 100 min after alum addition, clusters of two to three microspheres began to form, resulting in equal spheres with an approximate maximum diameter of 9.6 μm (data not shown). Since the particle injection duration within the experimental period was less than 50 min, it was safe to say that microsphere aggregation was almost unlikely to occur and 10 μm was the maximum size that could be encountered during the experiment if any.

Inspection of the breakthrough curves of the modified 10 μm particles showed an overall significantly lower breakthrough concentration of all conditions tested (Figure 5.2, B1). According to Yao's theory (Yao et al. 1971), removals of particles larger than 1 μm are enhanced by increased particle sizes. Larger ratios of the colloid to the median grain diameter were reported to cause greater deposition via straining (Bradford et al. 2007). The hindered transport of the 10 μm microspheres were most likely caused by the increased particle size when comparing to the breakthrough of 4.5 μm microspheres. Straining likely predominately retained the modified 10 μm particles. Microspheres were more readily retained physically in the pore spaces generated through grain-grain contact, instead of being affected by the chemical factors (alum and/or HA). Unlike those of the modified 4.5 μm microspheres, the deposition profiles of the modified 10 μm microspheres showed very concentrated retention close to the column bed inlet (Figure 5.2, B2-5). It was observed that a steeper slope of decreased experimental deposition

results (dots) occurred within the first half of the column bed. As the microspheres were travelling further towards the outlet of filter media, a reduced rate of deposition was observed, as the concentration of the deposited microspheres remained relatively the same (i.e. shallow slopes). Similar retention pattern was reported by Tufenkji and Elimelech (2004b, 2005b) where they observed a reduced slope close to the column outlet in the deposition profile using fluorescent polystyrene latex particles and *C. parvum* oocysts and found that this reduction of slope was most pronounced at low salt concentration (3 mM).It was reported that in the presence of repulsive DLVO interactions, the concurrent existence of favorable and unfavorable particle-collector interactions due to surface heterogeneity could induce different rate for deposition (Tufenkji and Elimelech 2004b). Lastly, the impact of secondary energy minimum was more pronounced for larger particles (Elimelech 1998). Therefore, in complementary to the physical straining, the modified 10 μm microspheres were also likely to deposit in the secondary energy well under conditions unfavorable for deposition, and get retained through polymer bridging.

5.4 Conclusions

In this study, we modified the surface properties of carboxylated polystyrene microspheres with similar diameter as of *Cryptosporidium*, and evaluated the filtration of the surrogates under effects of flow rate, alum addition and HA-coating on collector surface. The surfaces of the selected microspheres were modified using an alpha-1-acid glycoprotein with similar surface charges characteristics to *C. parvum* oocysts, and examined the size and surface electrokinetic potentials of the modified surrogates. Filtration experiments were performed in bench-scale columns filled with sand media

often used by water treatment utilities. Obtained sands were thoroughly cleaned following procedure previously reported in order to eliminate impurities that could affect the experiment. Three flow velocities were chosen to broadly represent the flow rates in natural aquifers and water treatment utilities. Selected experiments were performed in the presence of alum or HA coating or both to investigate the impact of chemical factors. DLVO interaction energy was calculated based on the modified 4.5 μm microspheres and the retention mechanisms were discussed.

The zeta potentials of modified 4.5 μm microspheres were more negative than the unmodified ones. But the size and zeta potential of modified microspheres were found to be similar to that of the viable *Cryptosporidium* measured in our study. This excellent resemblance indicated that the modified microspheres could be utilized as very suitable surrogates for *Cryptosporidium*. DLVO calculations predicted the presence of a shallow secondary minimum well and a huge energy barrier for experimental conditions where no alum was present. Our filtration results showed that flow rate increased the transport of microspheres regardless of solution chemistry (i.e. presence of alum) under increased hydrodynamic forces. When only alum was supplied as coagulant, the microspheres showed hindered travel ability. When microspheres were moving in the HA-coated porous media bed, enhanced transport was observed in comparison to when HA was absent, possibly due to site competition and increased repulsion (steric and electrostatic). When alum and HA were both affecting the fate of microspheres, their coupled effects were minimal as the fate of microspheres under such condition was almost the same to the condition when alum and HA were absent.

Lastly, the surrogates used in this study can be used as a cost-effective tool for understanding *Cryptosporidium* removal in porous media without having to pose extra stress on health risk management. However, limitations of these glycoprotein modified microspheres still exist. As such, the surface hydrophobicity and softness might be different from the viable *Cryptosporidium*. Therefore, further validation and development of these surrogates suitable for various conditions is also needed.

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Chapter 6. Conclusions

6.1 Summary of findings

Lots of research has been conducted to understand the factors that influence the fate and transport of microbial particles in different types of porous media under aerobic conditions. In contrast, transport processes under anaerobic conditions have been minimally explored. This dissertation aims at closing this knowledge gap by better understanding microbial transport under different environmental scenarios.

The first investigation determined the role of solution ionic strength on the transport of different microbial cells in porous media under strictly anaerobic conditions. Secondly, the role of oxygen concentration on the growth and transport of a pathogenic facultative bacterium in porous media was explored. Lastly, the filtration of a novel surrogate for *Cryptosporidium* under various conditions that are commonly encountered in a water treatment process was evaluated. Research findings are summarized below.

6.1.1. Chapter 3. Retention and transport of an anaerobic trichloroethene dechlorinating microbial culture in porous media

In this chapter, an anaerobic mixed culture capable of complete reduction of TCE to ethene was studied. Three distinct species within the mixed culture were tracked in the experiments: *Dehalococcoides*, *Geobacter* and *Methanomethylovorans*. The goal of the research was to understand how these three microbial species transport and deposit in anaerobic porous media and if they are equally sensitive to changes in solution ionic strength.

Firstly, reduced transport and increased cell deposition in the packed bed column was observed for all three species when ionic strengths increased from 1 to 10, and 100 mM. Interestingly and more importantly, the three species exhibited similar normalized breakthrough concentrations (C/C_0 of 0.95, 0.84, and 0.56 at 1, 10 and 100 mM, respectively) and travel times (i.e., breakthrough plateau reached after 2 pore volumes of cell suspension injection) in the columns and showed almost equal sensitivity to solution ionic strength changes. This observation differed from what we had hypothesized where the species would have different transport behaviors because of their distinct surface properties. One possible explanation for the observed transport behavior includes surface interactions (such as steric repulsion and polymer bridging) of these microorganisms with other microbial species in the culture, which can either hinder or enhance their travel ability. Overall, these microorganisms can travel at similar rates.

Similar transport and deposition behaviors among the microbial species in a mixed culture enhance the efficiency and success of in situ bioremediation of TCE contaminated plumes. This is especially true when successful bioremediation happens when the dechlorinators (*Dehalococcoides* and *Geobacter*) and other microorganisms that support nutrients for cell synthesis and electrons for redox reactions in the culture are all working together.

6.1.2. Chapter 4. Comparison of the transport and deposition of Pseudomonas aeruginosa under aerobic and anaerobic conditions

In this chapter, the role of oxygen tension on the growth and transport of a pathogenic facultative bacterium *P. aeruginosa* was evaluated under aerobic and strictly anaerobic

conditions at different ionic strengths. Firstly, *P. aeruginosa* growth under anaerobic conditions exhibited approxomately 1.19 and 1.23 times higher protein and polysaccharides content as compared to growth under aerobic conditions as determined by EPS analysis. This observation agreed with our hypothesis that growth under aerobic and anaerobic conditions would produce different surface properties. Secondly, *P. aeruginosa* transport was significantly retarded under aerobic conditions (C/C_0 = 0.89, 0.61 and 0.44 at 1, 10 and 100 mM, respectively) as compared to anaerobic conditions (C/C_0 = 0.90, 0.74, 0.54 at 1, 10 and 100 mM, respectively). In addition, the bacterium showed higher sensitivity to solution ionic strength changes under aerobic conditions as shown by the reduced C/C_0 when ionic strengths increased from 1 to 10, and 100 mM. These observations supported our previous hypothesis that the differences in surface properties would lead to different transport behaviors.

In practice, when predicting pathogen travel trajectory on a larger scale, it is important to account for the gradient of dissolved oxygen concentration in natural aquifers, and apply different deposition rates according to different oxygen concentrations.

6.1.3. Chapter 5. Filtration of Cryptosporidium oocysts surrogates in porous media: Effects of flow rate, alum, and humic acid

In this chapter, the surface properties of glycoprotein modified microspheres were examined to determine if they can be used as surrogates of viable *Cryptosporidium*. Then the filtration of these microspheres was explored under different flow rates, together with the effects of alum addition and HA adsorption on porous media surfaces.

As hypothesized, surface property measurement of the modified microspheres showed good resemblance of the surrogates to viable *Cryptosporidium*, thus implying the high possibility of utilizing these surrogates to perform studies to evaluate *Cryptosporidium* removal, while eliminating personnel health hazards. Based on our results, removal was best achieved when filtration was operated at a flow rate lower than 10.9 m/day, alum addition of 5 mg/L and no HA adsorption on collector surfaces. This agreed with our hypothesis.

Special attention should be paid when HA adsorbed on the collector surfaces because of the increased transport as compared to conditions where no HA was adsorbed. The HA in the filter bed may hamper the effective removal of *Cryptosporidium* during the filtration process, which is usually considered a key process for removing protozoa during water treatment.

6.2 Recommendations

The work in this dissertation points to several directions that deserve further investigation, as follows:

(1) For in situ bioremediation applications, the large diversity of environmental variables cannot be overlooked. Solution chemical composition, temperature, solution pH, and surface interactions with the concurrent indigenous bacteria, are all known to affect the fate of injected bacteria in the subsurface. In addition to the three species in the mixed culture examined in this dissertation, transport studies conducted with other microorganisms is also necessary for a more comprehensive understanding. Use of porous media collected from natural aquifers can also enrich

the study. It would be interesting to explore the transport of the culture in the presence of TCE at a longer trajectory and analyze the distribution of each species in the porous media.

(2) In this dissertation, the fate of pathogenic bacteria in soil and groundwater was based on *P. aeruginosa*; a wider selection of pathogens may be included when exploring the effect of oxygen concentration on the travel ability in the subsurface. Longer travel trajectory with continuous gradient of oxygen concentration and use of various types of porous media is also an interesting aspect for future investigation.

(3) In the water treatment application, the large diversity of environmental and engineered variables, such as surface interactions with the concurrent bacteria in raw water and filter bed, particulate matter, chemical composition and temperature of the raw water are also important factors that influence the filter efficiency. Studies evaluating these factors would also provide further validation of the surrogates. It would also be interesting to investigate the removal rate at pilot scale experiments in the presence of these variables.

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Appendices

Appendix A. Defined Mineral Medium (Chapter 3)

The medium had the following constituents per liter of ultrapure water:

(1) 10 ml of phosphate buffer (20.96 g of KH_2PO_4 per liter, 42.85 g of K_2HPO_4 per liter);

(2) 10 ml of salt solution (53.5 g of NH_4Cl per liter, 7.0 g of $CaCl_2 \cdot 6H_2O$ per liter, 2.0 g of $FeCl_2 \cdot 4H_2O$ per liter);

(3) 2 ml of trace mineral solution [0.3 g of H_3BO_3 per liter, 0.1 g of $ZnCl_2$ per liter, 0.75 g of $NiCl_2 \cdot 6H_2O$ per liter, 1.0 g of $MnCl_2 \cdot 4H_2O$ per liter, 0.1 g of $CuCl_2 \cdot 2H_2O$ per liter, 1.5 g of $CoCl_2 \cdot 6H_2O$ per liter, 0.02 g of Na_2SeO_3 per liter, 0.1 g of $A1_2(SO_4)_3 \cdot 16H_2O$ per liter, 1 ml of H_2SO_4 per liter];

(4) 2 ml of $MgSO_4 \cdot 7H_2O$ solution (62.5 g/liter);

(5) 1 ml of redox indicator stock solution (1 g of resazurin per liter);

(6) 10 ml of saturated bicarbonate solution (260 g of $NaHCO_3$ per liter);

(7) 10 ml of filter-sterilized vitamin stock solution (0.02 g of biotin per liter, 0.02 g of folic acid per liter, 0.1 g of pyridoxine hydrochloride per liter, 0.05 g of riboflavin per liter, 0.05 g of thiamine per liter, 0.05 g of nicotinic acid per liter, 0.05 g of pantothenic acid per liter, 0.05 g of *p*-aminobenzoic acid per liter, 0.05 g of cyanocobalamin per liter, 0.05 g of thioctic acid per liter, 1 g of mercaptoethanesulfonic acid [coenzyme M] per liter);

(8) 10 ml of an amorphous ferrous sulfide solution [39.2 g of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ per liter, 24.0 g of $Na_2S \cdot 9H_2O$ per liter] that had been washed three times with deionized water to remove free sulfide.

The bicarbonate, vitamins, and ferrous sulfide were added from sterile stock solutions after the medium had been autoclaved and cooled while being gassed with N_2-CO_2 (80:20 [vol/vol]) filtered with sterile 0.22 μm filter. The pH of the medium was usually between 6.8 and 7.0.

Appendix B. Detailed Procedure for DNA Isolation (Chapter 3)

A PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc.) was used.

1. To the PowerBead Tubes provided in the kit, add an average of 0.3 gram of porous media samples or an aliquot of 300 µL of liquid sample.
2. Gently vortex to mix.
3. Check Solution C1. If Solution C1 is precipitated, heat solution to 60 °C until dissolved before use.
4. Add 60 µL of Solution C1 and invert several times or vortex briefly.
5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex. Vortex at maximum speed for 10 minutes.
6. Centrifuge tubes at 10,000 g for 30 second at room temperature. Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing.
7. Transfer between 400 to 500 µL of the supernatant to a provided clean 2 mL Collection Tube.
8. Add 250 µL of Solution C2 and vortex for 5 second. Incubate at 4 °C for 5 minute.
9. Centrifuge the tubes at room temperature for 1 min at 10,000 g.
10. Transfer up to, but no more than 600 µL of the supernatant to a provided clean 2 mL Collection Tube. Avoid the pellet.
11. Add 200 µL of Solution C3 and vortex briefly. Incubate at 4 °C for 5 minute.
12. Centrifuge the tubes at room temperature for 1 min at 10,000 g.
13. Transfer up to, but no more than 750 µL of the supernatant to a provided clean 2 mL Collection Tube. Avoid the pellet.

14. Shake to mix Solution C4 before use. Add 1200 µL of Solution C4 to the supernatant and vortex for 5 second.
15. Load approximately 675 µL of the supernatant onto a Spin Filter and centrifuge the tubes at room temperature for 1 min at 10,000 g. Discard the flow through and repeat the centrifugation until all supernatant is processed.
16. Add 500 µL of Solution C5 and centrifuge at room temperature for 30 second at 10,000 g.
17. Discard the flow through.
18. Centrifuge again at room temperature for 1 min at 10,000 g.
19. Carefully place spin filter in a provided clean 2 mL Collection Tube. Avoid splashing any Solution C5 onto the Spin Filter.
20. Add 100 µL of Solution C6 to the center of the white filter membrane. Centrifuge the tubes at room temperature for 30 second at 10,000 g.
21. Discard the Spin Filter. Store the tube containing DNA at -20 °C.

Appendix C. Sequences of Targeted Genes from qPCR (Chapter 3)

Dehalococcoides

Targeting gene: chromosome 16S ribosomal RNA (16S rRNA)

Primer: 1f, 5'-GGA TGA ACG CTA GCG GCG-3'

259r, 5'-CAG ACC AGC TAC CGA TCG AA-3'

Size: 260 base pair

GGATGAACGCTAGCGGCGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCA

ATTAAGATAGTGGCGAACGGGTGAGTAACGCGTAAGTAACCACCTCTAAGTG

GGGGATAGCTTCGGGAAACTGAAGGTAATACCGCATGTGGTGGACCGACATA

TGTTGGTTCACTAAAGCCGTAAGGCGCTTGGTGAGGGGCTTGCGTCCGATTA

GCTAGTTGGTGGGGTAATGGCCTACCAAGGCTTCGATCGGTAGCTGGTCTG

Geobacter

Targeting gene: chromosome 16S ribosomal RNA

Primer: 73f, 5'-CTT GCT CTT TCA TTT AGT GG-3'

485r, 5'-AAG AAA ACC GGG TAT TAA CC-3'

Size: 413 base pair

CTTGCTCTTTCATTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC

CTTGGACTCTGGGATAACATCTCGAAAGGGGTGCTAATACCGGATAAGCCCA

CGATGGCGTAAGTCATTGCGGGAAAAGGGGGCCTCTGAATATGCTCCTGATT
CAAGATGAGTCTGCGTACCATTAGCTAGTTGGTAGGGTAAGAGCCTACCAAG
GCGACGATGGTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGA
CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGCGCAATGGGG
GAAACCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAA
AGCTCTGTCTAGAGGGAAGAAATGATAGTCGGTTAATACCCGGTTTTCTT

Methanomethylovorans

Targeting gene: chromosome 16S ribosomal RNA

Primer: 166f, 5'-AAA GCT TTT GTG CCT AAG GA-3'

413R, 5'-ATG GAC AGC CAA CAT AGG AT-3'

Size: 223 base pair

AAAGCTTTTGTGCCTAAGGATGGGTCTGCGGTCTATCAGGTTGTAGTGGGTGT
AACGTACCTACTAGCCTACGACGGATACGGGTTGTGGGAGCAAGAGCCCGGA
GATGGATTCTGAGACATGAATCCAGGCCCTACGGGGCGCAGCAGGCGCGAA
AACTTTACAATGCGGGAAACCGCGATAAGGGGACACCGAGTGCCAGCATCCT
ATGTTGGCTGTCCAT

Appendix D. Parameter Values used in DLVO Calculations

	Chapter 3	Chapter 4	Chapter 5
ε_0 (F/m)	8.85E-12		
ε_r (unitless)	79.99 (1 mM)		79.99
	79.89 (10 mM)		
	78.90 (100 mM)		
a_p (E-07 m)	2.71 (*Dehalococcoides*)	4.80 (aerobic)	24.5
	3.30 (*Geobacter*)	4.45 (anaerobic)	
	6.25 (*Methanomethylovorans*)		
κ (1/m)	1.04E+08 (1 mM)		1.04E+08
	3.28E+08 (10 mM)		
	1.04E+09 (100 mM)		
h (nm)	0-150		
ψ_p (E-02 V)	*Dehalococcoides*	Aerobic	-1.99 (modified)
	-2.53 (1 mM)	-4.14 (1 mM)	3.44 (modified+alum)
	-1.99 (10 mM)	-3.03 (10 mM)	
	-1.09 (100 mM)	-1.76 (100 mM)	
	Geobacter	Anaerobic	
	-4.07 (1 mM)	-3.87 (1 mM)	
	-2.70 (10 mM)	-3.07 (10 mM)	
	-1.33 (100 mM)	-1.52 (100 mM)	
	Methanomethylovorans		
	-4.97 (1 mM)		
	-3.82 (10 mM)		
	-2.53 (100 mM)		
ψ_c (E-02 V)	-5.10 (1 mM)	Aerobic	-3.53 (sand)
	-5.07 (10 mM)	-5.90 (1 mM)	2.62 (sand in alum)
	-2.97 (100 mM)	-5.65 (10 mM)	-4.64 (sand+HA)
		-1.76 (100 mM)	2.06 (sand+HA in alum)

		Anaerobic	
		-5.10 (1 mM)	
		-5.07 (10 mM)	
		-2.97 (100 mM)	
A (J)	4.16E-21		6.50E-21
λ (m)	1.00E-07		