Factors Affecting the Transport of Pathogens & Pathogen Surrogates in Saturated Porous Media:

Implications for Natural & Engineered Drinking Water Filters

by

Nicole McLellan

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of

Master of Applied Science
in

Civil Engineering

Waterloo, Ontario, Canada, 2013

©Nicole McLellan 2013

AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Three tiers of bench-scale experiments were conducted to evaluate the use of laboratory column investigations for studying the transport and removal of pathogenic microorganisms (i.e. disease causing viruses, bacteria and protozoa) and pathogen-surrogates (i.e. (bio)colloids) in saturated porous media (filtration). These experiments were used to explore the effects of individual and concurrent factors on the transport and removal of a suite of (bio)colloids at a range of environmentally relevant conditions typical of natural riverbank filtration and engineered drinking water filters. Several bench-scale column designs were investigated to elucidate laboratory-scale column size factors that may affect reproducibility of (bio)colloid passage through granular media filtration. The physical and chemical factors investigated for their individual and concurrent effects on the transport of a suite of (bio)colloids included: media grain size, media uniformity coefficient, ionic strength, and the presence of natural organic matter. The suite of pathogens and (bio)colloids utilized in this study included PR772 bacteriophage, *Escherichia coli* RS2g bacteria, *Salmonella typhimurium* bacterial pathogen, and two sizes of fluorescent polycarbonate microspheres (1.1 μm and 4.5 μm). In addition to *S. typhimurium*, pathogenic bacterial strains of *E. coli* and *Pseudomonas aeruginosa* were isolated and used in an experiment to investigate the effects of bacterial exposure to different environmental water matrices (impacted by various land-uses) on the transport of pathogenic bacteria. Additionally, the effects of bacterial exposure to the different water matrices on cell size and surface EPS composition of the suite of bacterial pathogens were investigated. Pathogen and (bio)colloid removal was assessed for the three experiments by plotting breakthrough curves and/or removal value from each trial, followed by ANOVA to determine the statistical significance of the effect of each parameter studied on (bio)colloid removal. The outcomes of this work have several implications for the use of bench-scale column studies in (bio)colloid transport investigations to improve the understanding of natural and engineered filter performance.

Laboratory bench-scale experiments using replicate glass columns proved to be a useful tool in investigating factors that affect (bio)colloid transport in saturated porous media. In contrasts to common recommendations for experimental design (e.g., column diameter (D) to collector diameter (d) ratio > 50), column and collector media designs with D/d between 15 and 116 did not have a significant effect on the reproducibility and removal of a suite of (bio)colloids in transport investigations using varying ionic strengths and flow velocities representative of natural subsurface environments. Accordingly, small scale column studies of (bio)colloid removal by filtration that are conducted at D/d < 50 should not be universally disregarded because of wall effects concerns.

Observations of (bio)colloid removal by granular media filtration were generally consistent with colloid filtration theory. Grain size, ionic strength and the presence of natural organic matter significantly affected the removal of a suite of (bio)colloids at values representative of natural field conditions. Interaction effects were also identified between the chemical factors of ionic strength and natural organic matter, as well as between physical media characteristics of grain size and uniformity coefficient. These results suggest that synergistic effects within physical and chemical factors known to effect pathogen transport in saturated porous media should be considered when assessing pilot- and full-scale filter performance demonstrations.

Differences in removal between the suite of bacterial pathogens investigated at conditions representative of subsurface filtration were small (<0.5 log), suggesting that nuances between the removal of various strains of bacteria that are present at the micro-scale may not be substantial at the macro- or field-scale. The effects of bacterial EPS on (bio)colloid transport may be more important in environments with profuse biofilm formation (unlike the "clean-bed" environments used in this study). Established and standardised methods for EPS extraction and characterization for a range of applications are necessary to improve our understanding of bacterial EPS production, and the effects of these compounds in a range of saturated porous media environments. A conceptual model was developed to encompass the current state of knowledge on bacterial EPS effects on bacterial removal and the results presented herein.

Acknowledgements

This dissertation would not have been possible without the support, guidance and humour of several knowledgeable and helpful individuals and organizations.

First and foremost, I would like to extend my utmost gratitude to Prof. Monica B. Emelko for her encouragement and provision of this work. Your enthusiasm for all things physico-chemical in water treatment research has been an inspiration, and your "big-picture" perspective, an asset. I am also grateful for your support of my international development work and that you were never reluctant to give me time off to pursue this passion. You've opened countless doors for me and provided me with numerous networking opportunities that enhanced my studies and career path - this I will never forget.

Dr. Michele Van Dyke and Prof. Peter M. Huck – you provided me with my first opportunity in drinking water treatment research. Thank you for believing in me and encouraging me to pursue graduate studies.

Thank you to Jesse Stimson and Maria Mesquita for your frequent huddles over experimental designs, approach, materials, methods, "next steps", data analysis, life advice and jokes. Your knowledge, honesty and fortitude were invaluable assets to this project.

Staff at the Canadian Water Network, especially Bernadette Conant, I am thankful for the learning and networking opportunities you provide and encourage for students like me in the field of water treatment.

The American Water Works Association deserves recognition for the networking events and awards made available to students that I was able to take advantage of. These opportunities lead to a more enjoyable experience for me as I worked through my degree and developed my career path.

Several individuals are to thank for keeping DWE 3505 an enjoyable place to work: Mark Sobon, Leslie Gray, Alex Chik, Mark Spanjers, Jin Chao, Phil Schmidt and Ryan Snider. Enormous gratitude to undergraduate researcher Jessica McLeod for your work ethic and meticulousness; without your input the quality of the data presented herein would have suffered.

Justin Davis – you win the award for most supportive husband. Meal deliveries and lab visits were just a few ways you made long lab days bearable; best of all, you were never phased by my irregular schedule. You celebrated in my successes and carried me through the struggles, and for that I am forever indebted.

To my parents, thank you for telling me I could do whatever I wanted when I grew up, and for standing by that statement.

Dad – you always believed in me and helped me to begin my studies. You are forever missed.

Dedication

To those without access to safe drinking water.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AG	Agricultural
ANOVA	Analysis of Variance
CER	Cation Exchange Resin
CFT	Colloid Filtration Theory
CFU	Colony Forming Units
D/d	Column Diameter (D) : Collector Diameter (d)
DLS	Dynamic Light Scattering
DOC	Dissolved Organic Carbon
EPS	Extracellular Polymeric Substances
ES	Effective Size
GS	Grain Size
GW	Groundwater
IS	Ionic Strength
LB	Luria-Bertani Broth
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
MS	Microspheres
NOM	Natural Organic Matter
PBS	Phosphate Buffered Saline
PCA	*Pseudomonas* Cetrimide Agar
PFU	Plaque Forming Units
PTFE	Polytetrafluoroethylene
PV	Pore Volume
R^2	Coefficient of Determination
RBF	Riverbank Filtration
SD	Standard Deviation
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
TSA	Trypticase Soy Agar
TSS	Total Suspended Solids
TOC	Total Organic Carbon
UC	Uniformity Coefficient
URB	Urban

USEPA	United States Environmental Protection Agency
WW	Waste Water
WWTP	Waste Water Treatment Plant
WTP	Water Treatment Plant
Ø	Inner Diameter

Chapter 1 Introduction

1.1 Introduction

Water filtration through granular porous media is an effective process to reduce the pathogen (i.e. disease causing organism) load to drinking water sources and the risk of waterborne illness. A multi-barrier approach to drinking water protection often includes filtration; through the subsurface in groundwater flow and recharge and/or through engineered filters in drinking water treatment plants. Subsurface filtration is impacted by both surface landscape activities and subsurface physico-chemical conditions (i.e. water quality, geology and local biogeochemistry), while plant performance is typically driven by treatment process design and operations. Improving pathogen removal by various types of granular media filtration processes requires a mechanistic understanding of pathogen transport through saturated porous media. At present, this understanding is relatively poor and non-predictive; it is limited by several factors, including available tools (e.g. column and pilot trials) for investigating pathogen transport and the use of surrogates for pathogens in place of etiological agents. Accordingly, production of safe drinking water requires further research into the transport of pathogens to: 1) evaluate the use of bench-scale column studies to represent larger-scale pilot-testing and performance demonstrations, 2) elucidate both individual *and* concurrent factors that affect pathogen transport/passage through natural and engineered filtration processes, and 3) recognize the effects of various land-uses on groundwater contamination. Improving the understanding of pathogen transport through porous media in these treatment scenarios will help to better inform watershed management and drinking water treatment decision making (e.g., assessment of groundwater under the direct influence of surface water [GUDI], quantitative microbial risk assessment [QMRA]), as well as granting of regulatory treatment credits for filtration processes.

Considering natural filtration processes, the United States Environmental Protection Agency's (USEPA) Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) recognizes riverbank filtration (RBF) as an effective treatment barrier to *Cryptosporidium* passage into treated water supplies (Federal Register, 2006). RBF is a natural, sustainable (due to low material and energy demands) and relatively low-cost drinking water treatment process that can be utilized to improve surface water quality (e.g. from a river or lake source) (Emelko et al. 2010; Tufenkji et al. 2002). Specifically, it is the active process of drawing water from a surface source through porous media in the subsurface and into an abstraction well. This method of treatment has been used for centuries in Europe (Kuehn and Mueller, 2000; Ray, 2008), and can effectively reduce the chemical and microbial contaminant load to subsequent water treatment processes (Bertelkamp et al. 2012; Ing, 2012; Schijven et al. 2002). With rapid urbanization and climatic events that are leading to increasingly variable source water quality, utilities can achieve more consistent

effluent water quality and benefit economically with the use of RBF (Emelko et al. 2010). RBF can be relied upon for consistent long-term production of improved water quality and has been cited to act as a barrier against contaminant shock loads resulting from precipitation (e.g. snow melt or heavy rainfall) and anthropogenically induced events (e.g. manure spreading) (Kuehn and Mueller, 2000). Nonetheless, further investigation into the mechanisms that drive pathogen transport in saturated porous media environments is required to evaluate RBF performance in various settings, and to assess the associated health risks due to potential pathogen passage into subsequent treatment processes (Emelko et al. 2010; Hiscock and Grischeck, 2002; Matthess and Pekdeger, 1985). The LT2ESWTR prescribes treatment credits for processes such as RBF and also allows performance demonstrations for processes seeking treatment credits not specified in the regulation (Federal Register, 2006). Field and laboratory studies have demonstrated the performance of RBF sites to remove pathogens (Kuehn and Mueller, 2000); including viruses (Schijven et al. 2000; Havelaar et al. 1995) and *Cryptosporidium* oocysts (Gollnitz et al. 2003; Weiss et al. 2005); however, this capacity can be highly site specific. Field studies are limited by poor consideration for groundwater flow, reliance on unproven "surrogate" parameters, non-detects in process effluents, and unsatisfactory sampling abilities. Variable flow paths and fluxes also complicate performance assessments of subsurface filtration (Unc and Gross, 2004). Issues such as inadequate reproducibility, experimental scale and variable subsurface settings/filtration media characteristics make it difficult to extrapolate laboratory outcomes to field/plant performance in both natural subsurface filtration and engineered filtration systems (Ray et al. 2003; Hiscock and Grischek, 2002).

Pathogen removal by RBF is dependent on several physico-chemical factors including contaminant loading and raw water quality; pumping-induced groundwater flow and retention time; media characteristics (e.g. porosity); and water chemistry (e.g. ionic strength, NOM content, temperature, pH, and oxygen concentration) (Kuehn and Mueller, 2000; Tufenkji et al. 2002; Abudalo et al. 2010; Emelko et al. 2010; Sen, 2011). Unlike conventional chemically-assisted filtration processes (that most heavily rely on chemical coagulants to enhance particle and pathogen removal by physico-chemical filtration), pathogen removal during RBF is dependent upon environmental conditions (such as source water quality and aquifer setting) that affect physico-chemical filtration and inactivation during the treatment process. Accordingly, it is important to determine the environmental and anthropogenic conditions that affect raw water quality and impact pathogen removal by filtration. This includes consideration of various land-uses that can greatly affect surface source water quality, which in turn can impact pathogen removal by RBF (Harvey and Harms, 2002; Unc and Gross, 2004; Gerba and Smith, 2005).

Land-uses can considerably impact the physical, chemical and biological characteristics of surface water and groundwater. Certain land-uses and aquifer characteristics increase the risk of source water

contamination (Eckhardt and Stackelberg, 1995; Honisch et al. 2002; Scanlon et al. 2005), particularly by pathogens (Jamieson et al. 2002). A substantial pathogen load can be introduced into water systems by heavy rainfall, snowmelt and climatic events; agricultural practices and manure application; sewage/septic system leaks and wastewater effluent discharges; urbanization and increased impervious land surfaces (Scanlon et al. 2005; Emelko et al. 2010; Emelko et al. 2011). Several types of waterborne pathogens threaten the safety of drinking water supplies and include strains of viruses, bacteria and protozoa.

Pathogen transport through porous media is often studied with the use of surrogate (bio)colloids (e.g. bacteriophage as virus surrogates; harmless laboratory strains of bacteria; polycarbonate microsphere particles of varying sizes), typically for ease of use in the laboratory and to prevent harm to the laboratory worker. Suitable surrogates must be selected carefully as substantial differences in transport can be observed between (bio)colloids of different sizes, shapes, origin, surface characteristics, and survival ability (Schijven et al. 2003; Brookes et al. 2005; Wilkes et al. 2009). It is often necessary to study the etiological agents themselves, or select suitable surrogates that have similar characteristics to pathogens (density, surface charge, etc), which are likely to affect their passage through, and retention within, porous media systems.

Relative to other types of pathogens, bacteria are of particular interest due to their detection in groundwater and surface water sources, and association with drinking water-associated outbreaks of human illness (Holme, 2003; Harvey and Harms, 2002; Macler and Merkle, 2000). Although bacterial pathogens in water are relatively easily disinfected, disinfection of groundwater (GW) supplies is not required in some jurisdictions (e.g. parts of the US) (Federal Register, 2006). Bacterial pathogens have been detected in subsurface water supplies (Ibekwe and Lyon, 2008), and sources that are susceptible to fecal contamination are often designated as groundwater under the direct influence of surface water (GUDI or GWUDI) (Clancy, 2012; Federal Register, 2006; Hiscock and Grischek, 2002). Bacteria can enter RBF sites in elevated concentrations during river bed disturbances (Hiscock and Grischek, 2002; Matthes and Pekdeger, 1985). Colloid filtration theory (CFT) describes that pathogens of bacterial size may be preferentially transported through saturated porous media (Gregory, 2006; Tufenkji and Elimelech, 2004; Yao et al. 1971); however, this theory has been shown not to hold true at the field-scale due to the effects of several factors including reversible attachment, hydrophobic forces, steric repulsion, and colloid-colloid interactions (Ford and Harvey, 2007; Tufenkji, 2006; Harvey and Harms, 2002; Shijven et al. 2002). In addition to size impacts (which affects colloid transport to the surface of media grain collectors in a filter), many factors can affect the removal of bacteria (i.e. attachment and retention), including their intricate surfaces and EPS characteristics. For example, bacterial surface characteristics can influence whether attachment to media grains occurs permanently or reversibly (Kim et al. 2009;

Camesano et al. 2007; Jucker et al. 1998). Consequently, not all ~1 μm colloids will be transported through and removed by porous media to the same extent.

Many bacterial strains excrete extracellular polymeric substances (EPS) onto their surfaces to various thicknesses and compositions. EPS is a complex matrix of polysaccharides, proteins, nucleic acids, and lipids produced by bacteria in response to environmental conditions. Changes in EPS production and composition can impact bacteria transport through porous media by altering cell surface electrophoretic mobility (i.e. zeta potential), charge density, hydrophobicity, as well as size and shape (Rozatos et al. 1998; Walker et al. 2005; Bolster et al. 2009). It has been demonstrated that the variation in EPS production in response to changing environmental conditions may impact bacterial transport and retention in porous media (Juker et al. 1998; Tsuneda et al. 2004; Liu et al. 2007; Long et al. 2009; Kim et al. 2009; Tong et al. 2010). Investigating the natural conditions by which bacteria exude EPS will contribute to a better understanding of bacterial transport in saturated porous media. Numerous studies have compared various physical, chemical or combined methods for the extraction and characterization of EPS, but a standardized method for bacterial cultures in aqueous suspensions has yet to be established. Limitations of existing methods include relatively low yields of EPS for characterization and interferences from chemicals used in extraction procedures (Jucker et al. 1998; Comte et al. 2007).

In addition to developing better pathogen characterization and enumeration techniques, developing reliable methods and techniques for evaluating pathogen transport in porous media environments will enable better extrapolation of laboratory results to circumstances encountered in the field. Pilot- and laboratory-scale column experiments are versatile and useful tools for determining design and operational factors that affect filtration efficiency; however, demonstrated reproducibility and experimental design guidelines are lacking. A design recommendation that column experiments are often limited by is the column diameter (D) to collector (i.e. media grain) diameter (d) ratio (D/d). It has been suggested that a low D/d (e.g. < 100) could cause variable flow paths across the column, where the porosity is possibly lower in the interior of the bed than next to the wall (Shankararman and Wiesner, 1993). A D/d of 50:1 or higher has been suggested for column experiments investigating rapid granular media filtration with backwashing (AWWA, 1982; Lang et al. 1993); however, the relevance of this ratio for investigations of (bio)colloid transport through natural or engineered filters has not been established. This limitation restricts efficient study design and necessitates increased material/infrastructure costs than would be required if investigations were conducted at a lower D/d.

1.2 Research Objectives

This study involved three research goals and associated tiers of experimentation and analysis to investigate a variety factors that impact pathogen transport through saturated porous media. They were designed to: 1) determine appropriate limitations to column experiment designs (D/d) for investigating (bio)colloid transport through saturated porous media; 2) investigate the concurrent effects of physico-chemical factors affecting pathogen and (bio)colloid removal by filtration; and 3) study the effects of different water matrices (impacted by various land-uses) on bacterial EPS production and composition, and elucidate the effects of these impacts on transport of bacterial pathogens through saturated porous media at environmentally relevant conditions. The specific research objectives for each of these research goals are outlined below.

1.2.1 Experiment 1: Effects of Column D/d Conditions on (Bio)Colloid Transport Investigations

1. To discern an appropriate, practically relevant lower-limit ratio of D/d conditions for investigating of (bio)colloid removal by granular media filtration at bench-scale.

1.2.2 Experiment 2: Concurrent Effects of Physico-Chemical Factors on (Bio)Colloid Transport in Saturated Porous Media Filtration

2. To evaluate the use of bench-scale column experiments for investigating factors effecting pathogen and (bio)colloid transport.
3. To investigate the independent and concurrent effects of ionic strength (IS), natural organic matter (NOM), grain size (GS), and media uniformity coefficient (UC) on the transport and attachment of a suite of (bio)colloids.
4. To observe the effects of a range of physico-chemical conditions on the transport of a suite of (bio)colloids.

1.2.3 Experiment 3: Water Matrix Effects on Bacterial Pathogen EPS, Size and Transport in Saturated Porous Media Filtration

5. To critically review the current state of knowledge on bacterial EPS production and effects on bacterial transport.
6. To critically review and evaluate methods for the extraction and characterization of bacterial EPS.

7. To identify the effects of bacterial exposure to a range of natural source waters (influenced by different land-uses) on bacterial size, surface characteristics, and transport of a suite of pathogenic bacteria.
8. To elucidate the significance of EPS production in natural environments and the degree of effects on bacterial attachment to media grains.

1.3 Research Approach

Three studies were designed to address the above-mentioned objectives related to microbial transport in saturated porous media environments. Laboratory bench-scale column experiments were utilized in all of the studies. Solute tracer tests were performed for each media type investigated to allow for accurate PV determination and to compare solute breakthrough with that of the (bio)colloids studied. (Bio)colloid breakthrough curves and/or box-and-whisker plots were graphed to compare removal levels observed (log or percent removal) for the various scenarios. When possible, ANOVA was used to quantitatively analyze the experimental results. In Experiment 3, bacterial cell size was also monitored to determine correlations between this factor and bacterial removal.

1.3.1 Experiment 1

Three paired column experiments were designed to utilize two columns (Ø 16 and 50 mm), two granular quartz media (0.43 and 1.1 mm D_{10}), two ionic strength conditions (0.01 and 10 mM KCl), and two loading rates (1 and 5.5 m/h) to investigate the effects of D/d design on (bio)colloid transport in saturated porous media. The resulting D/d ratios investigated for comparison were 37 and 116, as well as 15 and 45, which effectively span the commonly used values (between 50 – 100) and also challenge the use of designs resulting in D/d < 50. Four (bio)colloids were used concurrently to represent a suite of protozoan, bacterial, and virus particle sizes. They included 1.1 and 4.5 µm polystyrene fluorescent microspheres (spheres), *Escherichia coli* RS2g bacteria, and PR772 bacteriophage as a virus surrogate.

1.3.2 Experiment 2

To provide guidance for assessing the efficacy of RBF processes, a factorial experimental investigation on the concurrent impacts of the four parameters was conducted. Column studies were performed in duplicate to evaluate the transport and breakthrough of four colloids: two sizes of fluorescent microspheres (1.1 µm and 4.5 µm); *Salmonella typhimurium* bacteria, a known human pathogen; and PR772 bacteriophage.

1.3.3 Experiment 3

To determine bacterial pathogen responses (such as EPS production) to different water matrices (impacted by various land-uses) at environmentally relevant conditions, three pathogenic strains of bacteria were isolated from a surface water source (*E. coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*). These pathogenic bacterial strains were then passed through saturated porous media columns (at controlled physico-chemical conditions) and differences in removal were quantified. Free- and bound-EPS extracts were characterized for carbohydrate and protein content. In addition to column experiments and EPS characterization, the results of a thorough literature review on the current state of knowledge of bacterial surface EPS effects on bacterial transport in saturated porous media environments is reported herein.

1.4 Thesis Organization

Chapter two contains a literature review detailing background information on factors known to affect pathogen and (bio)colloid transport through saturated porous media; a thorough discussion of the current state of knowledge on the effects of bacterial EPS on (bio)colloid transport through saturated porous media; followed by a review of column experiment design considerations (i.e. D/d specifications and concerns). Experimental procedures, materials and methods are described in Chapter 3. The results from the three experiments described in the above Research Approach section are presented in Chapters 4, 5, and 6, respectively. Chapter 7 lists the conclusions drawn from these investigations, and Chapter 8 outlines recommendations for bench-scale investigations into (bio)colloid transport research and provides recommendations for future work.

Chapter 2: Literature Review

2.1 Colloid Filtration Theory

Understanding the interaction between pathogens and solid media surfaces in water filtration requires investigating and quantifying the transport of microbial pathogens ([bio]colloids) in saturated porous media environments and attachment to media grains. Colloids are described as particles in the size range of several nanometers to ten microns (Tchobanoglous and Schroeder, 1985). Colloids in water are of various shapes (i.e. rods, plates, spheres and other variations) (Gregory, 2006), and include pathogenic microorganisms such as viruses, bacteria, and most protozoa. Colloids are effectively removed by physico-chemical filtration when they are transported and attach, either permanently or reversibly, to the surface of collectors (i.e. media grains in packed beds of engineered filters or aquifers) (Yao et al. 1971). The mechanisms by which particles come into contact and attach to collectors are described by colloid filtration theory.

Classic colloid filtration theory describes the mass transfer of suspended colloids from bulk fluid suspensions to collector surfaces, and subsequent attachment to these surfaces (Yao et al. 1971). Suspended colloids can be transported to the surface of collectors due to sedimentation, diffusion, and interception, which are depicted in Figure 1 (Yao et al. 1971). Other mechanisms can also affect the colloid trajectory towards collector surfaces and include hydrodynamic deposition, inertia, mechanical straining in small porosity zones, and chance contact (Metcalf and Eddy, 1991). Hydrodynamic forces can transport colloids to collector surfaces as a result of the fluid flow path intercepting with media grains in a packed bed (Yao et al. 1971). The forces of diffusion and sedimentation can cause colloid movements to deviate from flow paths. Colloid transport due to diffusion, also known as Brownian motion, results from the random bombardment of molecules in the water that move due to thermal motion (Elimelech and O'Melia, 1990b; Gregory, 2006). Sedimentation describes transport due to gravitation, and is impacted by particle mass, density and velocity (Elimelech and O'Melia 1990b; Gregory, 2006). Fluid drag can retard the forces of diffusion and sedimentation, and is proportional to the projected area of the particle.

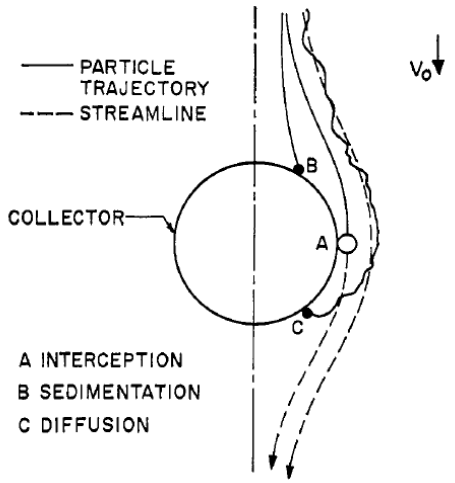


Figure 1: Three transport mechanisms illustrating colloid transport to a collector surface, describe by classic colloid filtration theory (CFT) (modified from Yao et al. 1971).

Colloid filtration theory helps describe how particle size contributes to trends in particle removal (Yao et al. 1971). The term single-collector efficiency is used to define the rate of particle contacts with collectors, relative to the rate of particle flow towards the area of a collector (Yao et al. 1971; Tufenkji and Elimelech, 2004). Colloid filtration theory indicates that the contact opportunities (with media grain collectors) of particles of about 1 μm are at a minimum, as depicted in Figure 2 (Yao et al. 1971; Tufenkji and Elimelech, 2004). Particles larger than 1 μm experience relatively more contact opportunities with increasing particle size due to the forces of sedimentation and interception (Yao et al. 1971), while particles smaller than 1 μm experience increased contact opportunities with decreasing particle size due to the forces of diffusion (Yao et al. 1971). Most pathogenic bacteria of concern in water sources are in the size range of 1 μm (Tchobanoglous and Schroeder, 1985; Gregory, 2006). Therefore, colloid filtration theory predicts that bacterial cells will possibly experience minimal removal in saturated granular environments due to fewer contact opportunities with collector surfaces (relative to smaller viral colloids and larger protozoan and spore colloids).

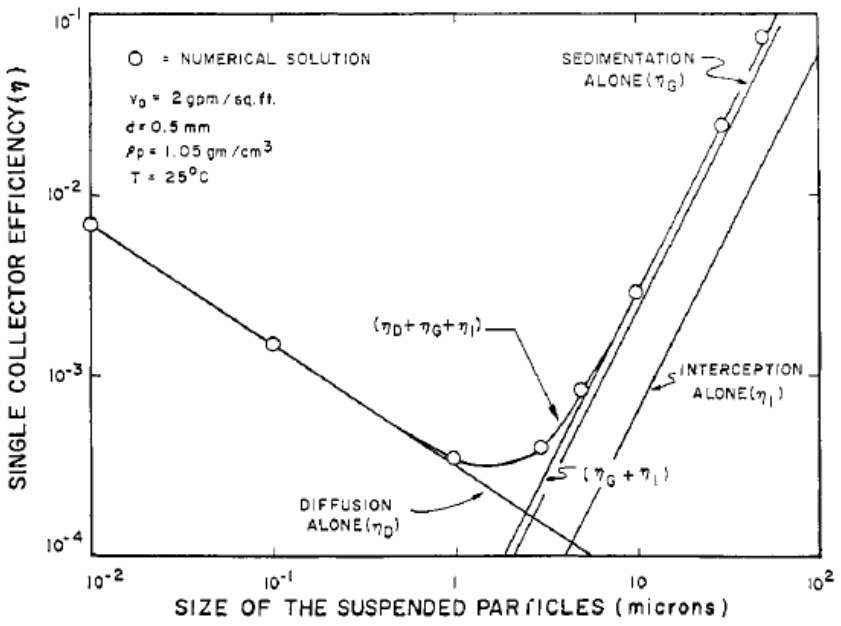


Figure 2: Single-collector contact efficiency model from Yao et al (1971) used to assess colloidal attachment relative to colloid size based on transport due to diffusion, interception and sedimentation alone.

Colloid interactions and attachment to solid surfaces are affected by colloid characteristics, including surface charge. Suspended colloid surfaces carry a charge that can be either positive or negative. The surfaces of biological colloids in natural waters are typically negative due to incorporated proteins that can ionize. Colloidal surfaces can also carry a charge due to functional groups with acidic or basic groups that can be affected by pH, isomorphuous substitutions and specific adsorption of ions at the surface of the colloids (Gregory, 2006).

The electric double layer is depicted in Figure 3, and describes the distribution of ions in solution and in contact with the surface of a colloid (Neubauer et al. 1998). The charge of a colloid surface creates a resulting layer of oppositely charged ions (i.e. counterions) in solution to maintain electrical neutrality. The "plane of shear" describes the separation between ions at the surface of a colloid that are fixed and the ions that are free to move in the liquid (i.e. diffuse layer). The strength of this layer is impacted by the degree of charge at the surface, and can be measured via electrophoretic mobility (from which zeta potential is calculated) (Gregory, 2006).

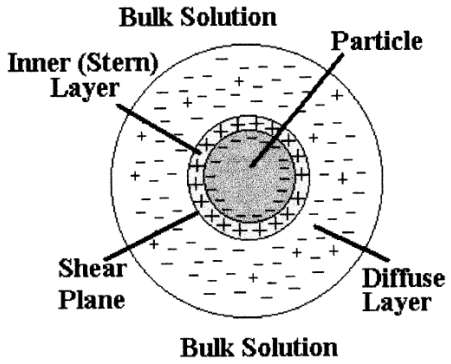


Figure 3: Electrical double layer model showing the inner stern layer of fixed ions and the outer diffuse layer of fluid ions in the bulk solution (modified from Neubauer et al. 1998).

The initial adhesion of pathogenic microorganisms onto solid surfaces in aquatic systems is generally thought to be similar to that of depositing colloidal particles (Grasso et al. 2002). The surfaces of colloids can interact with other surfaces (i.e. those of media grains) due to a variety of repulsive and attractive forces. These forces include attractive hydrophobic effects and polymer bridging; repulsive effects of hydration and steric interactions; and repulsive or attractive London-van der Waals (usually attractive) and electric-double layer forces (Gregory, 2006). London-van der Waals forces arise due to spontaneous electrical and magnetic polarizations that creates a fluctuating electromagnetic field within the particles and in the spaces between them (Gregory, 2006). Colloids and collectors are kept apart when their overall surface charges repel each other; while attachment requires that attractive forces overcome repulsive forces between the collector and the colloid (Yao et al. 1971).

The Derjaguain-Landau-Verwey-Overbeek (DLVO) theory describes the potential energy that a colloid would need to surmount to come in contact with a collector to allow attachment (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). This theory assumes that the interactions between particles is additive. DLVO accounts for the effects of London-van der Waals attractive dispersion forces, and electrostatic forces resulting from the double-layer of counter ions (Elimelech and O'Melia 1990a; Gregory, 2006) that can be attractive or repulsive depending on chemical structure, suspending medium properties and surface potential (Grasso et al. 2002).

Other non-DLVO forces that affect colloid attachment have been investigated and incorporated into various models. Forces associated with the hydration of ions at the colloid surfaces and the presence of adsorbed polymers can cause either be repulsive ("steric" interaction) (Ginn et al. 2002) or attractive ("polymer bridging") (Grasso et al. 2002; Kim et al. 2009; Kim et al. 2010). Repulsive hydration forces

arise when particles move closer together and the fluid between them must move out of the way – the repulsive force caused by this displacement of fluid is called hydrodynamic shear or drag (Elimelech and O'Melia, 1990b; Ryan and Elimelech, 1996). Repulsive interactions between biological colloids due to hydration can be particularly important because surface proteins and polysaccharides can retain high amounts of bound water (Gregory, 2006). Steric repulsion of polymers can also cause colloids to remain a finite distance from attachment sites, and prevent colloid interactions. Polymer bridging, however, can be an attractive type of interaction between biological colloids, where large amounts of polymers protruding from the cell wall form individual chains, usually of week strength (Grasso et al. 2002). Several models and approaches have been developed to describe electrostatic interactions between colloids and media grain collectors based on assumptions about colloid surface charge, potential and geometry (Bolster et al. 1999; Camesano et al. 1998; Tufenkji et al. 2007); however, the complex interactions between numerous factors affecting colloid transport to collector surfaces are not well understood which complicates the application and utility of such models.

Many investigations have made an effort to improve the understanding of pathogen attachment to porous media, and to account for some of the inherent limitations resulting from the assumptions made in colloid filtration theory (Murphy and Ginn, 2000; Tufenkji, 2007). Studies have been performed with the use of laboratory columns to artificially replicate and model natural groundwater environments (Yao et al. 1971; Ryan and Elimelech 1996; Tufenkji et al. 2003). Bradford et al (2002) found that the mechanism of straining (i.e. blocked pores acted as dead ends for the colloids) was a predominant bacterial removal mechanism in saturated sand, a mechanism not accounted for in classic filtration theory. In Yao et al (1971), the original derivation of colloid-filtration theory, the assumptions include that the filtration media is clean (or has no contaminants or particles attached); however, it has been shown that attachment rates can be affected by previously attached particles (Tufenkj, 2007). Several other factors have been found to contribute to deviations from colloid theory in natural groundwater environments, including colloid-facilitated transport and surface heterogeneities on colloid deposition and transport (Ryan and Elimelech, 1996); preferential pathways such as macropores and fractures (Stinton et al. 2005; Toran and Palumbo, 1992); microbial motility impacts (Ford and Harvey, 2007); and inactivation on the grain surface, in bulk solution, or during detachment from collectors (Tufenkji et al. 2003).

A variety of physicho-chemical factors can also significantly impact contact opportunities and subsequent attachment during colloid filtration. These factors include media grain characteristics and size distribution, the presence of organic matter, ionic strength, and pH. These factors are discussed in detail in the following sections, however this work does not discuss or investigate pH in detail; the impacts of pH on colloid filtration have been reviewed by others (Harvey, 1991; Stevik et al. 2004). Generally, higher

pH conditions reduce attachment of (bio)colloids in saturated granular media environments (Scholl and Harvey, 1992; Jewett et al. 1994; Kim et al. 2008; Kim et al. 2009), because cell surface charge can be affected by the dissociation of carboxylic and amino groups located on the cell wall. However, interferences such as those induced by changing ionic strength and different iso-electrical points for different bacterial species can confound this relationship (Stevik et al. 2004); for example, Jewett et al (1995) found that environmentally relevant changes in pH (from 5.5 to 7) did not significantly influence attachment of *Pseudomonas fluorescens* under a variety of ionic strength conditions.

2.2 Media Characteristics and Pathogen Transport

Media characteristics can affect pathogen removal in saturated porous media environments. Media grain size (GS) and uniformity coefficient (UC) are often used to describe granular media in subsurface and engineered filtration systems. Grain size can be described by the median (D_{50}) or effective (D_{10}) size of granular media used in filtration applications, representing the media grain diameter at which 50 or 10 percent of the media by mass are smaller, respectively. The uniformity coefficient of the media is described by D_{60}/D_{10}, or the ratio of the grain diameter of the 60^{th} percentile to the 10^{th} percentile. It provides an indication of the heterogeneity of the media grain sizes; a smaller UC is indicative of a more homogeneous media size distribution. In general, smaller grain size media has been shown to provide more removal of colloids (Bradford and Bettahar, 2006; Knappett and Emelko, 2008). Smaller grain size media provides greater surface area for attachment and more contact opportunities with collectors. Smaller grain sizes media also create smaller pore sizes that can enable additional removal by pore-exclusion or "straining", and more wedging niches (Bradford et al. 2004; Tufenkji et al. 2004). The retention of bacterial cells in porous media may not be affected greatly by media grain size due to the minimum transport efficiency of bacterial cells that are approximately 1 μm in size. In one study, Bolster et al (2001) found that changes from fine (0.42 to 0.50 mm) to coarse (0.707 to 0.850 mm) media grain sizes did not significantly affect bacterial retention.

The angularity and roughness of media grains can also substantially affect the pore size between media grains and impact contact opportunities between colloids and media grain surfaces (Bhattacharjee et al. 1998; Saiers and Ryan, 2005). For example, Tufenkji et al (2004) found that media with high angularity significantly reduced the pore sizes between the media grains, and increased the attachment of *Cryptosporidium* oocysts. Therefore, media size and uniformity should be important considerations when investigating transport of a suite of colloids through saturated porous media.

The impacts of uniformity coefficient on pathogen removal by filtration have not been investigated extensively. It has been argued that heterogeneous media (or media with greater UC) can have higher

total surface area for attachment, and can provide more regions for removal by straining (Silliman, 1995). Straining may only significantly impact attachment when investigating larger colloids and/or finer granular media. Bradford et al (2004), found that straining in finer sands in the vadose zone impacted the attachment of latex microspheres (common bacterial indicators) when the ratio of the colloid diameter was greater than 0.5% of the media grain diameter. The effects of different media distributions and media uniformity coefficient may be more apparent at lower loading rates and in groundwater environments, and require further investigation under environmentally relevant conditions (Harvey et al. 1993).

2.3 Ionic Strength and Pathogen Transport

Ionic strength is a measure if ion concentration in water and can significantly affect the transport and attachment of pathogens in subsurface environments (Scholl and Harvey, 1992). Typical ionic strength conditions used in pathogen transport investigations range between 1 and 100 mM; often, monovalent KCl is used to represent artificial groundwater (AGW) conditions. “High” and “low” ionic strengths are relative to what might be found in environmental waters. Here, “low” ionic strength is defined as below ~5 mM, which would represent environmental rainwaters and many high quality rivers, while higher values might represent typical groundwater or wastewater environments. Values closer to 100 mM and above can be representative of marine and some wastewater environments.

In its simplest form, DLVO theory describes the overall forces (i.e. the sum of all attraction and repulsion forces) acting on a colloidal particle as it approaches another particle, collector, or charged surface. The overall attractive or repulsive force between a colloid and a collector depends on the ionic strength of the suspending fluid. High ionic strength conditions reduce the size of the electric double layer on the surface of a colloid, thereby reducing electrostatic repulsion between two approaching surfaces. DLVO theory has been used extensively to model the effects of changes in ionic strength on the surfaces of colloids and collectors (Bradford and Torkzaban, 2012; Schijven and Hassanizadeh, 2000; Yee et al. 1999; Ryan and Elimelech, 1996).

In high ionic strength environments more ions in solution are available to reduce or dampen the electrostatic repulsive charge on colloid surfaces (Franchi and O’Melia, 2003). Accordingly, high ionic strength conditions generally favour colloid attachment. Conversely, in lower ionic strength environments fewer ions are available to neutralize the typically negative surface charge of the colloids; here the double layer is thicker and increases the electrostatic repulsion between colloids and collectors (Mills et al. 1994; Franchi and O’Melia, 2003). Consequently, fewer contact opportunities can occur between colloids and collectors in low ionic strength environments.

In natural environments, typically negatively charged colloids come into contact with each other at higher ionic strength conditions due to electrical double layer compression (Bolster et al. 2001). This has been demonstrated in a variety of granular media environments, including controlled laboratory column experiments (Mills et al. 1994; Jewett et al. 1994; Yee et al. 1999; Bunn et al. 2002; Haznedaroglu, 2009). Schijven and Hassanizadeh (2000) found that high ionic strength environments favoured virus attachment, while rainfall events (i.e. decreased ionic strength) were able to remobilize attached viruses. Jewett et al. (1994) determined that bacterial collision efficiency decreased by nearly 90 percent when ionic strength was decreased significantly from 10^{-1} to 10^{-5} M NaCl. Bolster et al. (2001) found that bacteria removal by porous media was consistent with DLVO theory with the use of clean silica sand (with predominantly negatively charged collector surfaces); decreasing ionic strength (from 10^{-1} to 10^{-2} M KCl) resulted in noticeably less attachment of bacteria to media grains. When sands with positively charged surfaces were used, changes in ionic strength had a minimal effect on bacterial attachment to media grains (Bolster et al. 2001).

Differences in retention of bacteria may not be significant as ionic strength is reduced in natural environments with collectors such as ferric oxyhydroxide-coated sediments (Bunn et al. 2002; Haznedaroglu, 2009) and quartz sand (Yee et al. 1999). It is thought that the majority of bacteria attach reversibly to sand grains, and that a decrease in ionic strength could release previously attached pathogens into suspension, causing bacteria to be transported further into a filter or aquifer (Redman et al. 2004); as was demonstrated in one study, which reported that decreasing the ionic strength of the suspension medium resulted in the release of the majority of previously deposited bacteria (Redman et al. 2004). Therefore, although high ionic strength environments are thought to favour colloid attachment, the effects of natural events and anthropogenic activities on water quality may effectively interfere with these impacts. These findings suggest that a holistic approach to investigating the physico-chemical conditions that can impact colloid transport should be taken to evaluating natural and engineered filter performance for pathogen removal.

2.4 NOM and Pathogen Transport

2.4.1 NOM Definition

Organic matter of natural origin is primarily derived from biological processes of plants and microorganisms. It is a complex mixture of compounds ranging from largely aliphatic to highly coloured organics having varying molecular sizes and properties. The complexity and heterogeneity of aquatic organics have made characterizing its structure and functional extremely difficult. Natural organic matter (NOM) content in water can be described by fractions of total organic carbon (TOC). TOC is the sum of

dissolved organic carbon (DOC) and particulate organic carbon (POC) (Thurman, 1985). POC is the organic carbon retained on a 0.45 µm pore-size membrane filter and DOC is the amount that passes through the membrane (Thurman, 1985). DOC is often used to characterize NOM in source waters (Christman and Gjessing, 1983; Thurman, 1985). In DOC measurements, filtration removes macroscopic particulate organic carbon (e.g. zooplankton, algae, bacteria and detrital organic matter from soil and plants), while viruses and some ultra-small bacteria pass through the filter and are enumerated with the dissolved component (Thurman, 1985). DOC concentrations can be determined by oxidation of the dissolved organic load to carbon dioxide, and quantification of carbon dioxide by infrared spectrometry (Thurman, 1985).

Ten to twenty percent of organic matter in natural waters (e.g. aquifer and river water) is composed of simple sugars, amino acids, fatty acids, and hydroxyl acids that are simple organic compounds from decomposition of plants and soils (Thurman, 1985). Polysaccharides make up roughly 0-25% of environmental organic matter (Sachse et al. 2005). The remainder of the DOC content is polymeric molecules, called hydrophilic acids, which are also derived from the decomposition of plant and animal tissues (Thurman, 1985), but are more persistent in the environment than their precursors (Gaffney et al. 1996). Fifty to seventy percent of the molecular DOC present in natural waters is composed of humic substances that are yellow polymeric organic acids (Sachse et al. 2005; Thurman, 1985).

Humic substances can be separated into the higher molecular weight humic acids, and lower molecular weight fulvic acids (Christman and Gjessing, 1983; Gaffney et al. 1996). Fulvic acids have a higher content of carboxylic acid, phenolic and ketonic groups which make them more soluble in water at all pH values (Gaffney et al. 1996). Humic acids are more aromatic, and when the carboxylate groups are protonated at low pH values they become less soluble in water (Gaffney et al. 1996).

2.4.2 NOM in Natural Waters

NOM is ubiquitous in natural waters. DOC values are typically higher in surface waters than in alluvial aquifers. DOC concentrations in North American surface waters have been reported from 0.1 to 50 mg/L (Christman et al. 1983), while deep groundwater concentrations have been reported from 0.1 to 10 mg/L (Gaffney et al. 1996). Average DOC values in river waters are between 2 and 10 mg/L, high quality streams typically range from 1 to 3 mg/L, and groundwater is typically less than 1.5 mg/L, with average values around 0.7 mg/L (Thurman, 1986). DOC concentrations in oligotrophic lakes are typically less than 2 mg/L while eutrophic lakes may be 10 mg/L or more (Thurman, 1985). Higher environmental DOC concentrations in water have been detected in wetlands, with values between ~10-60 mg/L, while peatlands have been shown to have the highest DOC concentrations (> 100 mg/L) (Moore et al. 2003).

Seasonal variations in the DOC content of surface waters may exist. Sachse et al (2005) found that a variety of surface waters in Germany had DOC concentrations 5 to 10 percent higher in the summer than in the winter. Additionally, polysaccharide content was twice as high in the summer, while humic fractions were higher in winter during discharge periods (Sachse et al. 2005). Biber et al (1996) surveyed North American surface waters and also found soil-derived organic matter was higher in winter and spring, and that aquagenic organic matter content was higher in summer months.

Climate change may affect environmental DOC concentrations and seasonal variations (Emelko et al. 2011). Drought and rewetting cycles may impact water quality as increased temperatures can enhance decomposition, and increased precipitation can cause flushing of organic matter into streams (Delpha et al. 2009). Increasing DOC has been documented in North American and European environments since the 1980's; however there is speculation as to whether this is caused by changes in climate or anthropogenic acidification of the atmosphere (Monteith et al. 2007). Changes in temperature, carbon dioxide, rainfall amount and intensity have been cited to affect DOC trends in the environment and source waters (Delpha et al. 2009).

2.4.3 NOM Effects in RBF

NOM impacts the efficiency and effectiveness of water filtration processes and the final effluent water quality. As water passes through the saturated subsurface in a RBF system, complex biological processes and adsorption cause changes in humic substance content to occur. Longer residence times in aquifers allow for more microbial degradation of organic carbon. For example, active subsurface bacteria can convert humic substances into methane or oxidize it into carbon dioxide (Thurman, 1986). Consequently, surface water drawn into RBF sites typically has DOC concentrations higher than that of the water in the aquifer. Nissinen et al (2001) found that large and intermediate humic fractions were dominant in lake and river samples, while artificially recharged groundwaters and natural groundwaters were predominated by intermediate and small humic fractions.

Humic substances are negatively charged and can adsorb readily to grain surfaces (Thurman, 1986). In RBF, DOC in the incoming surface water can adsorb onto previously positively-charged patches of the media grains (Wagai et al. 2009; Abudalo et al. 2010), and produce a net negative charge. Relatively low concentrations of NOM can cause charge reversal of media grains. The degree of cation saturation of the soil may indicate the amount of NOM adsorption (Theng, 1976). Abudalo et al (2010) used streaming potential measurements to show that DOC concentrations of approximately 1 mg/L fulvic acid could

cause charge reversal from positive to negative on ferric oxyhydroxide-coated sand. Over time, organic matter can continue to be adsorbed onto sand grains, further increasing the negative charge of media grains in the subsurface (Bixby and O'Brien, 1979; Abudalo et al. 2010).

The adsorption of NOM onto collector gains can reduce attachment of colloids (Ryan and Gschwend, 1992; Yan et al. 2010), protozoa oocysts (Dai and Hozalski, 2002; Abudalo et al. 2010), bacteria (Foppen and Schijven, 2006; Kim et al. 2008), as well as viruses and bacteriophage (Bixby and O'Brien, 1979; Pieper et al. 1997; Ryan et al. 1999; Zhuang and Jin, 2003; Foppen et al. 2006). NOM can also adsorb onto the surface of the colloids (Scholl and Harvey, 1992), further reducing attachment to media grains. The sorption of DOC onto mineral and colloid surfaces increases the thickness of the electrical double-layer. This creates greater repulsion between the collector and colloid surfaces (Ryan and Elimelech, 1996).

It has been speculated that the adsorption of organic matter onto collector grains, rather than onto colloids, is more likely the cause of reduced attachment of bacteria onto media grains in the subsurface (Johnson and Logan, 1996; Dong et al. 2002). Abudalo et al (2010) used microelectrophoresis to show that the surface charge of oocysts was not significantly impacted by the presence of DOC (increased from 0 to 20 mg/L). It was concluded that the enhanced transport of oocysts was caused by NOM coating ferric oxyhydroxides on the media grain surfaces, which reduced attachment through electrostatic repulsion (Abudalo et al. 2010). Scholl and Harvey (1992) demonstrated that organic matter competes for the same attachment sites that attenuate viruses in filtration. In natural subsurface environments DOC concentrations are usually higher than virus concentration, resulting in decreased virus attachment due to competition for favourable attachment sites (Scholl and Harvey, 1992). Bixby and O'Brien (1976) concluded that MS2 phage (a common virus surrogate) can become chemically bound to fulvic acid, which interferes with the attachment of virus in soils. Nevertheless, most pathogen surfaces are negatively charged at typical groundwater pH values (Harden and Harris, 1952; Schijven and Hassanizadeh, 2000), and these conditions can reduce the amount of contact opportunities between pathogens and collector grains (Bixby and O'Brien, 1979; Dai and Hozalski, 2002). These findings indicate that higher organic loads entering aquifers or engineered filters may coat media grains and/or bacteria and viruses and reduce the overall removal of pathogens by these processes. The relative magnitude of these effects, as well as the concurrent effects of other physico-chemical changes in filtration systems, require further investigation to better predict the risk of pathogen passage into filtered drinking water.

2.5 Bacterial EPS and Bacterial Transport

2.5.1 EPS Definition

The production of EPS by bacteria is both a natural metabolic activity (e.g. the excretion of waste) (Morgan et al. 1990), as well as a protective response to environmental stressors (e.g. antimicrobial agents, see Allison and Sutherland, 1987; Wingender et al. 1999; Vu et al. 2009). The literature exploring the biological role, characterization and various factors affecting EPS production by bacteria is vast. This is partly due to the fact that EPS contributes to biofilm formation, which is of interest in numerous fields of study. These include food production and processing (e.g. maintaining sterile manufacturing environments); healthcare and sanitation (e.g. studying *Pseudomonas* spp. in cystic fibrosis patients); river sediments and microbial mats; wastewater treatment and activated sludge reactors; engineered biofiltration in drinking water treatment; and bacterial transport in the subsurface (either for the protection of drinking water sources or bioremediation efforts).

In porous media filtration, bacterial transport is impacted by complex cell shapes and surface characteristics. Bacterial pathogens are often rod- or irregularly-shaped, and the lipopolysaccharides of gram negative bacteria may protrude more than 30 nm from the outer cell membrane into the suspension medium (Williams and Fletcher, 1996). EPS production on the surface of bacterial cell membranes has been implicated as a factor affecting the transport and attachment of bacteria in saturated porous media environments (Gancel and Novel., 1994; Tsuneda et al. 2004; Walker et al. 2004; Liu et al. 2007; Kim et al. 2009). These transport studies range from highly controlled clean-bed laboratory investigations using pure bacterial cultures (Jucker et al. 1997; Long et al. 2009; Tong et al. 2010), to complex field investigations of micro-colonies with complete biofilm formation (Gannon et al. 1991; Dong et al. 2002). The current literature describing EPS production responses to environmental conditions and subsequent effects on microbial transport in saturated porous media is inconsistent and often contradictory. This is likely because methods for extraction, quantification and characterization of EPS are numerous and lack standardization. The variability in EPS production and content by different bacterial strains and in different natural environments further complicates the development of an understanding of the role of EPS in filtration, and warrants further investigation.

2.5.2 Metabolism of EPS

The production of EPS by bacteria involves the transport of enzyme proteins and polysaccharides to the surface of the bacterial cell wall. Gram positive bacteria secrete EPS across the cytoplasmic membrane; and Gram negative bacteria secrete EPS across the inner and outer membrane (Madigan et al. 2003)

(Figure 4). Three main pathways have been identified for EPS secretion by bacteria. Type I is the adenosine triphosphate binding cassette (i.e. ABC pathway), Type II is the general secretory pathway (in Gram negative bacteria only) and Type III is the contact site dependent secretion pathway (developed in animal pathogens) (Wingender et al. 1999). A single bacterial species can utilize multiple pathways for EPS secretion. For example, strains of *Pseudomonas aeruginosa* have the ability to use all three pathways for various enzyme secretions (Wingender et al. 1999).

Bacteria require high amounts of carbon and energy input to produce EPS (Wingender et al. 1999) and EPS production has been described as a metabolic result of the internal production of amino acids and uptake of potassium ions (Wingender et al. 1999). Once secreted, EPS can remain situated permanently or transitory-bound to the cell surface structure. When EPS materials are released into the aqueous environment they are termed "cell free" or free-EPS (Wingender et al. 1999) and are also known as the colloidal fraction of EPS extracts (Aguilera et al. 2008).

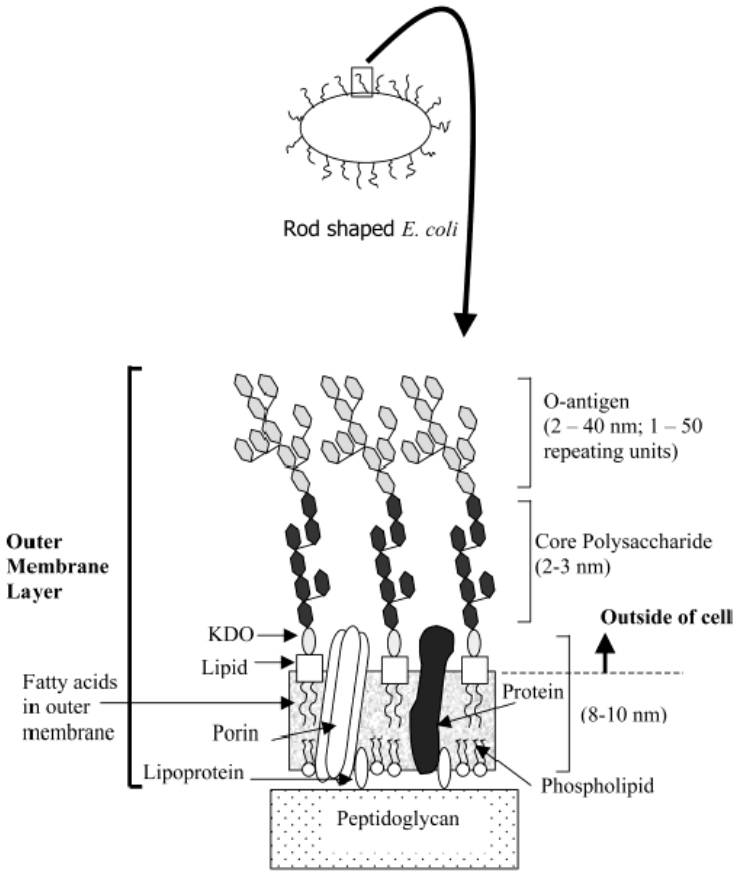


Figure 4: Schematic representation of a typical outer membrane of a gram-negative bacterium (Burks et al. 2003; Madigan et al. 2003).

2.5.3 Bacterial EPS Composition and Production

The composition of EPS material has been investigated in several studies, but inconsistencies in EPS extraction and quantification methods make it difficult to draw general conclusions. It has been suggested that the EPS produced by several bacterial strains is primarily composed of proteins (Long et al. 2009) and polysaccharides (Zhu et al. 2009; Frølund et al. 1996; Jucket et al. 1997; Jucker et al. 1998; Tsuneda et al. 2003; Kim et al. 2009); while the EPS of other bacteria may have a larger component of uronic acids (Blumenkrantz and Asboe-Hansen, 1973; Dade et al. 1990; Gómez-Suárez et al. 2002). The major composition of a given bacterial EPS coat may shift as a result of changing environmental conditions. Morgan et al (1990) found that protein was the major component of EPS from anaerobic sludge samples, while carbohydrates dominated in activated sludge samples. Dade et al (2009) found that the production of uronic acids by marine bacteria was correlated with nitrogen content of the growth media.

The composition and production of EPS varies between organisms (Fletcher, 1996), and correlations between surface phenotypes and gene expression have been difficult to establish. There may also be high variability between EPS from bacteria of the same genotype. Wijesinghe et al (2009) surveyed *E. coli* isolates in urban and rural sections of a mixed land-use watershed and found a 32.8% similarity in all DNA fingerprints (n = 271), while greater genetic diversity was found in secondary aquatic habitats. The genetic diversity of *E. coli* varied with season, flow conditions and sampling location (Wijesinghe et al. 2009). Yang et al (2004) were unable to identify a correlation between genotypic and phenotypic characteristics in 280 *E. coli* isolates of bovine origin. Yet, it was found that that *E. coli* strains that possessed gene *agn*43 (43% of the population) for motility were noted for the ability to form biofilm in an agar assay (Yang et al. 2004). These results are analogous with variability observed between human phenotypes. All humans are of the same species (i.e. *Homo sapiens*) but relatively small changes in DNA sequences can create great differences in a person's appearance (e.g. hair type, skin colour, height, etc). The surfaces of bacteria may also show a large degree of heterogeneity within natural populations due to small DNA substitutions.

The production and composition of EPS may be affected by environmental and growth conditions including temperature, pH, available nutrients, and carbon source (Gancel and Novel, 1994; Wingender et al. 1999). Vu et al (2009) found that the organism *Acidithiobacillus ferrooxidans* produced greater amounts of EPS in phosphorus depleted environments, possibly in response to stress. Bonet (1993) investigated the effects of several water chemistry factors on EPS production by the bacterium *Aeromonas salmonicida*, and found that EPS production was hindered when glucose, phosphate, magnesium chloride, or trace mineral components were absent from the medium. However, EPS

production increased with the addition of yeast. EPS production by this strain was not affected greatly by small changes in temperature (15-20 ^{o}C), but EPS production was at a maximum at neutral pH values and near the end of the logarithmic growth phase. Walker et al (2005) found that stationary cells of *E. coli* D21g were noticeably more adhesive than mid-exponential (i.e. log) growth phase, which was attributed to the high degree of heterogeneity on stationary-phase cells.

Slower-growing bacteria may produce relatively more EPS (Wingender et al. 1999). The presence of lactose and other sugars that decrease bacterial growth rate were found to increase surface polymer syhthesis on cells of *Streptococcus salivarius* ssp. *thermophilus* (Gancel and Novel, 1994). Omoike and Chorover (2004) found that slow-growing strains of *Bacillus subtilis* produced relatively large amounts of EPS, particularly in the stationary growth phase. A higher quantity of free EPS was also observed relative to cell-bound EPS from *B. subtilis*.

The amount of EPS produced by bacteria can be responsive to environmental stressors, including nutrient availability. Bacteria can use EPS to trap nutrients and ions to supplement those which might be deficient (Wingender et al. 1999). Additionally, the EPS matrix can retain a high amount of hydration at the bacterial cell wall surface (Wingender et al. 1999), which can be essential for biological activity and survival. The dynamic and transient nature of bacterial EPS production and content complicate the development of methods for extracting and characterizing EPS. Nevertheless, several methods have been explored to further characterize EPS in both microbial mats and pure suspensions.

2.5.4 Effects of Laboratory Bacterial Propagation Methods on EPS Characteristics

Bacteria appear to produce EPS in both stressful and favorable environments and it is possible that these two types of conditions may contribute to differences in EPS composition. In stressful environments, bacteria may have a greater likelihood of survival if they can secrete EPS to provide a protective shield and also to be transported to other, possibly more favorable, environments. Once a favorable location is reached, bacteria may produce EPS to encourage contacts with collectors to form micro-colonies. It is possible that the EPS produced during these different stages assists with re-location as well as attachment. Understanding the production of EPS by bacteria becomes complex when considering numerous biological responses and inter-strain variations, which further complicate developing an understanding of the importance of EPS in pathogen transport in porous media systems. Unfortunately, many studies have not made clear whether the bacteria used in porous media investigations were held in stressful, favorable or neutral conditions prior to inoculation. it may be necessary to develop a framework for these environmental classifications.

Controls in laboratory experimentations are important to ensure minimal error and high reproducibility, and to ensure fair organism representation in the case of bacterial EPS investigations. For example, methods that include routine centrifugation can have a significant effect on bacterial attachment experiments. Smets et al. (1999) found that centrifuged cells of *Pseudomonas fluorescens* showed only ~10% attachment while ~40% of non-centrifuged cells attached to glass beads in a packed column. These results were well correlated with resulting cell surface charges (i.e. zeta potential), because the centrifuged cells were more negatively charged than the non-centrifuged cells. The effects of cell preparation on surface charge and heterogeneity have yet to be elucidated and deserve further investigation to allow for extrapolation of laboratory results to environmental systems.

Castro and Tufekji (2007) demonstrated the importance of experimental controls and reproducibility when conducting transport experiments using of pure bacterial cultures in porous media packed columns. Specifically, they demonstrated that temperature differences (11 and 22 ^{o}C) significantly affected the transport of two non-toxigenic strains of *E. coli* and pathogenic *E coli* O157:H7; generally more attachment occurred at the higher temperature. Bacterial strains with various suspension acclimation times produced similar results when passed through porous media packed columns (Castro and Tufankji, 2007). These results underscore the importance of bacterial strain selection, experiment design controls, and the need for caution in extrapolation of laboratory results to field applications.

2.5.5 Bacterial EPS Extraction Methods

While free- or colloidal-EPS is relatively easy to collect (e.g. found within the supernatant of centrifuged biofilm samples), the capsular fraction of EPS consists of tightly bound compounds that require a more extensive extraction protocol (Hirst and Jordan, 2003). Several physical and chemical extraction methods have been explored for various applications where EPS characterization is prudent, and the general execution of each of these methods are presented in Table 1 and Table 2, respectively. No standardized method for EPS extraction has been established for various EPS-containing substrates to date (Comte et al. 2006; Liu and Fang, 2002). Typical substrates include EPS-containing biofilm, sludge from wastewater treatment plants (WWTP), or pure bacterial suspensions. Initially to isolate the EPS-containing substrates, samples are centrifuged (using the range of speeds and durations described in Table 3 and the pellet is suspended in an extraction buffer to conduct the individual extraction procedures. Following extraction, the suspension is often centrifuged again to collect the EPS containing supernatant and separate the extracted EPS from the extraction chemicals or particles.

Table 1. Physical EPS Extraction Methods

EPS Extraction Method	Procedure	Sources
	Physical and Chemical	
CER	Suspended in either DOWEX® CER 50x8, 2.5 g in 5 mL extraction buffer (2mM Na_3PO_4; 4mM NaH_2PO_4; 9 mM NaCl and 1 mM KCl, pH 7) and stirred for 30 min to 3 h at 4°C.	Aguilera et al. 2008; Comte et al. 2006; Azeredo et al. 2003; Liu and Fang, 2002; Frølund et al. 1996; Karapanagiotis et al. 1989; originally in Rudd et al. 1983
	Physical	
Sonication	Sonicated for 30s to 2 min (or 10 min in a sonication bath for ultrasonication; Brown and Lester, 1980) at 20 to 40 W with 13 mm probe immersed 40 mm into the liquid. Suspensions can be kept on crushed ice during sonication.	Pan et al. 2010; Gong et al. 2009; Comte et al. 2006; Azeredo et al. 2003; Kiff and Thompson, 1979
High-Speed Centrifugation	Centrifuged at 33,000 to 36,500*g* for 10 to 15 min at 4°C. The pellet can be re-suspended and centrifugation repeated.	Pan et al. 2010; Zhang et al. 1999; Brown and Lester, 1980; Kiff and Thompson, 1979; originally in Pavoni et al. 1972
Steaming	Steamed in an autoclave for 10 min and then centrifuged while still hot at 8000*g* for 10 min (temperature reduced to 15°C).	Zhang et al. 1999; Brown and Lester, 1980; originally in WPRL, 1971
Heating	Heated for 10 min to 1 h at 80°C (1 bar).	Comte et al. 2006; Karapagnagiotis et al. 1989; Kiff and Thompson, 1979
Homogenization	Blended in a homogenizer at maximum speed for 4 min.	Kiff and Thompson, 1979
Shear	Samples are extruded under pressure through the narrow orifice valve of a "Bel" cream-maker.	Kiff and Thompson, 1979
Lyophilization	Frozen (-80°C) for 15 min. Sample is then placed in a lyophilizer for a minimum of 6 h at -60°C at a low pressure setting (~60 mTorr). EPS pellet is suspended in 10 mL DI water and vortexed prior to characterization.	Gong et al. 2009

Table 2. Chemical EPS Extraction Methods

EPS Extraction Method	Procedure	Sources
Milli-Q™	Suspended in Milli-Q™ water for 3 h at 30°C.	Takahashi et al. 2009; Aguilera et al. 2008
NaCl / ASW	Suspended in NaCl (9 mM or 10% w/v) for 3 h at 80°C.	Aguilera et al. 2008; Klock et al. 2007; originally by Rougeaux et al. 2001
EDTA	Suspended in EDTA (100 mL of 2% EDTA added to 100 mL sample) for 3 h at 4 to 21°C.	Pan et al. 2010; Aguilera et al. 2008; Comte et al. 2006; Liu and Fang, 2002; Zhang et al. 1999; Brown and Lester, 1980; Staats et al. 1999; Nishikawa and Kuriyama, 1968
NaOH	EPS material is added to 2 volumes of 2 M NaOH. Samples are gently agitated for 5 h at 20 °C, and then diluted with water to the original sample volume.	Karapanagiotis et al. 1989; Brown and Lester, 1980; originally by Tezuka, 1973
Formaldehyde and NaOH	Exposed to 36.5% formaldehyde for 1 h at 4°C and then NaOH (1M) for 3 h at 4°C.	Pan et al. 2010; Comte et al. 2006; Liu and Fang, 2002; Zhang et al. 1999
Phenol	Treated with 100 cm^3 90% (w/v) phenol preheated to 70°C for 15 min. Samples are cooled on ice and centrifuged at 5°C. This is followed by 12 h of dialysis and centrifugation at 33,000*g* for 20 min at 5°C. Extracted EPS is recovered in the supernatant fluid.	Karapanagiotis et al. 1989
Ethanol	Ice-cold ethanol (96%) is added to EPS material up to a final concentration of 70%. The high molecular weight polymers are allowed to precipitate for 8 h at 4°C. Ethanol precipitation is repeated up to three times to increase precipitation and purity of EPS extract.	Gong et al. 2009; Klock et al. 2007

2.5.5.1 Comparison of EPS Extraction Methods

Table 3 lists studies that have explored and compared EPS extraction methods for various EPS-containing substrates and the results of these comparison studies are summarized below. Each study evaluated EPS extraction methods based on similar criteria, including total mass of EPS extracted, protein and carbohydrate concentrations achieved, protein:carbohydrate content, and/or the impact of each extraction on cell integrity. The quantity of total EPS extracted by different methods should not be used for comparison purposes due to the differences in mechanisms by which each method removes and isolates EPS (Gong et al. 2009; Aguilera et al. 2008; Liu and Fang, 2002; Rudd et al. 1983). Although, Rudd et al. (1983) suggests that while using the same method for EPS extraction, results from colorimetric measurement of EPS carbohydrates can be an effective approach for comparison of EPS from various substrates. In the absence of a standardized method, the selection of EPS extraction methods should not be limited to those that extract the largest quantity of EPS, but should also include consideration for contamination by extraction reagents (Pan et al. 2010; Comte et al. 2006). The compromise between quantity of EPS extract and cell integrity can be difficult (Azeredo et al. 2003).

Cell integrity has been assessed by a variety of methods, including DNA content (Zhang et al. 1999), intracellular protein detection (Klock et al. 2007), ATP presence (Takahashi et al. 2009; Azeredo et al. 2003), cell viability (Gong et al. 2009), TEM imaging (Takahashi et al. 2009), and cell wall integrity (Kiff and Thompson, 1979) following EPS extraction. Certain methods for assessing, quantifying and comparing cell lysis may be more effective than others, as nucleotides and proteins are excreted as cellular waste products and considered natural components of EPS content (Nishikawa and Kuriyama, 1968). EPS extraction methods that have been affiliated with higher cell lysis include NaCl (Aguilera et al. 2008) and Milli-Q^{TM} water (Aguilerat et al. 2008). Methods found to cause less cell lysis during EPS extraction include CER methods and EDTA (Aguilera et al. 2008); although Pan et al. (2010) found that EDTA contributed to cell lysis. Time sensitivity was evaluated by Frølund et al. (1996). Fang and Jai (1996) and found that extractions completed in less than 1 h reduced cell lysis (i.e. rupture), when compared to extractions conducted for 3 h or longer. Azeredo et al. (2003) found that *Pseudomonas* spp. biofilms were more susceptible to ATP release than *Alcaligenes* spp. biofilm, indicating that certain cellular cultures may be more susceptible to cell lysis by various EPS extraction methods.

Several studies support the use of CER either alone or in combination with high-speed centrifugation and/or sonication for EPS extraction from cellular cultures due to the pure extract obtained, easy separation of EPS extract, and low cell lysis (Sheng et al. 2010). Alternatively, Klock et al. (2007) recommended an optimized EPS extraction protocol that includes the use of fresh mat samples, triplicate

extraction with NaCl (15 min at 40^{o}C), triplicate ethanol precipitation (70%, final concentration) and dialysis against distilled water (3 X 3 h, 10,000 Da). By this extraction procedure, a high molecular weight fraction was obtained.

The presence of reagents in EPS extract could partially or completely prevent the determination of EPS characterization (Pan et al. 2006; Comte et al. 2006). Comte et al. (2006) and Azeredo et al. (2003) investigated the addition of gluteraldehyde (GTA) (pH 7.0, 0.01 M) prior to EPS extraction (incubation at 4^{o}C for 12 to 3.5 h with 50 mL 2 to 10 % w/v GTA). Azeredo et al. (2003) utilized this pre-treatment method to provide a protective effect against cell lysis during relatively aggressive extraction protocols such as sonication. Less adenosine triphosphate (ATP) was detected following sonication, indicating that GTA could prevent cell lysis where long periods of sonication were employed. However, Comte et al. (2006) demonstrated, with the use of infra-red analysis, that GTA cannot effectively be washed from EPS extracts and can substantially interfere with biochemical composition analysis. These findings highlight that the use of chemicals for EPS extraction, particularly GTA, should be re-examined for the potential to interfere with subsequent analysis methods, as well as reactions with EPS compounds that may modify their properties (Pan et al. 2010). Pan et al. (2010) recommend appropriate physical methods (e.g. low-intensity ultrasonication) be used to enhance cell yield, and that caution should be exercised with chemical methods as these reagents can considerably affect the interaction of EPS extracts and release intracellular components.

Another pre-treatment method that has recently been explored is low-intensity ultrasound. Pan et al. (2010) used an ultrasound generator to apply ultrasound to algal-bacterial biofilm samples following ultrasonication (40W for 2 min) and before sonication and centrifugation. It was found that this pretreatment method doubled the EPS yield without significantly modifying the composition of EPS. Ultrasound and other methods for pretreatment of EPS-containing substrates, prior to EPS extraction, should be explored to elucidate the beneficial effects of increased EPS yield, while monitoring for EPS composition and cellular integrity.

CER. CER cleaves EPS by removing divalent cations that form cross-linkages of charged compounds in the EPS matrix (Jahn and Nielsen, 1995). CER extraction has been shown to be the most effective and least disruptive method for EPS extraction when compared to other physical and chemical methods (Takahashi et al. 2009; Frolund et al. 1996; Karapanagiotis et al. 1989; Rudd et al. 1983). This method is also preferred because it combines chemical and physical mechanisms of EPS extraction and is relatively easily separated following extraction (unlike purely chemical methods) (Rudd et al. 1983). Extraction of EPS using CER is most commonly performed using DOWEX® resin; although crown ether CER was

used in one study (Wuertz et al., 2001) and was found be less effective at extracting EPS. CER extraction methods may preferentially cleave the protein component of EPS materials (Aguilera et al. 2008). EPS extracted from two types of activated sludge have been shown to have protein and carbohydrate content in the range of 3-5:1 (Frolund et al. 1996; Karapanagiotis et al. 1989). CER methods for EPS extraction have been shown to cause very low levels of cell lysis, even after 4 hours of agitation (Aguilera et al. 2008; Frolund et al. 1996); however, slightly more ATP was detected when samples were agitated with DOWEX® at 1000 rpm when compared to 600 rpm (Azeredo et al. 2003). The CER method has been shown to extract more EPS than methods using heat, NaOH, EDTA, Milli-Q™, and phenol (Aguilera et al. 2008; Karapanagiotis et al. 1989).

High-Speed Centrifugation. This method has been used in several studies for the extraction of EPS due to the high yield, low cell disruption, and absence of chemical addition (Brown and Lester, 1980; Kiff and Thompson, 1979). Brown and Lester (1980) found that high-speed centrifugation was the most effective EPS extraction method for pure cultures of *Kelbsiella aerogenes,* when compared to untrasonication, steaming and NaOH treatment. However, this method was not effective at extracting carbohydrates from activated sludge (Brown and Lester, 1980). Kiff and Thompson (1979) also support the use of this method for high EPS extract yields when compared to heating, homogenization, shear, and sonication. Although the results from high-speed centrifugation did not show a high level of reproducibility, this method provided relatively pure and intact EPS.

Sonication. Sonication has been found to produce low yields of EPS extract, relative to high-speed centrifugation and homogenization (Brown and Lester, 1980; Kiff and Thompson, 1979). This method was not effective at extracting carbohydrates from activated sludge (Brown and Lester, 1980). The effect of sonication on cell lysis is inconclusive; Brown and Lester (1980) and Pan et al. (2010) found that no substantial damage was caused to cell integrity with sonication, while Kiff and Thompson (1979) found that sonication increased cell breakage. These inconsistencies may be the result of various intensities of sonication employed. Brown and Lester (1980) may have found less cell breakage since the samples were agitated in a sonication bath at low voltage, and samples were centrifuged for separation at only 2000*g*, compared to 25,000g in Kiff and Thompson (1979). It is suggested that low-intensity sonication could be used as a pre-treatment in conjunction with other extraction methods, but this has yet to be elucidated (Pan et al. 2010; Brown and Lester, 1980).

Homogenization. This method has been shown to produce lower yields of EPS extract from activated sludge when compared to high-speed centrifugation and sonication (Kiff and Thompson, 1979).

Shear. The results from shear methods for the extraction of EPS from activated sludge showed lower yields than high-speed centrifugation, although the results were more reproducible (Kiff and Thompson, 1979).

Steaming. Brown and Lester (1980) found that this was the most effective treatment for EPS extraction from activated sludges, when compared to centrifugation, ultrasonication, and NaOH. Additionally, steaming was found to cause less cellular disruption than NaOH. However, steaming has been found to preferentially extract the carbohydrate component of EPS-containing materials and release intracellular proteins (Zhang et al. 1999; Brown and Lester, 1980).

Heating. This method has been shown to cause a high degree of depolymerization of EPS extract from activated sludge, and cause substantial cell breakage (Kiff and Thompson, 1979).

Lyophilization. This method of extraction was found to produce higher amounts of EPS extract when compared to sonication (Gong et al. 2009); however, the advantages of this method have yet to be compared to other methods available.

Milli-Q™. Exposure to Milli-Q™ water showed a high level of cell lysis during EPS extraction from activated sludge and benthic diatom mats (Aguilera et al. 2008; Takahashi et al. 2009).

NaCl or Artificial Sea Water (ASW). NaCl is through to promote EPS cleavage from the cell wall through cation exchange (May and Chakrabarty, 1994). This method has been shown to preferentially extract the carbohydrate component of EPS material. Additionally, a high level of cell lysis during extraction using NaCl has been observed (Aguilera et al. 2008).

EDTA. This method can increase the carbohydrate yield likely by chelating metal ions that formed interchain linkages between hexoses (Underwood et al., 1995). It has been found that EDTA causes the release of intracellular proteins, particularly when coupled with physical extraction methods (Pan et al. 2010), and interferes with protein analysis of EPS extracts by intensifying the blue colour produced bycolour produced by the reaction (Brown and Lester, 1980).

NaOH. NaOH or alkaline treatment ionizes carboxylic groups within the EPS matrix, and effectively dissolves EPS away from the cell membrane (Sheng et al. 2005). Treatment with NaOH for EPS extraction was found to cause extensive cellular disruption in both activated sludge and pure bacterial cultures, and preferentially extract the carbohydrate and protein components of activated sludge and pure cultures, respectively (Brown and Lester, 1980). Karapanagiotis et al. (1989) found that suspending

sewage sludge in 0.05 M NaOH was an effective pre-treatment for reducing an inherent "slime" layer that interfered with EPS extraction methods.

Formaldehyde and NaOH. The addition of formaldehyde reduces cell lysis that can be caused by NaOH (Liu and Fang, 2002). Liu and Fang (2002) determined that this method was the most effective in extracting EPS from activated sludge, with minimal cell leakage, when compared to sonication, CER, and EDTA. However, the use of confocal laser scanning microscope revealed that this method only extracted a small fraction of the available EPS for extraction. Additionally, the results of Comte et al (2006) show that these results (i.e. high observed protein and carbohydrate extracted) may be biased by the use of chemical formaldehyde, that can contaminate and artificially inflate colorimetric methods for biochemical characterization of EPS extracts.

Phenol. Phenol was found to interfere with protein extraction and produced substantially lower protein to carbohydrate ratio than other methods investigated (e.g. steaming and CER) (Karapanagiotis et al. 1989). Karapanagiotis et al. (1989) suggest that this method could be useful where only the carbohydrate fraction of EPS is of interest, but point out that this chemical can be rather complicated and hazardous for routine use.

Ethanol. Gong et al. (2009) found that this method produced higher EPS extract yields when compared to sonication, but the effectiveness of this method has yet to be compared against other available methods.

2.5.5.2 Purification of EPS Extract

Once extracted and isolated (via centrifugation or lyophilization), EPS samples can be enumerated directly or further "purified" using dialysis or ethanol precipitation, or filtration. Pan et al. (2010) report filtering supernatants (following ultrasonication and centrifugation) through 0.22 μm pore membranes. The filtrate was used as the EPS sample for further characterization. Kiff and Thompson (1979) report precipitation of polymeric materials with the addition of ethanol and refrigeration at 4^{o}C for 24 h. Purification of EPS extracts has been performed using dialysis for 24 h at 4 ^{o}C (Liu and Fang, 2002; Comte et al. 2006; Klock et al. 2007). In other studies, purification by dialysis for 12 h has been preferred (Pan et al. 2010; Karapanagiotis et al. 1989). Pan et al. (2010) found that EPS extracts that were dialysed with a 3,500 Dalton molecular cut off membrane to remove humic substances from interfering with EPS characterization. Lyophilization (i.e. freeze drying) has also been used to permit the weights of EPS extracts to be compared (Gong et al. 2009; Aguilera et al. 2008; Liu and Fang, 2002). Klock et al. (2007) reported freeze drying half of the EPS extract to determine the dry weight, while the other half was stored at -20^{o}C and used for partial chemical characterization. However, the mass of EPS from biofilm mats or

activated sludge is relatively large compared to that obtained from pure bacterial suspensions, which would not contribute to a high level of detection when using lyophilization for small quantities of EPS extract.

Table 3. Studies Investigating EPS Extraction Methods

Source	Substrate	Centrifugation Speed (*g*)	Centrifugation Time (min)	EPS Extraction Methods
Pan et al. 2010	Alga-bacteria biofilm (pond-sourced)	20,000	20	Centrifugation Ultrasonication EDTA Formaldehyde Formaldehyde and NaOH
Gong et al. 2009	*Salmonella pullorum* SA 1685	3700	15	Lyophilization Ethanol Sonication
Takahashi et al. 2009	Benthic diatoms *Navicula jeffreyi*	2000	15	Milli-Q™ CER: DOWEX® ASW Gluteraldehyde and Milli-Q™ Gluteraldehyde and CER: DOWEX®
Aguilera et al. 2008	Benthic eukaryotic biofilms	4000	15	Milli-Q™ NaCl EDTA CER: DOWEX® CER: Crown Ether
Klock et al. 2007	Cyanobacterial mats	4000 - 8220	15	EDTA Centrifugation ethanol NaCl and ASW
Comte et al. 2006	Two different activated sludges	4000	20	EDTA Formaldehyde and NaOH Gluteraldehyde Sonication CER: DOWEX® Sonication and CER Heating

Azeredo et al. 2003	*Pseudomonas fluoresens* and *Alcaligenes* denitrification biofiilms	5000	5	CER: DOWEX®[a] Sonication[a]
Liu and Fang, 2002	Aerobic, acidogenic and methanogenic sludges	20,000	20	EDTA CER: DOWEX® Formaldehyde and NaOH Ultrasonciation
Zhang, et al. 1999	Aerobic/sulfate reducing and nitrifying/denitrifying biofilm	6000	10	Centrifugation EDTA Steaming Formaldehyde
FrØlund et al. 1996	Two different activated sludges	2000	15	CER: DOWEX®
Karapanagiotis et al. 1989	Anaerobically digested co-settled sewage sludge	2000	30	CER: DOWEX® Heating NaOH Phenol
Brown and Lester, 1980	Activated sludge and *Klebsiella aerogenes*	2000	20	Centrifugation Sonication Centrifugation and Sonication Steaming NaOH
Kiff and Thompson, 1979	Activated sludge biomass	-	-	Centrifugation Heating Homogenization Shear Sonication

[a]After pre-treatment with gluteraldehyde

2.5.5.3 Characterization of Free- and Bound-EPS

Investigating the quantity and/or composition of free-EPS should not be ignored, as this may provide more information regarding EPS production for comparison purposes. Zhang et al. (1999) found that free-EPS contributed to between 5 and 18% of total carbohydrate in EPS material. Additionally, Aguilera et al. (2008) found that extracted Free-EPS of benthic eukaryotic biofilms had higher concentrations of carbohydrates than extracted capsular EPS. Accordingly, direct measurements of protein and carbohydrate concentration in free EPS can lead to a better understanding of bacterial EPS production and characterization.

2.5.6 EPS and Bacterial Attachment: Introduction

An important role of EPS production by bacteria is to promote cell adhesion to solid surfaces. This can allow for microcolony (i.e. biofilm) formation, cell-to-cell adhesion, and cell-to-cell communication (Allison and Sutherland, 1987; Vu et al. 2009). This is particularly evident when bacteria are introduced to new environments containing sediments or substrates, where bacteria have been observed to produce excess EPS to initiate colony formation (Allison and Sutherland, 1987; Gancel and Novel 1994, Quintero and Weiner 1995; Liu and Li, 2008).

Initial bacterial attachment to solid surfaces may not be affected by EPS presence, but rather the differences in amount and composition of bacterial EPS can impact the rate and type of biofilm formed following initial attachment (Allison and Sutherland, 1987). Dade et al. (1990) found that marine bacteria produced more EPS following attachment to fine sands, and suggested that this was a protective response to shear stress. The use of cell staining techniques has helped to demonstrate that EPS is involved in the formation of biofilms. Vandevivere and Kirchman (1993) investigated EPS production by environmental strains of bacteria (isolated from a river drilling site in South Carolina) that were all Gram-negative, nonmotile, mucoid producing bacteria. This well controlled study demonstrated that bacteria produced more EPS (higher carbohydrate and polysaccharide content) when media grains were introduced to the suspension. Moreover, EPS production decreased when these surface-grown cells were re-suspended in grain-free media. Evans et al. (1994) found that attached cells of *Staphylococcus epidermidis* produced significantly more EPS than suspended counterparts. Zolghadr et al. (2010) found that two strains of *S. solfataricus* produced more EPS when attached to a glass surface (possibly due to the surface being hydrophilic), and that the form of EPS secreted changed during three days of attachment to the solid surface.

Other surface characteristics of bacteria, such as surface pili or flagella for motility, may affect attachment to media grains in saturated environments. For example, Zolghadr et al. (2010) found that cells lacking either flagella or pili were unable to attach to various surfaces in shaking cultures, but that attached cells showed increased expression of pili genes (ups-A and -B). Accordingly, porous media environments with favorable conditions for attachment (e.g. low porosity, higher ionic strength, and/or low organics) may contain thicker biofilms due to a larger number of bacteria attaching and subsequently contributing significant amounts of EPS to form biofilm mats and micro-colonies.

2.5.6.1 EPS: Effects on Bacterial Transport and Attachment in Porous Media

Investigating the effects of bacterial surface EPS production on the transport and attachment of bacteria in porous media environments can help describe filter performance in a variety of saturated environments. Although it is generally believed that bacterial EPS production and characteristics may affect pathogen transport in porous media filtration, a conceptual model of cellular surface EPS effects on bacterial transport in saturated porous media is lacking. The results to date have been contradictory; both in demonstrating whether EPS typically enhances or prevents attachment and in elucidating the mechanisms of EPS interaction with solid surfaces. Jucker et al. (1998) underscored that the quantification and characterization of polymer interactions was limited by the poor sensitivity of methods determining the physical and chemical properties of bacterial envelopes. Some method advancements have been achieved in recent years for various substrates and bacterial surface characterization has improved (Camesano et al. 2007).

Assessing the effects of bacterial EPS factors on cell attachment in saturated porous media can be confounded by the physico-chemical properties at the surface of the collector grains. Gómez-Suárez et al. (2002) investigated bacterial attachment onto materials with different hydrophobicity for EPS-producing (zeta potential of -9 mV) and –nonproducing strains of *Pseudomonas* (zeta potential of -16 mV). Both strains adhered slightly faster and in slightly higher numbers to the hydrophobic surfaces, and detachment of the EPS-producing strain was greater than the EPS-deficient strain (38 and 28% detachment, respectively). Additionally, the highest initial- and re-adherence was for the non-producing bacterium. In this work, cells were suspended in a buffer, (PBS) which may have altered their surface characteristics; possibly in a manner that is not representative of natural groundwater conditions.

Typical approaches to determining the affects of EPS on bacterial transport in through porous media filtration processes include physical or chemical removal of EPS; adding or promoting EPS for comparison purposes; using mutant (EPS deficient), wild-type (typical EPS production) and “mucoid” (excess EPS producers) strains; and modeling bacterial transport based on measured changes in surface properties (e.g. hydrophobicity, polymer interactions, and surface charge). These approaches may help to elucidate the attachment mechanisms affected by the presence of EPS on bacterial surfaces; however, they may not be practical when evaluating the significance of bacterial surface EPS on bacterial retention in saturated porous media environments.

Pathogenic and non-toxigenic strains of *Pseudomonas* and *E. coli* bacteria have been used extensively in porous media investigations because they are well-characterized, easy to use, generally present in water sources and contribute to biofilm formation (Liu et al. 2007; Castro and Tufenkji, 2007; Bolster et al.

2009; Kim et al. 2009). Laboratory strain organisms are commonly used because they are easy to work with and are well studied; however, it can be argued that considerations for inter-strain variability, surface heterogeneities of soil pathogens and natural physico-chemical conditions are not fairly represented in many controlled studies. Investigating differences in attachment between a variety of laboratory and environmental isolates would benefit the extrapolation of controlled laboratory study findings to environments such as those found in aquifers and drinking water filters.

Inter-strain (i.e. within the same serotype) variability in phenotypes may result in differences in bacterial transport in saturated porous media (Yang et al. 2004; Morrow et al. 2005; Bolster et al. 2009). For example, Castro and Tufenkji (2007) found that three strains of *E. coli* exhibited different attachment efficiencies to quartz sand – highlighting that serotypes of the same bacterial strain can exhibit different transport in saturated porous media under highly controlled conditions. Bolster et al (2009) demonstrated that genetic diversity and pathogen source (i.e. human, bovine and poultry derived) contributed to differences in bacterial attachment to media grains. Twelve strains of *E. coli* were utilized in a well-controlled laboratory study using a 10-cm deep backed bed of clean quartz sand. The percent recovery of bacteria measured in column effluents ranged from 1.7 to 95% (>1 log); differences were also significant between bacterial strains that were all isolated from bovine hosts. It was found that the percentage of bacteria passage through the columns significantly correlated with cell width and sphericity. The authors suggested that variation in surface characteristics including EPS production and characterization also contributed to the observed differences in *E. coli* passage through the filtration columns; however, neither correlated well with percent passage of bacteria. Hydrophobicity and surface charge (similar to those found by Morrow et al. 2005) were substantially different between the various bacterial strains investigated; hydrophobicity ranged from 0.6% to 61% and electrophoretic mobilities (i.e. zeta potential) ranged from -3.5 mV to -49 mV. EPS content also was measured (following extraction using a formaldehyde-NaCl solution, centrifugation, and cold ethanol-KCl exposure). Total protein in the extracted samples varied slightly (from 7.4 to 11.7 mg per 10^{10} cells) and carbohydrate content ranged from 0.074 mg to 0.22 mg per 10^{10} cells. Overall, these studies emphasize that choice of bacteria may be a critical consideration when evaluating microbial transport through saturated porous media.

Variability in transport between bacterial strains may be caused by differences in bacterial phenotypes (De Kerchove and Elimelech, 2008) resulting from genetic diversity (e.g. presence of pili and flagella, cell size and shape, EPS characterization differences). Gannon et al. (1991) investigated bacterial transport in saturated soil with ten strains of bacteria, including those of *Enterobacter, Pseudomonas, Bacillus, Achromobacter, Flavobacterium* and *Arthrobacter* sp. The transport experiments were conducted at 3 ^{o}C and involved passage through 5 cm of packed sand. Bacteria less than 1 μm in size

showed the most transport through the column. These results are consistent with Bolster et al (2009) and colloid filtration theory, in which bacterial size governed retention in sand. No correlations were found between bacterial retention and hydrophobicity, net surface charge, the presence of flagella or capsules, nor genera of the bacterial strain (Gannon et al. 1991). Dong et al. (2002) investigated the differences in simultaneous attachment between two labeled, indigenous soil strains of bacteria in intact soil cores from Oyster, VA. The surfaces of the two bacteria were described as "adhesion-deficient", gram-negative, hydrophilic, and negatively charged. Among the cell properties analyzed, cell length and diameter were statistically related to passage into column effluent. Specifically, only 45% of the shorter, larger-diameter cells (*Comamonas* sp.) attached to the porous medium, while 70% of the longer, smaller-diameter cells (*Erwinia herbicola*) attached. Modeling results suggested that the long, narrow shape of the *E. herbicola* strain allowed for lower pore velocity, higher attachment and lower detachment rates (Dong et al. 2002). These results suggest that factors that alter bacteria size and shape may be primary drivers of transport in subsurface environments, regardless of nuances in surface differences between strains of bacteria.

Several bioremediation studies have investigated the feasibility of removing bacterial surface EPS to enhance bacterial transport further into the contaminated subsurface thereby enhancing bioremediation efficiency (Long et al. 2009; Liu et al. 2007; Bell et al. 2005). Liu et al. (2007) investigated bacterial transport through porous media and deposition of three strains of *Pseudomonas*, each with different EPS production. The presence of EPS on two mucoid strains significantly increased bacterial adhesion when compared to an EPS deficient strain, despite similarities in measured surface charge. Abu-Lail and Camesano (2003) found that the removal of lipopolysaccharides from the surface of *E.coli* cells resulted in ~10% less attachment than wild-type cells. Bell et al. (2005) examined batch retention of wild-type *Pseudomonas putida* treated with cellulose (an enzyme that is known to break down the linkages of EPS molecules) onto glass, and the treatment of *Pseudomonas putida* cells caused less attachment to occur. Tong et al. (2010) used *Pseudomonas* sp. (QG6, motile) and *E. coli* cultures treated with cation exchange resin (Brown and Lester, 1980, Aguilera, 2008) to remove the EPS, and produced cells with comparable surfaces charges for transport investigations. Bacterial strains were transported through columns packed with 20 cm of quartz sand, and bacteria that had EPS intact (untreated) were removed between 5 and 25% more than cells without EPS (treated), at a range of ionic strength conditions (2.5 and 20 mM NaCl, and 5 mM CaCl at pH 6.0). Further investigation by elution of attached cells (using an identical, but colloid-free, wash) demonstrated that the presence of EPS on cell surfaces increased cell deposition in quartz sand, regardless of cell type and motility. These results suggest that EPS on the surface of a variety of bacterial surfaces may increase bacterial attachment onto porous media and that the removal of EPS (by

various mechanisms) can reduce bacterial attachment. However, further investigation into the effects of various EPS-removal methods on the surface characteristics of bacteria is required.

The presence of EPS on bacteria cell surfaces has also been shown to hinder bacterial attachment to granular media (Rijnaarts et al. 1999; Prince and Dickenson, 2003; Kim et al. 2009a; Kim et al. 2009b). Rijnaarts et al. (1999) found that a suite of bacterial strains were hindered from attachment to negatively charged glass surfaces at high ionic strength conditions, and attributed these findings to steric repulsion between surface macromolecules and the solid surface. Prince and Dickenson (2003) found similar results between polysaccharide and non-polysaccharide producing strains of *Staphylococcus* sp. Surface characterization suggested that the hydrophobicity and surface charge of both strains were similar (hydrophilic); however, the use of optical tweezers and evanescent wave light scattering revealed that steric repulsion occurred between the EPS-producing strain, while the non-mucoid strain indicated net attractive forces (Prince and Dickenson, 2003). It has been suggested that the presence of EPS can hinder cell deposition due to electrostatic repulsion; however, these results may be an artifact of the methods used to artificially produce the EPS-deficient strain. Cells of *E. coli* O157:H7, with EPS partially removed by the proteolytic enzyme proteinase K, exhibited greater removal than the EPS producing wild-type strain when ionic strength was increased to above 1mM KCl. Kim et al (2009) reported that, following proteinase K treatment to remove or reduce EPS content from the surface of the wild-type strain, cells were more negative in electrophoretic mobility at ionic strengths less than 1 mM KCl, less hydrophobic, and 22% smaller than untreated cells. Additional artifacts of the EPS removal method may have altered the cells from accurately representing EPS-free or -deficient strains that may be present in natural environments.

When bacteria detach from collector surfaces they may leave behind free-EPS, also known as EPS "footprints". These footprints can be valuable tools for tracking the trajectory of bacterial transport in saturated porous environments (Vandevivere and Kirchman, 1993). The "footprint" left by an EPS-producing strain was found to be more heterogeneous, thick and irregular than that of an EPS-deficient strain; EPS covering was up to 32 nm thick from the EPS-producing strain, and just 9 nm thick from the non-producing strain after one cycle of bacterial passage over a glass substratum (Gómez-Suárez et al. 2002). The potential effects of EPS footprints in subsurface environments are numerous, and may contribute to reducing porosity (particularly near column inlet during laboratory investigations) and biofilm formation. The biological role of these footprints is likely to assist with initial attachment of bacteria in new environments to form micro-colonies and biofilms (Liu et al. 2007).

2.5.6.2 EPS: Mechanisms of Attachment to Porous Media

EPS can affect bacterial interaction mechanisms such as electrostatic interactions, steric repulsion and polymer bridging. Liu et al (2007) investigated attachment of mucoid and EPS-deficient strains of *Pseudomonas aeruginosa* (with similar surface charges) in saturated columns filled with glass beads, and found that attachment of the mucoid strain was significantly higher than that of the EPS-deficient strain, at a variety of ionic strength conditions. These behaviours are not explained well by DLVO theory, and suggested that a combined interaction between DLVO forces, LPS-associated chemical interactions, and the hydrodynamics of a system dictated bacterial attachment exist in these complex systems (Walker et al. 2004).

The amount of EPS produced by a particular strain may impact transport and attachment in porous media. The attachment of EPS-poor strains can be inhibited by electrostatic interactions between charged bacterial surface proteins and collector surfaces; while the attachment of EPS-rich strains can be enhanced by attractive polymeric interactions. Tsuneda et al. (2003) used 27 heterotrophic strains of bacteria isolated from a wastewater treatment plant reactor and compared cell adhesion onto glass beads in a column experiment. Through measurement of protein and sugar content in the EPS matrices, it was found that EPS-rich strains had high levels of sugar, and sugar content correlated well with cell attachment trends. These results suggest that the "sticky" nature of sugar-rich EPS on the surface of some bacteria cells may play a role in increasing attachment to collector surfaces. Bacteria proliferating in wastewater treatment plants may preferentially produce "sticky" or thick biofilm and thrive in this nutrient-rich environment. Nevertheless, EPS can alter the physico-chemical characteristics of the surface of bacteria and influence steric and polymeric interactions and adhesion onto solid surfaces. The relative significance of these effects under a variety of natural physico-chemical conditions found in the subsurface and engineered filters has yet to be thoroughly elucidated.

Electrostatic and steric interactions. In low ionic strength environments, it is possible that steric interactions (i.e. stabilization) and/or polymer bridging drive attachment; while the abundance of counter-ions in high ionic strength conditions disguise the effects of EPS-interactions. It is hypothesized that EPS-proteins contribute to electrostatic interactions that can hinder attachment, particularly in EPS-poor strains (Walker et al. 2004). The transport of three mutants of *E. coli* K12 in a column packed with quartz sand was investigated, and cell attachment generally correlated well with ionic strength conditions (i.e. attachment was related to electrostatic double-layer interactions). However, one strain with a lengthy, uncharged O-antigen portion experienced higher attachment than the other strains, particularly at low

ionic strength conditions. This outcome may be the result of increased cell size caused by long EPS molecules.

Correlations between ionic strength, cell size and EPS molecules have been identified. Cell size is an important bacterial parameter that dictates colloid interaction mechanisms (Gannon et al. 1991), and can be affected by EPS production. EPS in low ionic strength conditions can be longer and extend further into the bulk fluid; while increased ionic strength can cause polymers to coil and remain close to the cell wall (Frank and Belfort, 2003). Relatively long EPS molecules have been identified using TEM images (ranging from 5 to 100 nm) and could cause an increase cell size by 20% (Rijnaarts et al. 1999; Walker et al. 2004). Camesano and Abu-Lai found that polymer chains remained extended in low ionic strength and DI water suspensions. Furthermore, Burks et al. (2003) investigated the transport of three *E. coli* strains with different LPS lengths in glass-bead filled columns and found that attachment increased with the length of LPS at low ionic strengths. Additionally, at low ionic strength conditions, zeta potential was not well correlated with cell attachment. These results suggest that steric repulsion and polymer bridging may be significant factors affecting bacterial attachment at low ionic strength conditions.

Polymer interactions. Attachment between the EPS on bacterial surfaces and quartz sand surfaces can be enhanced by polymeric interactions, which are not considered in classic DLVO theory. The abundance of polysaccharides and O-antigens on EPS-rich strains can both disguise protein charge and increase surface heterogeneity and roughness to enhance colloid interactions and contribute to attachment (Walker et al. 2004). Hydrophobic groups, such as non-polar groups on EPS-proteins, may allow closer contacts between cells and collectors, which can lead to conformational changes in surface polymers and encourage attractive polymeric interactions and bridging (Fletcher, 1996; Long et al. 2009).

Bacterial capsular material has also been shown to reduce attachment to solid surfaces. Rozatos et al (1998) investigated the differences in surface material composition on *E. coli* cells and found that the overproducer of capsular material repelled attachment, which is possibly due to the higher negative charge density of this capsular material. Prince and Dickinson (2003) studied capsulated and capsule-free strains of *Staphylococcus aureus* (that had similar surface charges), and found that encapsulated strains experienced steric repulsion when approaching glass slides, while attachment of capsule-free bacteria was two orders of magnitude higher. This metabolic response may help bacteria to resist attachment in unfavorable conditions and promote attachment in favorable environments (e.g. with higher nutrient availability).

Hydrophobicity. Many researchers investigating the affects of EPS on colloid attachment mechanisms have found that factors other than hydrophobicity drive colloid interactions (Gannon et al. 1991; Gross

and Logan, 1995; Tsuneda et al. 2003), although good methods for measuring the hydrophobicity of bacterial cell surfaces are lacking (Camesano et al. 2007). Chen and Walker (2007) found differences in surface hydrophobicity between groundwater bacteria and a marine strain that were caused by both the high polysaccharide content in the EPS of the groundwater strains and also by the suspension salt (cell were more hydrophobic when suspended in $CaCl_2$ versus KCl). These findings suggest that EPS makeup may affect bacterial attachment to collector surfaces due to changes in hydrophobicity.

Hydrophobic cells are generally expected to adhere to negatively charged media grains. Van Loosdrecht et al. (1987) demonstrated that hydrophobic cells experienced greater adhesion than hydrophilic cells to polystyrene coated disks. Rijnaarts et al (1999) investigated the attachment of eight bacterial strains onto polytetrafluoroethylene (PTFE; Teflon®) and glass and found the EPS of the various bacterial strains extended between 5 and 100 nm into the surrounding medium which caused the surfaces of the various bacteria to have different hydrophobicities. While bridging was observed between PTFE and hydrophobic bacteria, generally higher polar anionic polysaccharide coatings on cells surfaces impeded attachment to both PTFE and glass (Rijnaarts, et al. 1999). The hydrophobicity of a solid surface can have a substantial effect on bacterial attachment. Camesano et al. (2007) found that bacteria generally attached less to glass surfaces than to metal-oxide surfaces (coated with Fe). These findings suggest that the hydrophobic nature of the collector surface may be an important consideration when interpreting bacterial attachment data. Clear distinctions between studies that use glass and quartz sand, for example, are necessary.

2.5.6.3 Summary of Bacterial EPS and Attachment Mechanisms

The various mechanisms by which EPS may affect transport and attachment result in great uncertainty as to what extent EPS affects bacteria attachment to media grains (Jucker et al. 1997). The interaction between EPS on bacteria cell membranes and collector grains may be further complicated by chemical composition and cell shape (Xu and Logan, 2005). Due to the contradictions in attachment trends of bacteria with excess EPS in recent studies, it seems a unifying theory presently cannot be created to explain the affects of EPS on bacterial surfaces (Camesano et al. 2007). Further research investingating the transport behaviour of a variety of bacterial strains from different environments will help to elucidate the effect of EPS on bacterial attachment to porous media at a range of physico-chemical conditions.

Investigations into factors that affect bacterial attachment in saturated porous media should critically evaluate the balance between highly controlled experiments and field studies. Field investigations are complicated by site-specific characteristics, but these types of studies are necessary to provide evidence that laboratory outcomes are relevant for field applications. Characterization of bacterial EPS from cells

suspended in natural waters is just one example of a controlled study that can incorporate both laboratory control and environmentally relevant conditions.

2.6 Wall Effects in Saturated Porous Media Columns

Appropriate and practically relevant recommendations for D/d ratios have yet to be developed for (bio)colloid filtration studies conducted at various water quality and flow conditions. Several investigations using various types of columns have recommended D/d ratios of 50 or higher (e.g., Shankararaman, and Wiesner, 1993; Lang et al. 1983; AWWA, 1982); however, this value is not supported for all column experimentation purposes. An examination of the published literature investigating (bio)colloid removal by filtration with the use of laboratory column studies yields numerous instances that violate this recommendation, but also demonstrate acceptable reproducibility in (bio)colloid removal by filtration (Table 4). These findings suggesting that variable flow paths arising from the D/d may not be a significant factor with respect to (bio)colloid passage through granular media filtration processes. Accordingly, universal recommendations targeting D/d ratios of 50 are not appropriate for all experimental investigations; particularly those focused on quantifying (bio)colloid removal and filtration mechanisms, in which traditional operational data such as headloss and filter effluent turbidity evaluation are not required.

A review of the relevant historical literature investigating D/d ratios and wall effects is presented in Table 5. The applications are numerous and include heat transfer in chemical reactors, chromatography and solute transport, aerated filters, as well as head loss . Both laboratory and modeling approaches have been utilized to discern “wall effects” on a variety of column studies, however few have investigated these effects with respect to (bio)colloid transport in various saturated granular media environments, such as those used to study drinking water filters or the subsurface with different flow conditions and physic-chemical properties.

Rose (1950) was among the first to recommend D/d ratios of 50 or greater when using column studies to investigate headloss in filters. It was shown through experimentation that the Reynolds number was impacted by D/d ratios of 20 and lower and that head loss error from wall effects was >0.05 (results shown in Lang et al. 1993). However, D/d ratios between 20 and 50 were not included in these studies, and are commonly used in laboratory investigations (Table 5). Lang et al. (1993) has been highly referenced for supporting these recommendations in drinking water filtration pilot studies. It is important to note that the recommendation of 50 or larger was based on observations of impacts to head loss at loading rates of approximately 7 m/h. When modeling filtration to describe head loss, Tobiason and Viagneswaran (1994) found that the porosity of pilot filters operated at 5 m/h did not affect predicted

colloidal particle removal (0.27 – 10 μm). Lang et al. (1993) state that the mean rate of head loss build-up, effluent turbidity and particle counts for the filters studied were not significantly impacted by D/d from 26 to 6000. The design recommendation for D/d of 50 or larger was due to the variance in the estimate of head loss accumulation (which was higher in smaller diameter filters), but not based on observations of particle breakthrough or removal.

Economical experimental designs that do not cause "wall effects" or other biases that may compromise full-scale representation, can be highly useful many applications. Specifically, smaller column design for investigations into filtration performance for the removal of (bio)colloids to pilot engineered or natural filtration systems have numerous uses; including investigating the effects of various physic-chemical factors (e.g. media characteristics, NOM, IS) on the transport and removal of a suite of (bio)colloids. Furthermore, it is imperative that studies performed to elucidate factors such as wall effects are conducted in well controlled environments, and demonstrate that the outcomes are relatively reproducible (Cohen and Metzner, 1981).

It is generally agreed that, assuming a relatively uniform grain size distribution, smaller D/d ratios may result in greater porosity in regions within the vicinity of the column wall (Lang et al. 1993; Shankararaman and Wiesner, 1993; Cohen and Metzner, 1981). In a cylindrical packed column, media grains must conform to the shape of the column and the voids in one horizontal layer would be filled by grains in the following horizontal layer, which would reduce the occurrence of long preferential flow paths and relatively high-porosity regions (Figure 5). After analyzing various flow and packing configurations, Shankararaman and Wiesner (1993) found that larger porosity areas near column walls were limited to relatively small areas within the bed and suggest that they may only become significant for D/d lower than 12. To an extent, larger grains would not likely create greater voids that extend the length of the column, or create short-circuiting that could significantly alter colloid breakthrough.

Table 4: (Bio)Colloid Transport Investigations and the Respective D/d Designs

Source	D/d	Column Diameter (mm)	Media Size D_{50} (mm)	Granular Media	Media Depth (cm)	(Bio)Colloids
Shellenberger and Logan, 2002	11 - 16	8	0.50 - 0.75	glass beads	n/a	*E. coli* and *Declorosoma* spp. Bacteria, and latex microspheres (1 µm) [c]
Castro and Tufenkji, 2007	13	10	0.76	quartz sand	9 - 30	*E. coli* 0157:H7
Abudalo et al., 2010	15 - 18	15	0.84-1.00	quartz sand	10	*C. parvum* oocysts
Tufenkji et al., 2004	43	10	0.23	glass beads	7.1	*C. parvum* oocysts
Tufenkji et al., 2004	48	10	0.21	quartz sand	7.1	*C. parvum* oocysts
Redman et al. 2004; Walker et al., 2004	49	10	0.21	quartz sand	n/a	*E. coli* K12
Kim et al. 2008	63 - 76	38	0.5-0.6	glass spheres	10	silica powder particles (< 5 µm)
Li et al. 2004; Tong et al. 2008	64 - 91	38	0.42 - 0.60	glass beads	20	latex microspheres (several sizes) [c]
Tobiason, 1989	88	35	0.4	glass spheres	25	latex microspheres (4 µm) [c]
Li et al. 2005	91	38	0.42 - 0.60	glass beads	20	latex microspheres (1.0 and 1.1 µm) [c]
Bradford and Bettahar, 2005; Bradford et al. 2002	139 - 357	50	0.71 - 0.15	quartz sand, glass beads	10	latex microspheres (1.0 - 3.2 µm) [c]
Gupta et al., 2009	408	10	0.25 [a]	river sand	300	*C. parvum* oocysts, three strains of *E. coli* bacteria, MS2 and PRD1 bacteriophage

[a] D_{10}; [b] surfactant free polystyrene; [c] fluorescent carboxylated polystyrene

Table 5: Recommendations for D/d from Various Column Experiments and Models

Recommendation	D/d Investigated	Loading Rate	Metric	Source
≥ 50	26, 50, 66, 105, 189, 5938	5 m/h	headloss	Lang et al., 1983
>50	1, 2, 4, 10, 20, 50, 100	n/a	Renolds number;	Rose, 1950
>50	100, 50	0.06, 1.9 m/h	colloidal particles, microspheres	Grolimund et al., 1998
> 50	n/a	range	mathematical model	Shankararaman, and Wiesner, 1993
>50	7 - 91	n/a	mathematical model, particle counts	Mehta and Hawley, 1969
> 30	10-200	n/a	mathematical model for fluid flow	Cohen and Metzner, 1981
>7	7 - 37	5 m/h	phenol adsorption on GAC	Arbuckle and Ho, 1990
>8	6, 8	10-20 ml/min	acetone adsorption, chromatography	Knox and Parcher, 1969
>30	range	range	velocity profile for gases	Schwartz and Smith, 1953

Higher porosity regions created next to the column boundary may have a greater effect on (bio)colloid passage at relatively higher flow rates (e.g. > 5 m/h). The hypothesized increased flow rate at these higher porosity areas (next to the wall) may be slowed by the friction caused against the continuous barrier (Arbuckle and Ho, 1990). Therefore, flow rate becomes an important consideration when designing column experiments and providing recommendations for D/d ratios. As water passes through a filter column it travels between sand grains following tortuous and dynamic pathways. It has been suggested that flow paths in regions near column walls may be less tortuous (Cohen and Metzner, 1981) when lower D/d ratios are utilized. In contrast, the water and colloids that enter at the boundary of the column wall will likely eventually travel back through the center of the column (Lang et al. 1993). Here, collector contacts are unchanged, or as numerous as they would be in a larger D/d design. Therefore, it is possible that D/d conditions lower than 50 allow for no fewer contact opportunities between the colloids and the

collectors than conditions performed at higher D/d ratios. Provided that column length allows for these contact opportunities to occur, reasonably lower D/d conditions should not significantly impact colloid transport or removal. Accordingly, in addition to design recommendations for D/d, column length should also be considered.

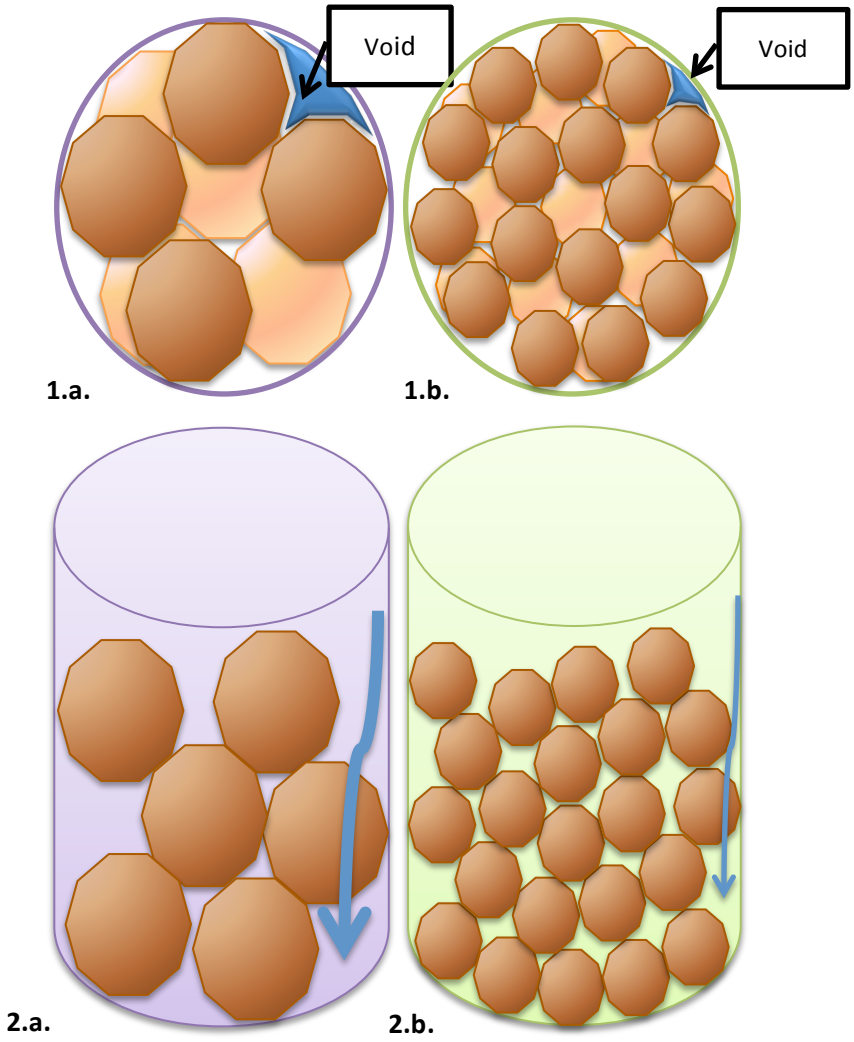


Figure 5: Conceptual diagram of different packing configurations as a result of different D/d conditions, showing the difference in void space at the column wall barrier. Visuals from the top of a column are shown in (1a and 2a) and from the side in (1b and 2b). Lower D/d conditions are shown in section (1, purple), while higher D/d conditions are shown in section (2, green). Note, the image is not to scale, but exaggerated to make a point.

Chapter 3 Materials and Methods

3.1 General Materials and Methods

In general, transport investigations were approached by suspending a suite of (bio)colloids in an AGW suspension with the appropriate physic-chemical conditions, representative of each experimental design, passing this solution through replicate columns packed with the appropriate granular media material, and calculating the log or percent removal of (bio)colloids achieved during passage.

3.1.1 Granular Media

Media source and characteristics. Four high purity silica sand blends (99.7% quartz; Best Sand Corporation, Chardon, Ohio), with median grain sizes (d_{50}) ranging from 0.2 to 1.2 mm (Figure 6) and uniformity coefficients ranging from 1.5 to 2.2, were sieved and mixed in different proportions to achieve five target sand blends with particular effective size (ES; d_{10}) and uniformity coefficients (Table 6). This sand is relatively irregularly shaped and is partially coated with iron oxyhydroxide as is noted by the slight orange tinge in Figure 6. This type of sand was selected because geochemically heterogeneous surfaces are important in representing and determining the transport of colloidal particles in heterogeneous aquatic environments, such as RBF (Johnson et al. 1996).

Table 6: Quartz Sand Media Blend Properties Used for Each Experiment

Property	Experiment 1		Experiment 2				Experiment 3
Code	Lg	+/-	+/-	+/+	-/-	-/+	+/+
Effective Size (D_{10} mm)	1.1	0.4	0.4	0.5	0.1	0.1	0.5
Grain Size (D_{50})	1.7	0.6	0.6	0.9	0.22	0.2	0.9
UC*	1.6	1.7	1.7	1.9	1.7	2.2	1.9
Porosity	0.40	0.40	0.40	0.35	0.38	0.38	0.35

*Uniformity Coefficient

Sieve Analysis. Media effective size (ES, D_{10}) and uniformity coefficient (D_{60}/D_{10}) are commonly used to characterize granular media used in filtration. These parameters are ascertained by sieve analysis (ASTM, 2001). A known mass of dried sand (100^{o}C for 24 h) was passed, using a mechanical agitator, through a nested column of sieves (i.e. sieve set) with gradually smaller mesh openings (from top to bottom). The

resulting mass of aggregate on each graduation was recorded and was divided by the total weight, to give a percentage retained on each sieve. The results can be shown graphically and can be used to determine effective size, median size, and uniformity coefficient (Appendix A). It should be noted that the media blend coded +/+ did not achieve the target UC value of 2.2, but was still elevated above that for media blends -/- and +/- (Table 6).

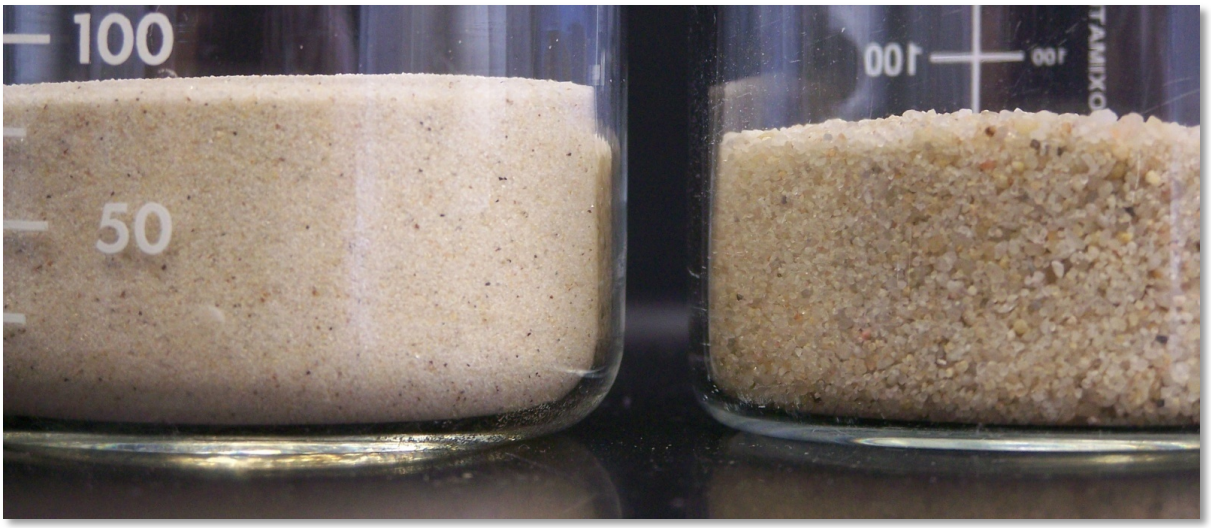


Figure 6: Small (-/-) (left) and large (+/-) (right) GS quartz media.

Media porosity determination. Sand porosity was determined using a 100 mL graduated cylinder. The cylinder was packed with dry and wet sand, and the following weights were recorded: i) mass and volume of the column, ii) mass of the column with water only, iii) mass of the column and saturated sand (achieved by dropping the media into water in a column and agitating any trapped bubbles free), iv) mass of column and dry sand. The porosity was calculated by determining the ratio of the volume of void space (the difference between wet-sand and dry-sand weight) and the total volume of material (the difference between the weight of the column when empty and when filled with water) (Equation 1). It was assumed that when water was present, all voids were filled with water and optimal packing conditions were mimicked. The resulting porosities of the four sands ranged from 35 to 40% as is shown in Table 7.

$$\boldsymbol{Porosity} = \frac{\left((\boldsymbol{Mass}_{column+sand+water}) - (\boldsymbol{Mass}_{column+dry\ sand})\right) \boldsymbol{X\ Density}_{water}}{\boldsymbol{Volume}_{total}} \quad \textbf{Equation 1.}$$

Media preparation. For Experiment 2, each sand mixture was washed at least 5 times in high-purity water (greater than 10 MΩ cm resistivity, Milli-Q™) to remove fines, and was autoclaved before use (not acid washed). For experiments conducted in the presence of NOM, sand was soaked in a 5 mg/L solution of NOM (fulvic acid; Suwannee River, Nordic Aquatic Inc.) for 24 h prior to use.

For experiments 2 and 3, each sand was acid-washed prior to use as described in Litton and Olson (1993) to provide uniform and consistent filtration media surfaces, and to avoid confounding transport due to interactions between metal deposits and (bio)colloids at the media grain surface (Abudalo et al. 2005; Metge et al. 2011). Briefly, batches of each sand type were soaked in 2M HCl for at least 24 hours, and then washed in Milli-Q™ water for up to a week to reach a pH greater than 5. The sands were then baked at 200°C for 20-25 minutes. Finally, the sands were saturated with Milli-Q™ water and sterilized by autoclaving, while effectively saturating the media and releasing air pockets.

Table 7: Media Porosity Determination

Calculation	*Units*	Media Code			
		-/-	**-/+**	**+/-**	**+/+**
$Mass_{column}$	(g)	115.1	108.9	108.9	108.9
$Mass_{column+dry\ sand}$	(g)	197.3	191.1	192.5	194.4
$Mass_{column+sand+water}$	(g)	216.1	209.9	212.6	212.0
$Mass_{dry\ sand}$	(g)	82.2	82.2	83.6	85.5
$Mass_{water}$	(g)	18.8	18.8	20.1	17.6
$Volume_{water}$	(mL)	18.8	18.8	20.1	17.6
$Volume_{total}$	(mL)	50.0	50.0	50.0	50.0
Porosity	(-)	**0.38**	**0.38**	**0.40**	**0.35**

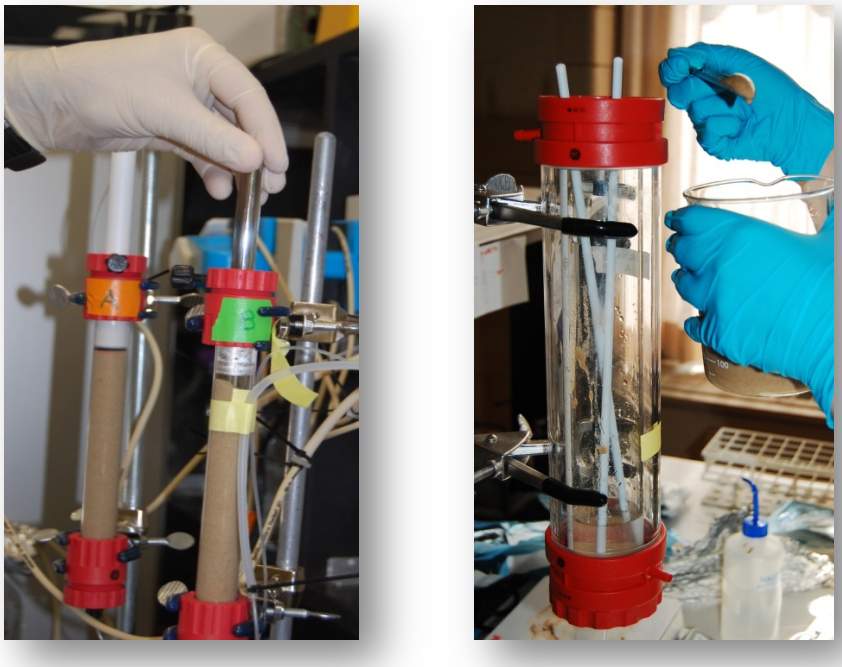
3.1.2 Glass Columns

Three glass filtration columns (GE Healthcare Bio-Sciences, Uppsala, Sweden) were used in this study. Two columns with an inner diameter (Ø) of 16 mm were used for replication in Experiments 2 (replicates denoted Column A and Column B) and 3 (replicates denoted 1 and 2). One of those columns (X) and a larger column with Ø 50 mm (Y) were used in Experiment 1 (Figure 7). Columns were fitted with steel

mesh at the effluent opening to prevent filter media exiting the bottom of the filter, and were sealed at the top with a plunger and rubber o-ring.

3.1.3 Column Packing

All materials were sterilized prior to use and a Bunsen burner was lit nearby to ensure that a sterile environment was maintained during column packing. Each column was packed with evenly mixed sand to a total depth of 15 cm. Saturated sand was added in a slurry in approximately 2 cm-deep increments. A minimum of 2 cm water height was maintained above the sand in the column at all times during the packing procedure. After each addition, layers were stirred together using a glass rod, and the column was agitated externally by tapping or using a handheld massager to compact the sand and release any entrapped air bubbles. Care was taken to avoid stratification within the media during agitation.



Scale: 1 cm –

Figure 7. Small (left; Ø 16 mm) and Larger (right; Ø 50 mm) Glass Columns.

3.1.4 Solute Tracer Test

A tracer test was performed with a bromide solution each time the columns were packed to ensure that reproducible packing was achieved. A sterile feed solution having a concentration of 100 - 200 mg/L Br^- was simultaneously introduced to each column, at the target loading rate, until breakthrough was sustained (~2 pore volumes [PV]). The tracer was then washed from the columns using sterile Milli-Q™ water. Effluent samples were collected in glass test tubes using a CF-1 Fraction Collector (Spectrum Laboratories Inc.; Houston, TX, USA). For Experiment 2, samples were analyzed for Br^- concentration using ion chromatography (ASTM D4327-11, 2003). For Experiments 1 and 3, bromide samples were analyzed using a conductivity meter using a HACH HQ40d meter with standard probe CDC40101 (APHA 2510-B, 1998). Bromide solute tracer test results were compared to theoretical PV calculations (Appendix A).

3.1.5 Pathogen and (Bio)colloid Selection, Propagation and Enumeration

Several (bio)colloids and bacterial pathogens were used in this study to represent the transport of pathogenic viruses, bacteria, and protozoa. For Experiments 1 and 2 these included two sizes of microspheres, PR772 bacteriophage, and two bacterial strains – a lab strain *Escherichia coli* RS2g (Experiment 1) and a pathogenic environmental strain of *Salmonella typhimurium* (Experiment 2). For Experiment 3, the same strain of *Salmonella typhimurium* was used, along with two additional environmentally indigenous pathogenic strains of bacteria – *Pseudomonas aeruginosa* and a strain of *E. coli*.

3.1.5.1 Microspheres

Selection of Microspheres. Surfactant-free, carboxylated, fluorescent-dyed polystyrene microspheres (MS or spheres) were used as non-biological surrogates of bacterial and protozoan transport and attenuation by physico-chemical attachment. The bacterial-sized microspheres have an average diameter of 1.1 µm (FluoSpheres, Invitrogen Corp., Carlsbad, CA, USA) and contain the fluorescent dye Nile Red (NR) that has a broad range of excitation and emission bandwidths. The protozoan-sized microspheres had an average diameter of 4.358 µm (herein referred to as 4.5) with coefficient of variation of 7% (FluoresBrite, Polysciences Inc., Warrington, PA, USA) and contain a proprietary chemical (YG) that has a maximum excitation at 441 nm and maximum emission at 486 nm**.** Both types of microspheres used have a density of 1.05 g/mL. These typical sizes of MS are commonly used in (bio)colloid transport investigations as surrogates for pathogenic bacteria (~1 µm) and protozoa (~3-10 µm) (Passmore et al. 2010; Metge et al. 2007; Emelko and Huck, 2004).

Enumeration of Microspheres. MS were enumerated via microscopy using a direct-count method (Emelko et al. 2003). Samples containing microspheres were thoroughly vortexed and diluted in PBS. Aliquots between 0.5 and 10 mL were filtered through 0.4 µm nominal porosity polycarbonate, 25 mm diameter membranes (Whatman Inc., Florham Park, NJ, USA), supported by 8.0 µm nominal porosity nitrocellulose membranes (Millipore, Billerica, MA, USA)at a vacuum pressure of 123 mm Hg. Subsequently, the filters were mounted on glass slides using DABCO in glycerol as a mounting medium. Spheres were enumerated manually using an AXIOSKOP 2 Plus microscope (Zeiss, Oberkochen, Germany) by excitation from a FluorArc epifluorescent light source at a total magnification of 100X and 200X for the 4.5 µm and 1.1 µm spheres, respectively (Figure 8: 1.1 µm NR spheres (left) and 4.5 µm YG spheres (right) at 200X magnification.). An absolute counting method was used (i.e. all microspheres on each slide were enumerated by scanning each filter), and counts between 30 and 300 spheres/slide were targeted (Emelko et al. 2008).

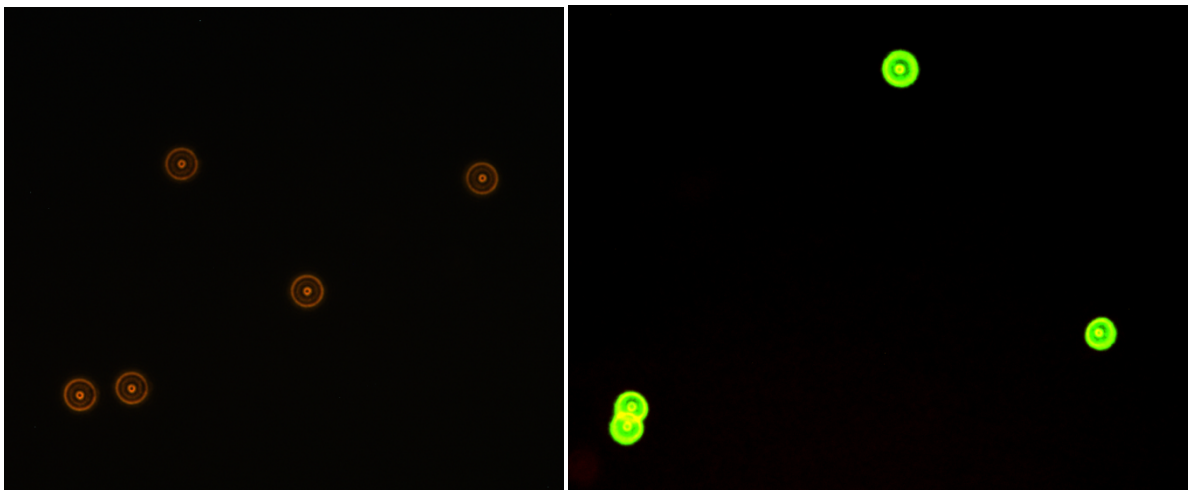


Figure 8: 1.1 µm NR spheres (left) and 4.5 µm YG spheres (right) at 200X magnification.

3.1.5.2 Bacteriophage

Phage selection. PR772 (ATCC BAA-769-B1) obtained from the American Type Culture Collection (Manassas, VA, USA) and the host bacterium *Escherichia coli* K-12 (ATCC BAA-769) were selected for use in this study. PR772 is an icosahedral DNA phage with an average physical diameter of 63 nm (Lute et al. 2004) and an isoelectric point of ~3.8-4.2 (Brorson et al. 2008). PR772 is closely related to PRD1, a commonly-used surrogate for subsurface viral fate and transport studies because of its stability in

aqueous and geologic media (Yahya et al. 1993a; Straub et al. 1992) and its structural and functional similarities to adenoviruses (Belnap and Steven, 2000). PR772 and PRD1 are both *Tectiviridae* viruses, which are 97.2% identical in nucleotide sequence (Lute et al. 2004), and have nearly identical isoelectric points, due to similar capsid structure (Brorson et al. 2008). In granular porous media, PR772 is transported similarly to pathogenic viruses due to its size, shape and surface characteristics and is considered a conservative surrogate (Schijven and Hassanizadeh, 2000). A transmission electron microscope (TEM) image of this phage is shown in Figure 9.

Propagation and Purification of PR772 phage. A high-titer PR772 stock suspension of ~10^{10} plaque forming units (PFU)/mL was prepared using the propagation and purification methodological sequence described by Mesquita et al (2010). Briefly, a double-layer agar plating method was used with large 100 mm x 15 mm Petri dishes, Trypticase Soy Agar (TSA) and Trypticase Soy Broth (TSB) media (BD; NJ, USA) with NaCl. For purification, the suspension was centrifuged using a Sorvall RG 5B plus and a SS34 rotor at 8000 *g* for 15 min. The supernatant was filtered through 1.2, 0.45, and 0.22 µm membrane filters (Whatman, London, UK) and then subjected to dual PEG treatment followed by Vertrel®(DuPont™ Canada, Mississauga, ON, Canada) lipid extraction. A purified suspension with a mean phage titer of 1 x 10^{11} PFU/mL was obtained and stored at 4°C until further use.

Enumeration of PR772 phage. PR772 were enumerated by the double layer agar plaque-forming method (Adams, 1959). The *E. coli* K12 host was grown in TSB at 37 °C for 12-18 hrs. Phage plaques were enumerated in 10 cm Petri dishes using 1.5% TSA with NaCl in the bottom layer and 0.7% TSA with NaCl in the top layer as described by Mesquita et al (2010). Negative controls (TSA and host) were included and the results expressed as PFU/ mL. Countable plaque numbers of 30-300 PFU/plate were targeted, although counts lower than 30 were also included in calculations (Emelko et al. 2008).

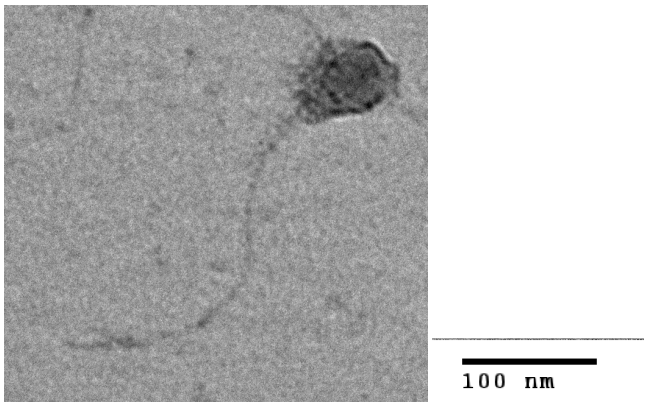


Figure 9: TEM image of PR772 phage. (Credit: A. Bhakta, McGill University)

3.1.5.3 *Escherichia coli* RS2g

Selection of *E.coli* RS2g. *E. coli* RS2g is a well characterized bacterial isolate frequently used in transport investigations as a surrogate for pathogenic bacteria (Passmore et al. 2010). This strain is resistant to the antibiotics kanamycin and rifampicin, and is modified to produce green fluorescent protein (Figure 10); a visually selective and differential identifier when grown on media containing these antibiotics and illuminated with UV light at 365 nm. *E. coli* RS2g is a rod shaped, Gram negative bacterium with dimensions of approximately 2.5 by 0.8 µm (Passmore et al. 2010).

Propagation and Enumeration of *E. coli* RS2g. Stock cultures of *E. coli* RS2g were maintained at -80 ^{o}C in Luria-Bertani broth supplemented with antibiotics (LB) and glycerol (80%). Propagation of stationary phase *E. coli* RS2g was achieved by inoculating 20 ml of LB broth with 0.1 ml of thawed freezer stock and incubated for 18-20 hours at 37 ^{o}C. The overnight culture was washed by repeat centrifugation at 5000 *g* and suspension in the target background ionic strength solution (0.1 or 10 mM KCl). Stock concentrations were estimated at 10^{8} colony forming units (CFU) / ml. Freshly propagated cells were used promptly for each experiment. Samples were enumerated via the spread pate technique (Clark, 1971) using 0.1 ml aliquots spread across LB agar plates and incubated at 37 ^{o}C for 24 h. Fluorescent isolated colonies were enumerated under UV light and recorded as CFU / ml after accounting for plating volume and dilutions.

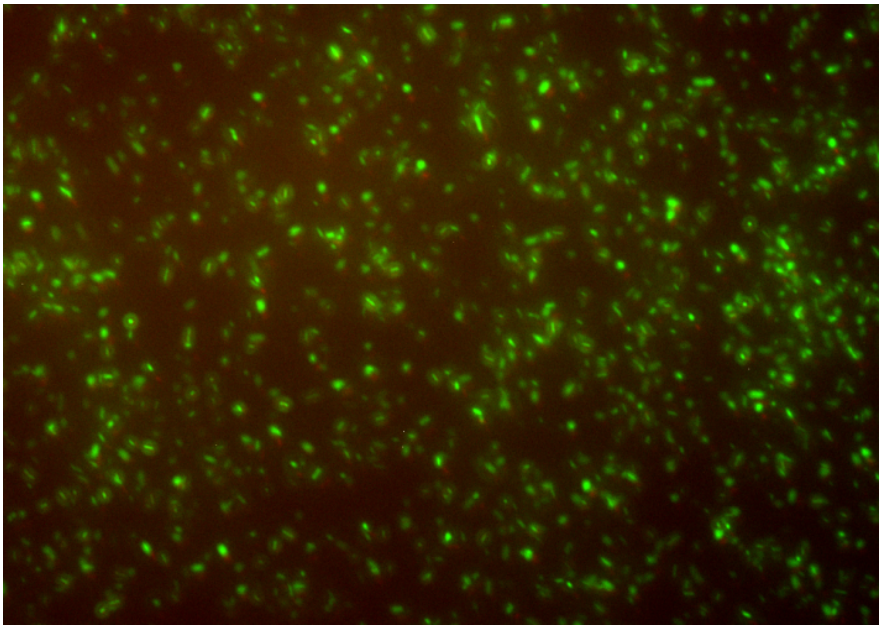


Figure 10. *E. coli* RS2g at 1000X magnification.

3.1.5.4 *Salmonella typhimurium* Bacteria

Selection of *S. typhimurium*. *Salmonella* is a facultative anaerobic, gram negative, rod shaped bacteria of approximately 1.5 µm by 0.5 µm (Yabuuchi et al. 1986; Figure 11). The genus belongs to the Enterobacteriaceae family (Madigan et al. 2003) and is a leading cause of human gastroenteritis worldwide (Pedley et al. 2006). The selected strain of *Salmonella* was chosen due to the direct link to human illness in the Grand River watershed (PHAC, 2007) and recognized persistence in surface water sources (Cho and Kim, 1999; Lemarchand and Lebaron, 2003). Untreated surface waters cannot be ignored as potential sources of salmonellosis outbreaks in humans (Lemarchand and Lebaron, 2003). An environmental isolate of *S. typhimurium* from Canagagigue Creek was used in this study (P.M. Huck, Civil and Environmentsl Engineering, University of Waterloo). Species identification was confirmed using multiplex PCR and PCR primers specific for *S. typhimurium*. Canagagigue Creek is a surface water source impacted by agricultural land use that is connected to the Grand River Watershed, Waterloo Region, Ontario. The species *Salmonella typhimurium* is motile with peritrichous flagella and is similar in shape and size to other, commonly studied pathogenic bacteria, such as *Salmonella typhi* and *Escherichia coli* (Pedley et al. 2006). A transmission electron microscope (TEM) image of this strain of *Salmonella typhimurium* is shown in Figure 11.

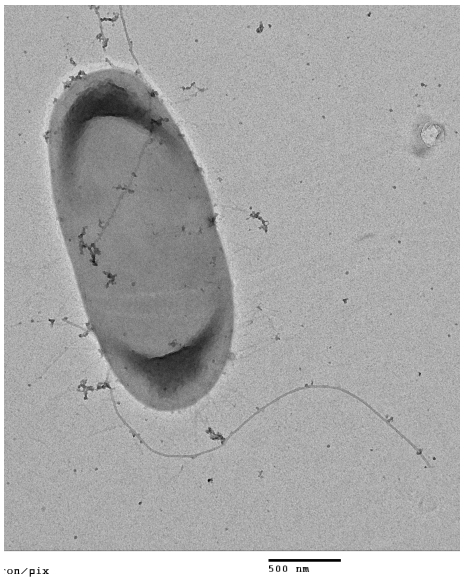


Figure 11: TEM image of *S. typhimurium*.

Propagation of *S. typhimurium*. *S. typhimurium* was propagated from a -80 °C freezer stock in nutrient broth and glycerol by inoculating 20 mL of nutrient broth (Difco Laboratories, subsidiary of Becton, Dickinson and Company, Sparks, MD 21152 USA) with 0.1 mL of thawed stock culture and incubating at 37 °C for 16-18 hrs. Cultured cells were pelleted by centrifuging for 10 min at 7500 *g* and then washed by decanting the supernatant, suspending the pellet in the desired ionic strength solution (0.01 or 10 mM KCl), and repeating the centrifuging/suspension step once.

Enumeration of *S. typhimurium*. Viable and culturable cells of *S. typhimurium* in the column effluent, expressed as colony forming units (CFU), were enumerated within 24 hours of collection using the spread plate technique (APHA, 1998; Clark, 1971). To this end, 0.100 mL aliquots of column effluent were spread on *Salmonella–Shigella* Agar (HiMedia Laboratories Pvt. Ltd., India) and incubated for 18 - 24 hrs at 37 °C. Negative controls were completed with each assay. Although countable colony numbers of 30 to 300 CFU/plate were targeted whenever possible, all counts below 30 were also included (Emelko et al. 2008).

3.1.6 Feed Suspensions

The solutions used to make the (bio)colloid seed suspensions were prepared using Milli-Q^{TM} water and the pH was adjusted to 7.0 +/- 0.2 using 1M NaOH. The desired ionic strength concentration was achieved using the addition of potassium chloride (KCl) at 0.01 or 10 mM KCl in Experiments 1 and 2; and at 1 mM KCl in Experiment 3. In Experiment 2, where NOM was present, NOM was added at a concentration of 5 mg/L fulvic acid. Feed suspensions were continuously stirred and maintained at room temperature for the duration of each experiment. Each feed suspension was sampled in triplicate at the beginning and termination of each experiment (i.e. 6 samples).

One feed suspension was prepared for each experiment and used to feed two columns simultaneously. For Experiments 1 and 2, all four (bio)colloids were suspended in the feed suspension together; while in Experiment 3, the three pathogenic bacterial strains were suspended and introduced individually to the filter columns. Each (bio)colloid stock was diluted to the target concentration in the feed suspension solution. The feed suspensions were maintained at room temperature (21 ± 2 ^{o}C) and continuously stirred using an immersed stir bar and a magnetic stir plate.

3.1.7 Column Experiment Operation

Columns were oriented vertically, operated in a down-flow mode and fed with a peristaltic Masterflex® L/S® Easy-Load® 3 pump head (Cole-Parmer Instruments Co., Montreal, QC, Canada) with L/S® 13 Chem-Durance® tubing (Figure 12). For Experiment 1, two pumps were used to accommodate the different flow rates required for each column size. Columns were flushed with at least 3 pore volumes (PV) of sterile Milli-Q™ water (pH .0 +/- 0.2) prior to colloid introduction. Suspensions were introduced at a rate of 1.2 mL/min (+/- 0.2 mL/min) (GW velocity of 22 m/d; loading rate of ~1 m/h) for all trials in Experiments 2 and 3. Experiment 1 included an additional trial operated at 6.5 mL/min (GW velocity of 119 m/d; loading rate of ~5 m/h). The respective GW velocities (m/d) and loading rates (m/h) for all experimental trials are detailed in Appendix A. Effluent samples from both columns were collected with a CF-1 fraction collector (Spectrum Chromatography, Houston, TX), at regular intervals. Feed suspensions were introduced to the columns until it was anticipated that pseudo-steady state breakthrough was achieved (~5 PV). At the termination of each experiment, columns were emptied, washed, sterilized with 70% acetone to remove any residual microspheres followed by several thorough washes with sterilized Milli-Q™ water.

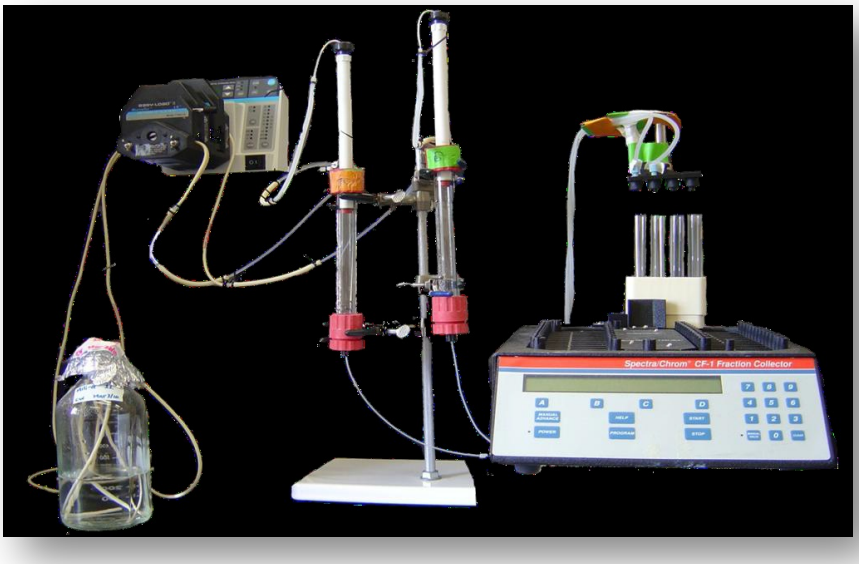


Figure 12: Column experimental apparatus; showing the peristaltic pump (left), two replicate glass columns (Ø 16 mm) (center), and the fraction collector (right).

3.1.8 Data Analysis

(Bio)colloid breakthrough curves and/or box-and-whisker plots were used to examine the experimental data. Breakthrough curves are commonly used to depict (bio)colloids removal by filtration. They were plotted as normalized concentration (log C/C_o, Equation 2) over time to show trends in transport as (bio)colloids were detected in the column effluents. Pseudo steady-state (bio)colloid removal values were calculated as either log removal (Equation 3) or percent removal (Equation 4), depending on whether the differences in removal were >99% or < 99% (i.e. 2 log), respectively. The removal of each colloid under each tested condition was calculated and these results were graphically presented as box-and-whisker plots, which help to illustrate the spread of the results and also visually compare means between colloids and test conditions. The "whiskers" of the plots show the adjusted minimum and maximum removal values, representing 1.5 times the interquartile range (difference between the 75^{th} and 25^{th} percentile). The bars of the box show (from bottom to top) the 25^{th}, 50^{th} (median), and 75^{th} percentile for each data set. Extreme values are shown with asterisk/star (*), representing values greater than 1.5 times the interquartile range.

A univariate statistical analysis of variance (ANOVA) using SPSS (IBM Corp., 2011) was performed to compare the means of the removal values for each (bio)colloid from each tested condition to see if there were any statistical differences among them (Tabachnick & Fidell, 2007). An ANOVA is a common statistical procedure that examines the relationship between the manipulation of independent variables and these effects on dependent variables. A univariate ANOVA allows one to identify causal relationships between variables, where the data set includes scenarios where one variable of interest (the independent variable) has been deliberately manipulated, while the others have been controlled or held constant (the dependent variables). Additionally, univariate ANOVA is used when the data only describe the variable by one dimension (Griffin, 1962); as only the removal of (bio)colloids was assessed herein. The ANOVA provides F-ratios (i.e. Fisher distribution), or variance ratios, and corresponding *p*-values (i.e. probability values) (Griffin, 1962). The F-ratio is the test statistic used for assessing the significance of each parameter on colloid removal using ANOVA, and is reported in each ANOVA table in the results section. The *p*-value, or statistical significance level, indicates the probability of obtaining a test statistic similar to the one that was observed. If the null hypothesis is true, the F-ratio would be ~1, as both mean squares would be similar between and within groups. If the F-ratio is much smaller than 1, the null hypothesis is false. The shape of the F distribution is affected by the degrees of freedom (df) within and between groups; where the lower the degrees of freedom, the larger the F valued required to indicate a significant difference between means (Griffin, 1962). *P*-values < 0.05 (5%) (Experiment 1) and <0.001 (0.1%)

(Experiments 2 and 3) were considered to indicate that a significant difference was detected between means tested.

$$\frac{C}{C_o} = \frac{\textit{Concentration of colloids in a sample collected from the filter effluent } (\frac{colloids}{mL})}{\textit{Concentration of colloids in the feed suspension } \left(\frac{colloids}{mL}\right)} \qquad \textbf{Equation 2}$$

$$\textit{Removal } (log) = -\left(Log_{10}\left(\frac{C}{C_o}\right)\right) \qquad \textbf{Equation 3}$$

$$\textit{Removal } (\%) = \frac{C_o - C}{C_o}\ x\ 100\ \% \qquad \textbf{Equation 4}$$

3.2 Experiment 1: Design

Three column experiments (denoted a, b, and c) were designed to investigate the effects of different D/d column designs on (bio)colloid transport in saturated porous media (Table 8). Different physical (GS, UC) and chemical (IS, NOM) factors known to impact colloid transport were investigated to identify conditions at which the D/d ratio may impact (bio)colloid transport in granular porous media. The range of values investigated included parameters selected to represent realistic conditions that may be encountered in both natural (e.g. RBF) and engineered filtration environments. Column experiments were conducted using two ionic strengths (0.01 and 10 mM KCl), two loading rates (approximately 1 and 5.5 m/h), and two granular quartz media with different grain sizes, but similar UC. The resulting D/d ratios that were investigated using the small (X) and larger (Y) columns are shown in Table 8.

Table 8: Experiment 1 - Design

Parameter		Units	Trial		
			a	b	c
Grain Size		(D_{10}, mm)	1.1	0.43	0.43
Uniformity Coefficient			1.6	1.6	1.6
Ionic Strength		(mm KCl)	0.1	0.01	10
Loading Rate		(m/h)	1	1	5
D/d	X		15	37	116
	Y		45	37	116

3.3 Experiment 2: Design

Sixteen experiments were carried out to complete a factorial designed experiment to determine the concurrent effects of four water quality or porous medium parameters (at two different values for each parameter) on the transport of four bio-colloids (Table 9). The dark shading in Table 9 indicates where a value was changed from "high" to "low". It can be noted that Trials #10 and #12 were performed using values for factors thought to increase transport to collector grains (and increase removal); where concurrently small media effective size, high ionic strength, and the absence of NOM were studied.

Table 9: Experiment 2 - Design

Trial #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ES[a]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
UC[b]	2.2	2.2	1.7	1.7	2.2	2.2	1.7	1.7	2.2	2.2	1.7	1.7	2.2	2.2	1.7	1.7
IS[c]	10	10	10	10	.01	.01	.01	.01	10	10	10	10	.01	.01	.01	.01
NOM[d]	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0

[a] Effective Size in mm; [b] Uniformity Coefficient; [c] Ionic Strength in mM of KCl; [d] NOM in mg/L of fulvic acid added.

3.4 Experiment 3: Design and Execution

Studies performed to investigate the effect of bacterial EPS on bacterial transport and removal in saturated porous media environments commonly have taken the approach of comparing the removal of bacteria with excessive EPS presence (i.e. mucoid) to the removal of bacteria with artificially removed or reduced EPS presence. In contrast, this study attempted to investigate various pathogenic bacterial strains after exposure to a range of environmental aquatic environments with the expectation that the physico-chemical nature of the different water matrices (e.g. nutrient availability) could cause natural changes to bacterial surface EPS that may result in differences in pathogen passage through porous media under otherwise controlled conditions. A schematic of the exposure of the three bacterial pathogens to the four water matrices is presented in Figure 13, while the subsequent procedures for the exposed bacterial strains are shown in Figure 14; transport through saturated porous media columns and EPS extraction and characterization. Additionally, characterization of extracts of free- and bound-EPS could provide an indication of the cause of differences observed in the exposed-bacterial strains (Figure 14).

3.4.1 Water Matrix Selection

Four natural source water matrices were used to expose the bacterial isolates prior to use in column experiments. Two matrices were collected from surface water sources (one from a river and one from a pond), one from a groundwater source, and another from a domestic wastewater treatment facility to represent a range of land-use impacted environments. The four matrices are depicted in Figure 15 and are denoted as groundwater (GW), agriculturally-impacted river water (AG), urban-impacted pond water (URB), and wastewater (WW). All water samples from the four matrices were collected in the spring months of April and May. The river water matrix samples were collected from the Nith River, where the banks are mostly formed of farm fields within the Regional Municipality of Waterloo in Southwestern, Ontario, Canada, located West of the city of Waterloo (Liss et al. 1996). This matrix was used to represent surface water impacted by agricultural activities (G). The urban-impacted water matrix sample was collected from Victoria Lake in Victoria Park, Kitchener, Ontario, prior to the structural improvements made in 2012 (URB). This urban surface water source has a large population of ducks and geese, and receives urban surface runoff from nearby roads and driveways. The groundwater matrix samples were collected from a municipal well within the Region of Waterloo (GW). Finally, the wastewater matrix samples were collected from a raw water holding reservoir at a waste water treatment facility in the city of Waterloo.

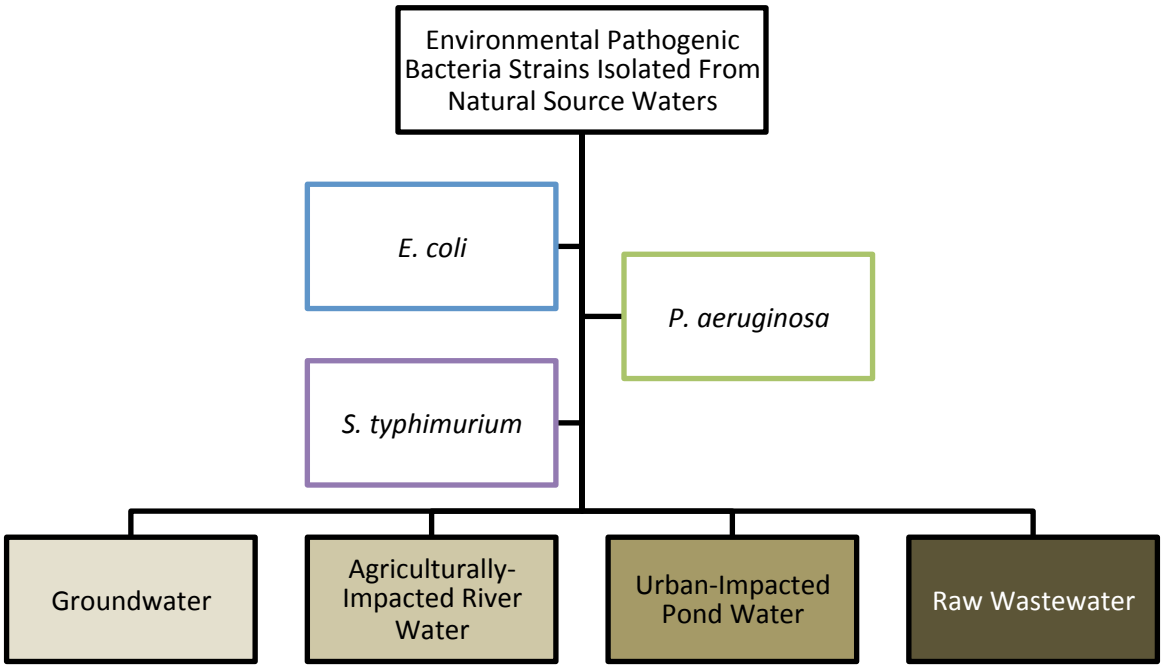


Figure 13: Experiment 3 - Environmental pathogenic bacterial pathogens and four water matrices.

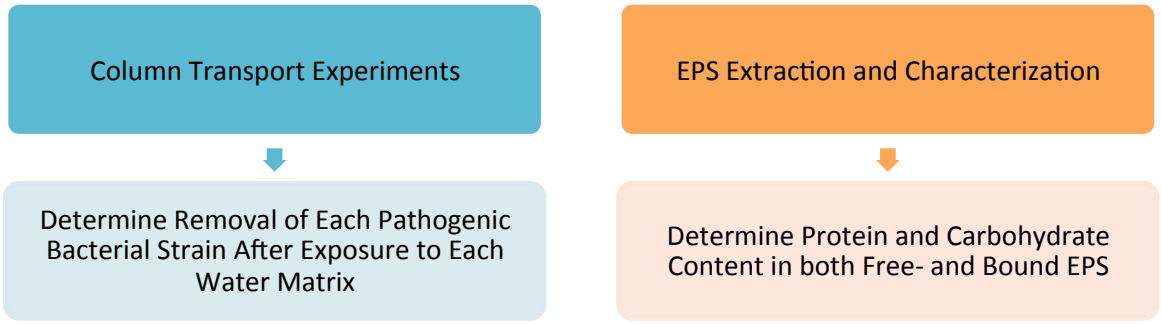


Figure 14: Experiment 3 - Column experimentation and EPS characterization tasks.

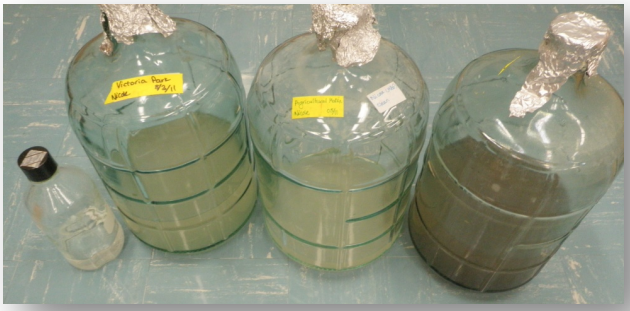


Figure 15. Four Water Matrices: GW, URB, AG, and WW (left to right).

3.4.2 Water Matrix Characterization

The resulting water matrix characteristics are reported in Table 10. All measurements were recorded in duplicate (TOC, pH, and nitrates) or triplicate (turbidity, TSS, conductivity, and salinity) and according to Standard Methods (APHA, 1998). Turbidity was measured using a HACH 2100N benchtop laboratory turbidity meter kit (Loveland, CO). Total suspended solids (TSS) were measured because this parameter can be used as a general indicator of the overall water quality, and were measured using Standard Methods 2540B (APHA, 1998). Briefly, glass-fiber filtration disks (Whatman AH-934) were dried thoroughly in a dessicator and placed in aluminum drying dishes. Well-mixed water samples of 100-500 mL were filtered using a vacuum manifold. Filters were dried in an oven at 100^{o}C for 24 h. The weight of the dried sample was recorded and TSS was calculated based on Equation 5. Conductivity and salinity

were measured with an Orion portable conductivity meter (Thermo Scientific, Waltham, MA). Samples for TOC analysis were diluted 10 X in Milli-Q™ water where necessary, and processed using a Schimadzu Analyser (Model TOC-5050A) and a Schimadzu high sensitivity catalyst (P/N 630-00996). Nitrates were measured because they can indicate where fertilizers or sewage discharge has contaminated a water source (Smith, 2008; Kasahara and Hill, 2006; REF). Nitrates were measured using NitraVer 5 Nitrate Reaagent AccuVac Ampules and measured using a DR/890 Portable HACH Colorimeter (Loveland, CO). Coliforms were enumerated by plating (membrane filtration) 1 – 100 mL of water sample onto MF-C agar and enumerating green-blue colonies. pH was measured using a Hanna Instruments 110 series pH meter (Laval, QC), after calibrating with standard buffers.

$$\boldsymbol{TSS} = [\boldsymbol{Weight\ of\ the\ filter + dish\ after\ drying}] - [\boldsymbol{weight\ of\ the\ filter + dish\ before\ filtration}] \quad \textbf{Equation 5}$$

Table 10: Water Quality Parameters of Four Selected Environmental Water Matrices

Parameter	Units	Water Matrix[b]			
		GW	AG	URB	WW
Turbidity	(NTU)	4.2	9.9	21	138
TSS	(mg/L)	0	212	247	464
Conductivity	(µS/m)	367	417	467	1897
Salinity	(%)	0.2	0.2	1.3	2.1
Nitrates	(mg/L)	0.27	3.9	0.6	8
TOC	(mg/L)	2	9.5	8.6	13.1
Coliforms	(CFU/mL)	0 (nd)[a]	1300	81	5000
pH		7.9	6.9	7.4	8.5

[a] none detected; [b] Groundwater (GW), Agriculturally-Impacted Water (AG), Urban-Impacted Water (URB), and Wastewater (WW) matrices

3.4.3 Bacterial Selection and Isolation

The same environmental isolate of *Salmonella typhimurium* as described above (Section 3.1.5.4) was used, while environmental strains of *Escherichia coli*, and *Pseudomonas aeruginosa* were isolated from natural source waters and used in the following experiments to realistically represent bacterial strains present in natural environments. The *E. coli* and *P. aeruginosa* strains were isolated from water collected

from Silver Lake in Waterloo Park, Ontario, Canada, which is occupied by a large duck and geese population. Once obtained and identified, all isolates were grown to log-growth stage and stored in 20% glycerol at -80^{o}C. These three pathogenic strains are all Gram negative, aerobic, rod-shaped bacteria that are relatively persistent in surface water sources and have well established isolation methods (Bolster et al. 2009; Gong et al. 2009; Liu and Li, 2008; Bradford and Bettahar, 2005; Zoblotowicz et al. 2001). Additionally, *Pseudomonas* spp. are known to develop profuse biofilms (Haznedaroglu et al. 2009; Bell et al. 2005), while *E. coli* is widely studied and commonly used to indicate the presence of fecal contamination (Edberg et al. 2000).

Isolation of an environmental strain of *E. coli*. The *E. coli* isolate was obtained using the fecal coliform membrane filtration procedure (APHA, 1998). A 100 mL sample of surface water was filtered through a sterile 0.45 µm pore, 45 mm diameter, gridded, filter membrane, rinsed with sterile DI water, and plated onto M-FC agar media plates with the use of sterile metal forceps, glass filter units, and a vacuum pump. Negative controls were used to help ensure that positive colonies were not the result of contamination. Plates were incubated for 24 h in a water bath held at 44.5 ± 0.5 ^{o}C. Colonies of fecal coliform bacteria on M-FC agar are various shades of blue. Five presumptive colonies were counted on the agar plate. Four were selected for confirmatory biochemical testing.

Confirmatory testing included the EC-MUG media test and Gram-staining; oxidase, and catalase (Madigan et al. 2003) tests were also performed. One of the five isolates selected for confirmatory testing appeared to test positive for an *E. coli* isolate (fluorescent blue and gas formation on EC-MUG, pink rods in the Gram-stain, negative for the oxidase test, and positive for the catalase test), and was shipped to Laboratory Services at the University of Guelph for microbial identification by 16S/18S rRNA procedures (Appendix E).

Isolation of environmental strain of *P. aeruginosa*. *P. aeruginosa* was initially propagated in a glass test tube with 50 mL selective Malchite green (MCG) broth base media (5 g/L peptone, 3 g/L beef extract, 0.37 g/L di-potassium hydrogen phosphate, with 0.3 mL filter sterilized 0.15 g of malachite green oxalate in 90 mL DI water addition following autoclaving) inoculated with 5 mL of surface water and incubated for 24 h at 42 ^{o}C to eliminate the possibility of *Klebsiella* growth. Aliquots of 0.1 mL media broth were spread plated (APHA, 1998) onto selective *Pseudomonas aeruginosa* cetrimide (PAC) agar and incubated for another 24 h at 42 ^{o}C along with negative controls of *Salmonella typhimurium* and *E. coli*. A total of eight fluorescent green colonies were selected for further confirmatory biochemical testing by Gram-staining and the catalase test (Zablotowicz et al. 2001). One positively identified colony (Gram-negative and positive for catalase testing) was confirmed by genetic microbial identification (Appendix E).

3.4.4 Bacterial Propagation

Bacteria propagation. All bacterial strains were handled separately, but as consistently as possible through the course of these experiments. The three bacterial strains were propagated; an aliquot of each freezer stock was streaked onto Tryptic Soy Broth (TSB) agar plates and incubated for 24 h at 37 ^{o}C. These plates were maintained at 4 ^{o}C. Isolate colonies were selected and used to inoculate 100 mL vials of TSB, which were incubated for 24 h at 37 ^{o}C. These stock cultures of each bacterium were used to inoculate 2 L containers of TSB, which were shaken at 200 rpm at 37 ^{o}C until early stationary growth phase (~10-16 h).

3.4.5 Exposure of Bacterial Strains to Water Matrices

Bacterial cultures were isolated from the growth media via repeat centrifugation at 8000*g*. Pellets were suspended in the selected water matrices and placed on a shaker for up to 7 days. The concentration of each batch ranged between 10^9 and 10^{10} CFU/mL. This resulted in 12 batches: each of three bacterial cultures individually exposed to four different water matrices (Figure 13). After ~120 h (5 d) samples were collected from each batch for 1) column experiments (i.e. un-treated), and 2) EPS characterization (i.e. treated). The exposure of bacteria to each water matrix was conducted at room temperature (~25^{o}C).

3.4.6 Bacterial Size Measurements

The size of the three bacterial pathogens studied in Experiment 3 was evaluated using dynamic light scattering (DLS) (Zetasizer Nano Series, Malvern Instruments Ltd., Worcestershire, United Kingdom). Triplicate measurements were obtained at 10 s intervals. Size measurements were included in this investigation as size has been indicated by others to be impacted by EPS production, and correlated with bacterial removal in saturated porous media environments (Bolster et al. 2009; Yao et al. 1971).

3.4.7 Bacterial Preparation for Column Experiments

After exposure to each water matrix, a 1 mL sample was collected from each batch and filtered through a 0.45 µm membrane. It was washed three times with 100 mL of sterile 5 mM KCl at pH 7 (the background matrix for the column experiments) to capture the suspended bacterial cells, but dilute the background matrix water. This membrane was then suspended in 10 mL of the 1 mM KCl and agitated by gentle vortexing to suspend the bacterial cells. An aliquot of 500 µL of this suspension was used to inoculate 300 mL of 1 mM KCl (for a target concentration of 10^4 CFU/mL), which would be used as the feed suspension for the column experiment. The method of membrane filtration for the separation of bacteria from the background water matrix was utilized instead of centrifugation. Membrane filtration with a

vacuum was thought to be less destructive to cell integrity and surfaces, and centrifugation has been implicated in altering or removing EPS materials on bacterial surfaces (Smets et al. 1999).

3.4.8 Bacterial Enumeration

All bacteria samples were enumerated in triplicate using the spread plate technique. Serial dilutions were performed where necessary using sterile 1X PBS with 1% Tween 80 to help prevent cell clumping, and 0.1-1.0 mL of sample volume was plated on selective agar. The plating agars used were as follows: m-FC ager for *E. coli*, PCA for *P. aeruginosa*, and *Salmonella* Selective Agar for *S. typhimurium*. Plates were incubated at 37^{o}C for at least 24 h. Selected plates for counting were limited to those with less than 300 colonies.

3.4.9 EPS Extraction and Characterization

EPS extraction was conducted with the use of centrifugation and a cation exchange resin (CER) that have been shown to maintain the integrity of bacterial cell walls (Azeredo, et al. 2003; Frølund et al. 1996). The CER used was DOWEX®, 50 x 8, 20-50 mesh, in the Na^{+} form (Aldrich-Fluka 44445), and extraction was carried out according to Azeredo et al.(2003) and Frølund et al. (1996). Each bacterial suspension (400 mL) was centrifuged at 5000*g* for 10 min, to remove the cells from the suspension matrix. For analysis of free-EPS (or EPS that had naturally sloughed off), 40 mL of supernatant was stored at 4^{o}C for subsequent EPS characterization. The pellet was suspended in 40 mL of DOWEX® extraction buffer (400 g/L DOWEX® in 2mM Na_3PO_4; 4mM NaH_2PO_4; 9 mM NaCl and 1 mM KCl, pH 7) and shaken at 600 rpm for 2 h at room temperature (~25^{o}C). DOWEX® was removed by high-speed centrifugation at 12,000 rpm for 20 min, and the resulting supernatant (containing the extracted EPS materials) was stored at 4^{o}C prior to EPS characterization.

3.4.10 EPS Characterization Methods

EPS extracts and free-EPS samples were characterized based on protein and carbohydrate content using colorimetric methods. Protein and carbohydrate measurements were performed in duplicate and results were expressed as mg/10^{10} bacterial cells. Acid-washed, sterile glass colorimetric tubes were used for all molecular analyses.

For protein quantification, five reagents were prepared according to the Lowry method (Lowry et al., 1951). Reagent 1 (R1) contained 100 mL water with 0.57 g NaOH and 2.24 g $NaCO_3$ (143 mM NaOH; 270 mM $NaCO_3$). Reagent 2 (R2) contained 10 mL water with 0.09 g $CuSO_4$ (57 mM $CuSO_4$). Reagent 3 (R3) contained 10 mL water with 0.24 g Na-tartrate (124 mM Na tartrate). All chemicals obtained were

reagent grade or higher. Reagent 4 (R4) was created by mixing 98 mL of R1, 1 mL of R2, and 1mL of R3 (100:1:1 ratio, respectively), immediately prior to use. R5 was created by mixing 10 mL Folin Reagent (source) with 12 mL water (5:6 ratio, respectively). For protein measurement, 1 mL of EPS extract or free-EPS was added to a spectrophotometer glass vial (~10 mL, Ø 12 mm). The sample was then vortexed with the addition of 1.4 mL of R4. Then 0.2 mL of R5 was added and the sample was vortexed. Samples were capped and allowed to sit at room temperature for 45 min. Standards were prepared from 2 g/L Bovine Serum Albumin Standard (Thermo Fisher Scientific Inc., Rockford, IL, USA) and diluted in sterile Milli-Q^{TM} water to concentrations of 0, 5, 10, 25, 50, 100, 150, and 200 mg/L to create a standard curve. Absorbance was measured with a spectrophotometer at 660 nm.

The procedure for carbohydrate quantification was carried out according to Dubois et al. (1956). This sulfuric-acid method was selected because it has been shown to have greater sensitivity and produce higher yields over the alternative anthrone method (Brown and Lester, 1980). Briefly, 0.5 mL of EPS extract or free-EPS was added to a glass vial. In a fume hood, 0.5 mL of 5% phenol solution (from loose crystals, Sigma-Aldrich) was added, immediately followed by the addition of 2.5 mL of sulfuric acid. The vials were vortexed and allowed to incubate at room temperature for 10 min. Next, samples were placed for 20 min in a shaking water bath held at 25^{o}C. A stock solution of sterile 200 mg/L glucose was used to prepare standards with concentrations of 0, 10, 25, 50, 75, and 100 mg/L glucose. Absorbance was measured at 490 nm.

Chapter 4 Results and Discussion: Experiment 1

The objectives of Experiment 1 were to assess if D/d ratios lower than 50 substantially affect (bio)colloid removal (percent) by granular media filtration at bench-scale. To this end, three paired column experiments were conducted (each using a small column [X] and a larger column [Y]) and investigating the differences between removal of a suite of (bio)colloids (representing viral, bacterial and protozoan pathogens) when two values representative of those that may be found in RBF systems for media effective size (0.43 and 1.1 mm) (Table 6), ionic strength (0.01 and 10 mM KCl), and loading rates (~1 m/h and ~5 m/h) were utilized. The resulting D/d ratios investigated were 37 and 116, as well as 15 and 45 (Table 8).

4.1. Solute Tracer Test

Bromide breakthrough curves are presented in Figure 16 from experiments operated at the two different loading rates. The bromide breakthrough curves were consistent between experiments using the small (X) and large (Y) columns, as part of Experiment 1. These results help to verify that columns were packed consistently between experiments, and indicate that the correct adjustments for pump flow rate were made for each column size. The passage of one pore volume (PV) of water through the column took ~2 and 15 min for loading rates of 5 m/h and 1 m/h, respectively. The experimental observations and the expected theoretical breakthrough time (2.0 and 11 min, respectively) were reasonably consistent (Appendix B).

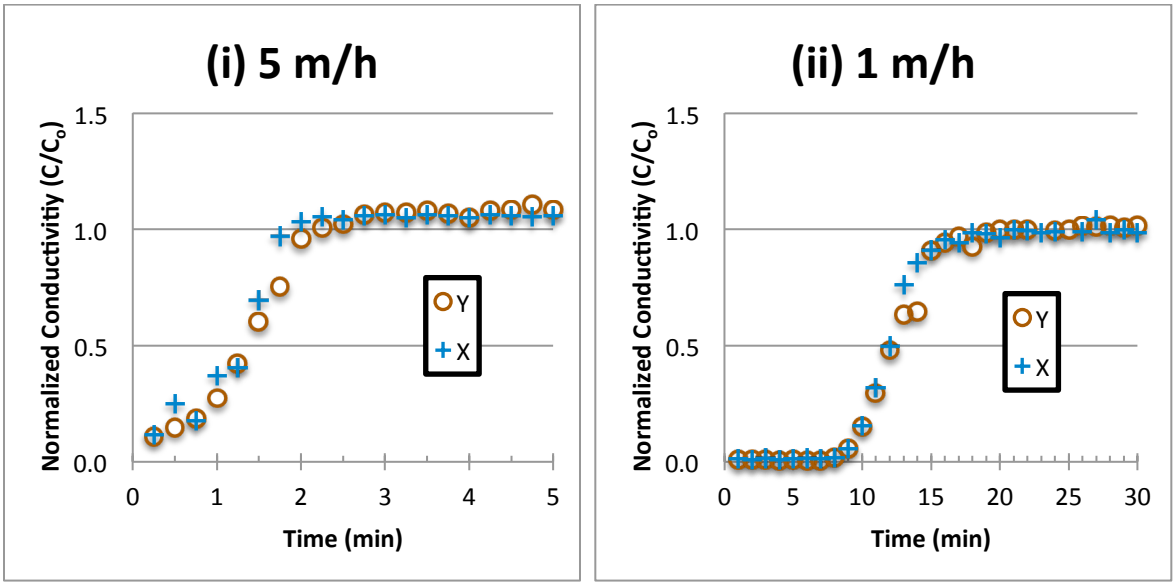


Figure 16: Bromide breakthrough curves from columns X (Ø 16 mm) and Y (Ø 50 mm)

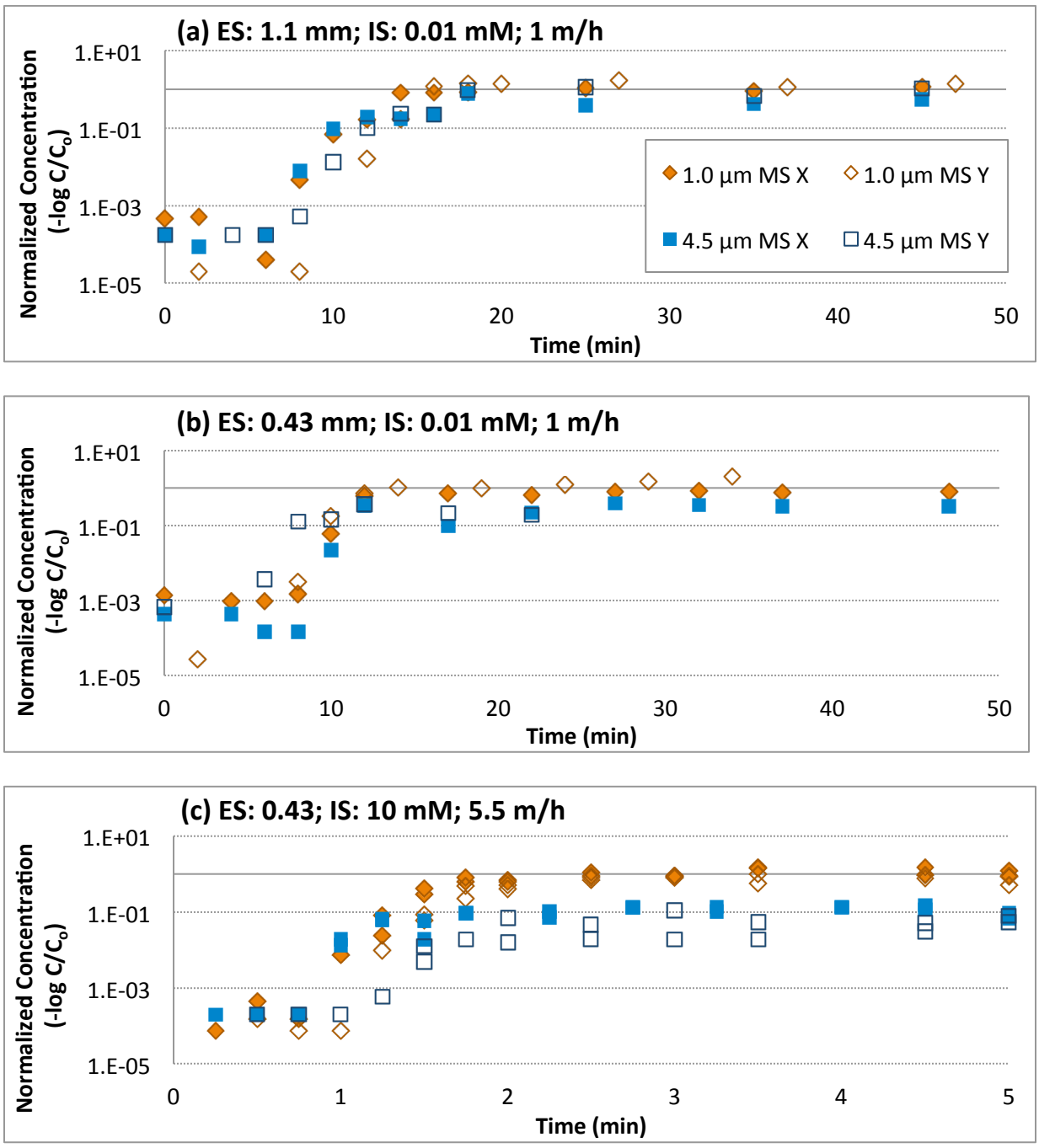


Figure 17: Microsphere breakthrough curves from column X (solid markers) and Y (open markers) from Experiment 1. Note the y-axis time label changes for trial (c).

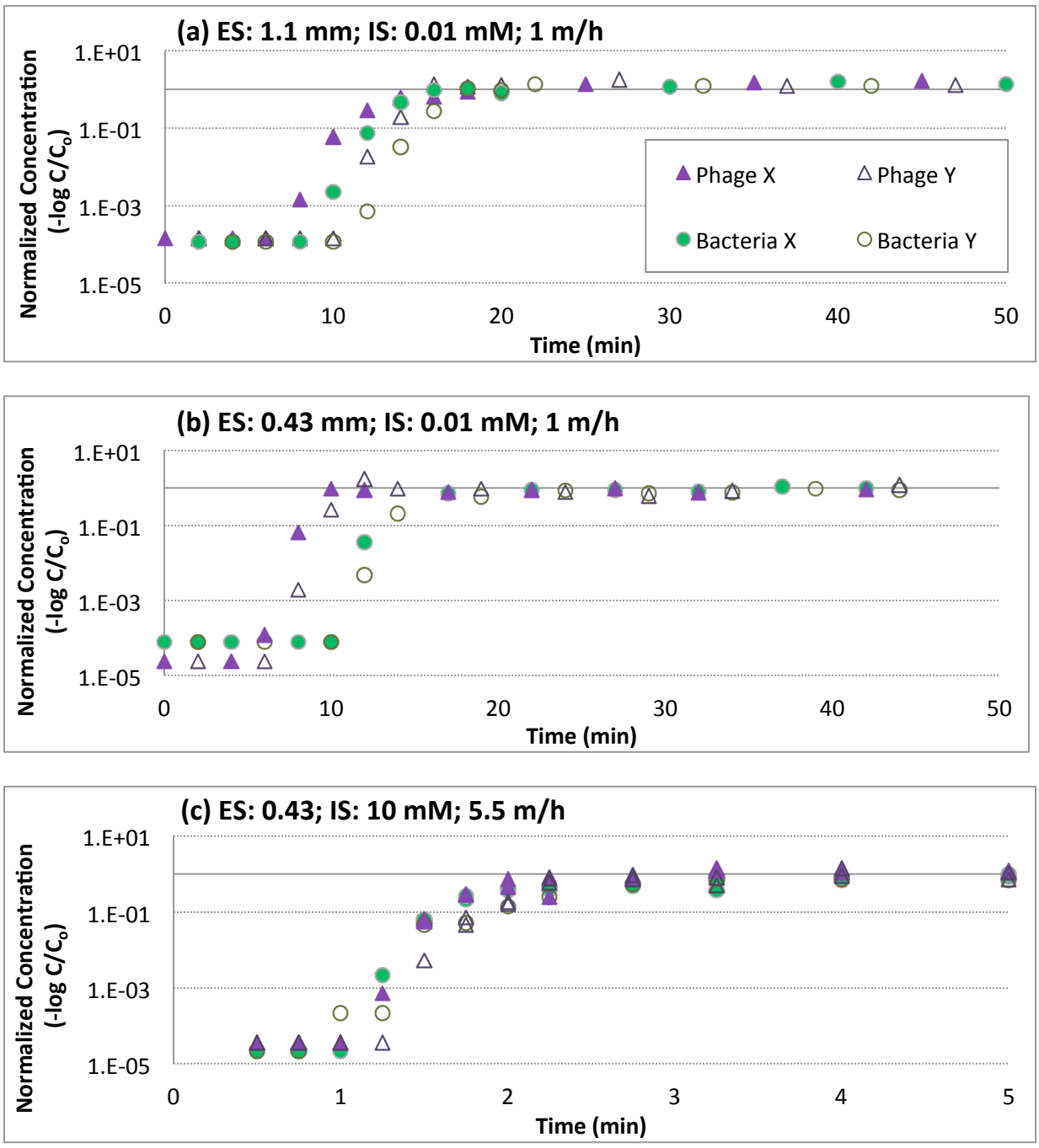


Figure 18: (Bio)colloid breakthrough curves from column X (solid markers) and Y (open markers) from Experiment 1. Note the y-axis time label changes for trial (c).

4.2 Breakthrough Curves

Breakthrough curves representing the various experimental conditions, (bio)colloids, and column diameters are presented in Figure 17 and 18. In general, at the physico-chemical conditions studied, the virus and bacterial pathogen surrogates showed <0.5 log removal, while the protozoa surrogate (4.5 µm spheres) showed greater removal, particularly in trial *c* where small grain size was paired with high ionic strength (Appendix B). Additionally, a difference between the removal of 4.5 µm spheres by columns X and Y is observed at the higher loading rate in trial *c*. Specifically, greater removal (~0.5 log) was observed for the larger column (Y).

Breakthrough of all (bio)colloids studied typically occurred at ~10 min and ~1 min for experiments conducted at 1 m/h and 5.5 m/h, respectively. When comparing the breakthrough curves of the tracer and the colloids, it can be seen that in some cases the colloidal particles traveled faster than the conservative tracer; this observation is not uncommon. Grolimund et al. (1998) reported that colloidal particles travelled much faster than nitrate through granular porous media in a column. This is because tracer ions can diffuse into the small pores within the aggregates, whereas mobile colloids are excluded from small crevices within the collectors, thereby possibly resulting in relatively reduced time to breakthrough in some cases. In general, solute tracers may experience more hydrodynamic dispersion than colloidal particles (Grolimund et al. 1998).

4.3 (Bio)colloid Removals

Box-and-whisker plots in Figure 19 show the respective removal (percent) for each colloid during experiments *a, b* and *c* from both columns X and Y. Removal of PR772 bacteriophage was consistently low in all experiments (~0 - 50%). Removal of *E. coli* RS2g bacteria and 1.1 µm spheres were fairly consistent when ionic strength was low (~0-30%), and increased when ionic strength was higher (~40 – 60 %). Removal of 4.5 µm spheres was higher than that of the other colloids (typically >50 %), and increased when experiments were conducted with smaller media grain size and higher ionic strength. Complete breakthrough of *E. coli* bacteria, PR772 and the 1.1 µm spheres was observed at almost all experimental conditions at which they were used (Table 8; *a*, *c*, and *d*).

The (bio)colloid removal results observed from these trials are consistent with colloid filtration theory. The bacterial pathogen surrogates, *E. coli* and 1.1 µm spheres were removed to the least extent by the granular porous media, as theory would suggest because these surrogates are in the size range that experiences the least transport to the surface of collectors. In contrast, higher removal of the virus surrogate PR772 bacteriophage was observed, which may be attributed to relatively increased transport to

collector surfaces via Brownian motion. The protozoan surrogate 4.5 µm spheres were removed by granular porous media to the greatest extent, which also may be explained by relatively increased transport to collector surface due to sedimentation and interception.

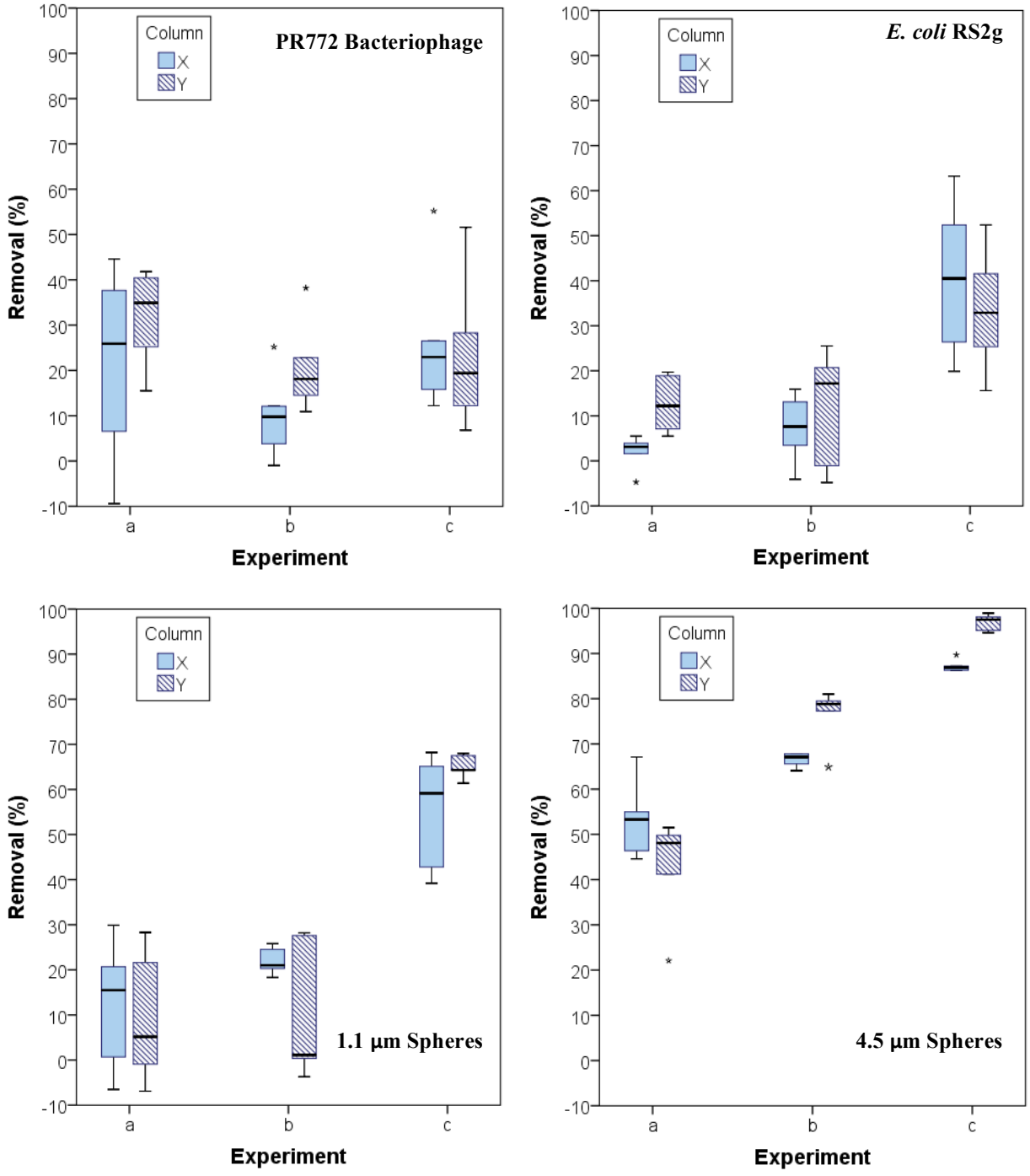


Figure 19: Box-and-whisker plots of (bio)colloid removal during trials a, b and c of Experiment 1 (n=5 for each box-and-whisker plot).

4.4 Comparing Results Between Different D/d Conditions

Substantive differences in (bio)colloid removal between columns with different D/d ratios were not observed, particularly at the low loading rate of 1 m/h (Figure 19). Differences in (bio)colloid removal between D/d conditions arose for 4.5 μm spheres at higher ionic strength conditions (experiment *b*) and more so when the columns were operated at a higher loading rate (experiment *c*). Overall, the differences in removal of 4.5 μm spheres from columns with different D/d designs are within an acceptable range (<15%).

The differences in percent removal of the various (bio)colloids at the investigated D/d ratios and loading rates were evaluating using an ANOVA ($p < 0.05$) and the results are presented in Table 11. In particular, the potential for differences in (bio)colloid removal due to wall effects was evaluated at D/d ratios of 37 and 116, as well as 15 and 45 (Table 8), which are outside of the commonly recommended ranges of D/d (AWWA, 1982), using media with different GS and operated with different IS. The different D/d column designs studied were found to have no significant effect on removal of the suite of (bio)colloids studied ($p >> 0.05$, Table 11). This analysis demonstrates that within the range of conditions investigated, filtration columns designed with D/d values between 15 and 116 should not show substantial differences in (bio)colloid removal by granular media filtration due to D/d design (or "wall effects"). As expected, and consistent with numerous studies (Haznedaroglu et al. 2009; Bolster et al. 2001; Yee et al, 1999; Jewett et al. 1994; and Mills et al. 1994), ionic strength was found to have a significant effect on the removal of *E. coli* RS2g, 1.1μm spheres and 4.5 μm spheres by granular media filtration ($p < 0.000$). Grain size was found to have a significant effect on PR772 removal ($p = 0.036$), however these results are suspect due to the overall low removal of PR772 found in all experiments and the low F-value results found for this interaction (F = 4.8). Additionally, ionic strength was not found to significantly affect the removal of PR772 in porous media filtration under the conditions investigate ($p = 0.130$). Furthermore, grain size was not found to have a significant effect on the removal of *E. coli* and 1.1 μm spheres in porous media filtration under the conditions investigated ($p = 0.648$ and $p = 0.246$, respectively).

Table 11: ANOVA Table for Effects of D/d on (Bio)Colloid Removal

(Bio)Colloid	Source	df	Mean Square	F_o	Significance	R Squared
PR772	D/d	1	289.295	1.457	.236	.233
	GS	1	954.028	4.804	.036[a]	
	IS	1	479.915	2.416	.130	
	Error	33	198.603			
***E. coli* RS2g**	D/d	1	40.716	0.344	.561	.658
	GS	1	25.125	0.212	.648	
	IS	1	5316.572	44.954	.000 [a]	
	Error	33	118.268			
1.1 µm spheres	D/d	1	0.290	0.002	.964	.796
	GS	1	190.460	1.390	.246	
	IS	1	10100.870	73.730	.000 [a]	
	Error	35	136.998			
4.5 µm spheres	D/d	1	18.088	0.473	.497	.919
	GS	1	2889.075	75.596	.000 [a]	
	IS	1	2577.868	67.453	.000 [a]	
	Error	29	38.217			

[a] 5% significance detected where $p < 0.05$ ($F_{obs} > F_{0.05}$)

Chapter 5 Results and Discussion: Experiment 2

The objectives of Experiment 2 were to evaluate the use of bench-scale column experiments for investigating the effects of chemical (IS, NOM) and physical (GS, UC) factors at environmentally relevant values for natural and engineered filters on the removal of a suite of (bio)colloids (i.e. pathogen surrogates). A factorial experiment design, consisting of 16 experiments conducted in duplicate, was utilized to determine the independent and concurrent effects of the four selected factors. The results include replicate tracer breakthrough curves, (bio)colloid breakthrough curves, (bio)colloid removal (log) shown as box-and-whisker plots, and an ANOVA, followed by a discussion of the independent and concurrent effects observed.

5.1 Tracer Test

Bromide breakthrough curves are shown in Figure 20 for trials 1 through 4 of the factorial study. Here, a Br^- free wash was passed through the column for 30 min, following 30 min of Br^- injection. These tracer results show that it took ~ 12 min for one PV to pass through a column, which was consistent with the theoretical calculations that determined that 1 PV would take ~11 min to reach the center of the advective flux (Appendix A). Additionally, the breakthrough curves of both columns, and the different media types, show excellent reproducibility.

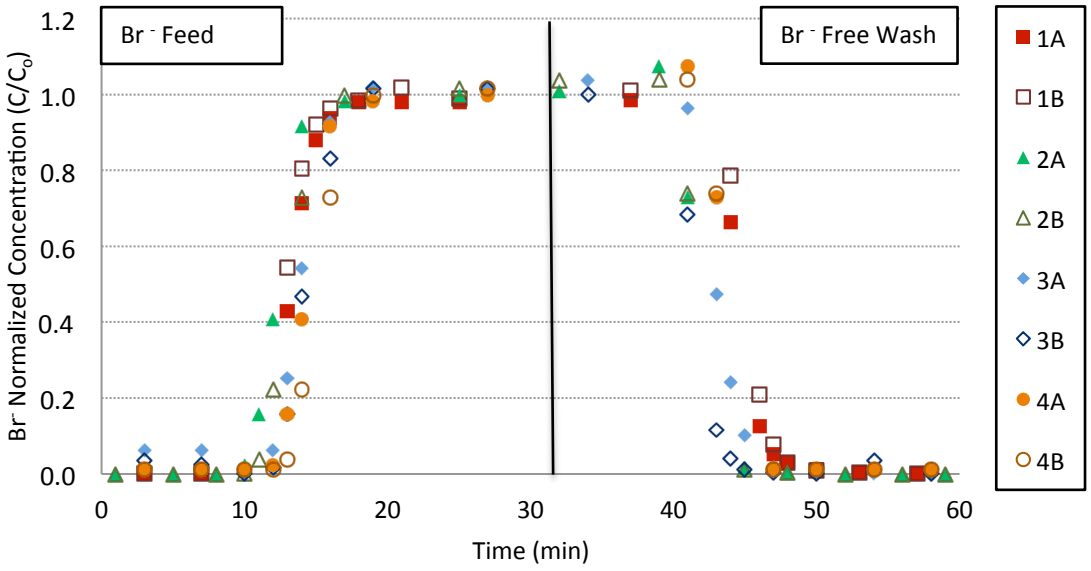


Figure 20: Bromide breakthrough curves for each media type used in the factorial trials of Experiment 2.

5.2 Feed Suspension Concentrations

A summary of the (bio)colloid concentrations in the feed suspensions measured in each of the 16 experiments is reported in Table 12 (Appendix C). One feed suspension was utilized to feed both replicate columns (a and b) for each of the 16 trials in Experiment 2, and each feed suspension was sampled 3 times (plated in duplicate) to determine the concentration of each (bio)colloid used. The target concentrations were well represented within the data, and also were not elevated above a concentration of 10^8 colloids/mL, which has been implicated to cause shielding within colloid transport investigations using granular porous media (Haznedaroglu et al. 2009; Bradford and Bettahar, 2006; Tufenkji et al. 2003). It can be seen that the standard deviation (SD) between the measured (bio)colloid concentrations from each experiment were the most variable for the *S. typhimurium* concentration results, while the results for the 4.5 µm spheres were the least variable (Table #). The variability between the *S. typhimurium* concentrations may be attributed to the lack of attention that was paid to *S. typhimurium* growth time (ranging between 18 to > 24 h) as a result of delays in experiment start up time. These delays ranged from air pockets in the filter columns, draining in the filter columns (resulting in incomplete saturation), necessary pH adjustments of the feed suspension and other common laboratory setbacks (e.g. lack of DI water supply, spills, material labeling, etc). Furthermore, variability *in S. typhimurium* concentrations could be attributed to non-conservative losses in the enumeration method during serial dilution preparation and spread plating (Emelko et al. 2008; LeChevallier et al. 1980) or due to possible viable but non-culturable (VBNC) cells (Caro et al. 1999). The less-variable measurements of the 4.5 µm sphere concentrations is likely due to the fact that sphere "viability" is not a concern as these particles are inert, and the same stock suspension was utilized for all experiments and the initial concentration was determined prior to all 16 experiments, by performing numerous replicate measurements. However, losses in serial dilutions and plating may still have occurred during these enumeration procedures for the 4.5 µm spheres.

Table 12. (Bio)Colloid Concentrations in Feed Suspensions used in Experiment 2

(Bio)Colloid	Units	Feed Suspension Concentration*				
		Target	Mean	SD	Max	Min
PR772	PFU/mL	10^7	6.0×10^6	2.7×10^6	9.98×10^6	1.2×10^6
S. typhimurium	CFU/mL	10^6	5.5×10^6	6.1×10^6	2.0×10^7	2.7×10^5
1.1 µm spheres	MS/mL	10^7	4.5×10^6	2.9×10^6	1.2×10^7	9.7×10^4
4.5 µm spheres	MS/mL	10^5	7.4×10^5	2.5×10^4	1.4×10^5	3.9×10^4

*n = 16

5.3 Breakthrough Curves

The breakthrough curves resulting from the 16 trials comprising Experiment 2 conducted to complete the factorial study are shown in Figure 21 for experiments conducted using media with a smaller grain size, and Figure 22 for experiments conducted using media with a larger grain size. Only breakthrough curves from column A are shown (experimental results from column A and B can be found in Appendix B). These figures demonstrate that, in general, the time to reach plateau (indicating a slowly-rising limb) was later in the even-numbered trials, possibly due to the concurrent effects of high IS conditions and the absence of NOM.

It should be noted that the ES of the granular media used in Experiment 2 was smaller than that of the media used in Experiment 1 (see section 3.1.1; Table 6). The overall removal of the bacterial sized surrogates, and also to some extent the virus surrogate, used in Experiment 2 were removed to a greater extent (typically >0.5 log, Figure 23) than what was observed in Experiment 1 (typically <0.5 log, Figure 19). These results further emphasize that grain size media can substantially affect the removal of pathogens and pathogen surrogates; where smaller grain size media provides more removal than larger grain size media.

It is important to note that the time-scale (x-axis) of each breakthrough curve is not identical; this is because the experiments conducted (i.e. columns were fed colloid-containing suspensions) until pseudo-steady state (bio)colloid breakthrough was achieved. The length of time required to reach pseudo-steady state breakthrough varied between experimental conditions (e.g. when IS was high, plateau was reached much later in the experiment run) (Appendix C).

The time-to-reach-plateau for each colloid is presented in Table 13. It can be seen that (bio)colloids broke through the columns (i.e. were detected in effluent samples) much later in trials 10 and 12, relative to other trials conducted. In trials 10 and 12 the experimental conditions consisted of smaller GS media, higher IS, and the absence of NOM. Similarly, the time to reach plateau (i.e. slowly-rising limb) was retarded in trials 10 and 12, as well as in trials 2 and 4 (in which GS was larger, but ionic strength was still high and NOM was absent) for all (bio)colloids investigated.

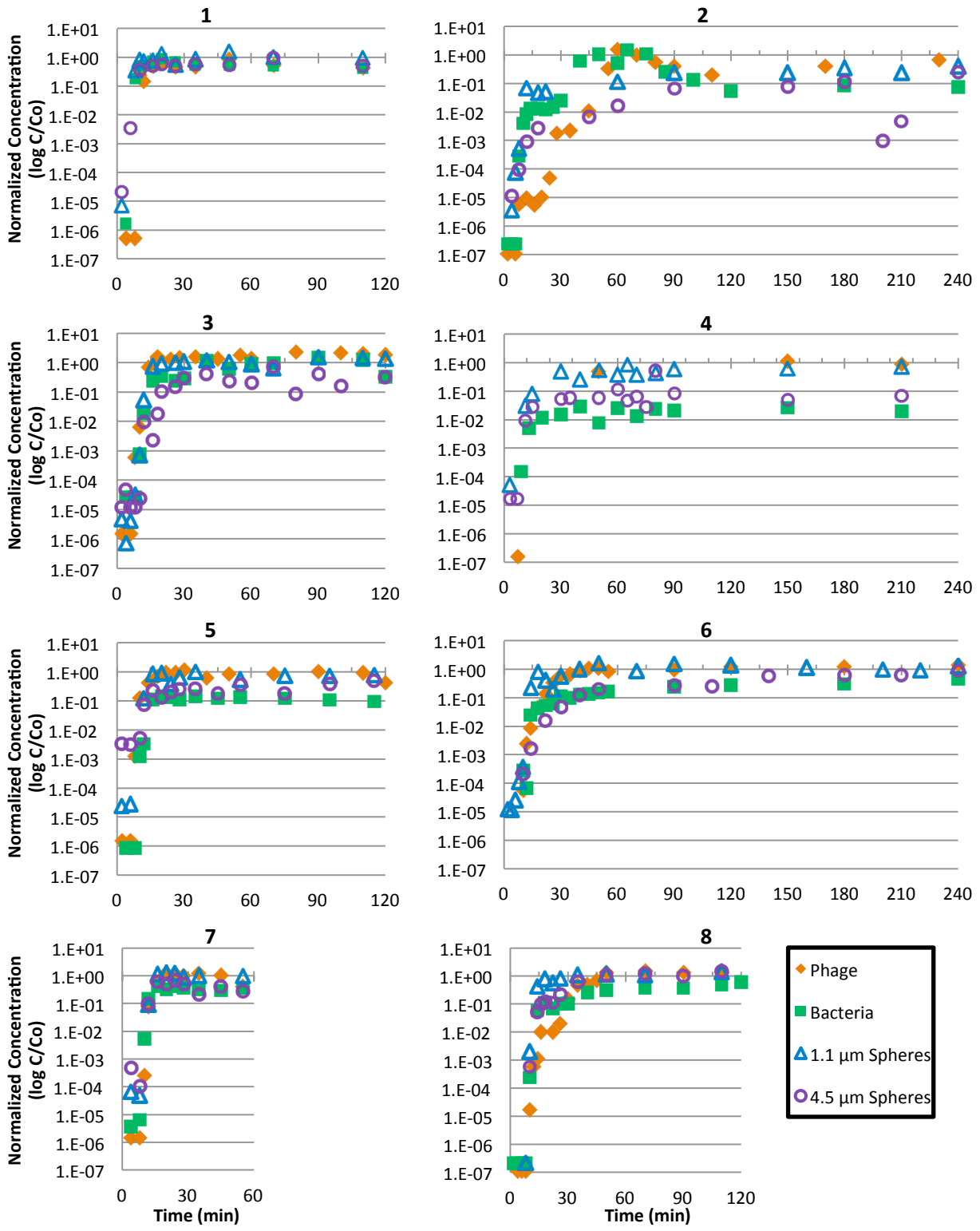


Figure 21. Breakthrough curves for all (bio)colloids from Experiment 2 conducted with larger media grain size (1-8).

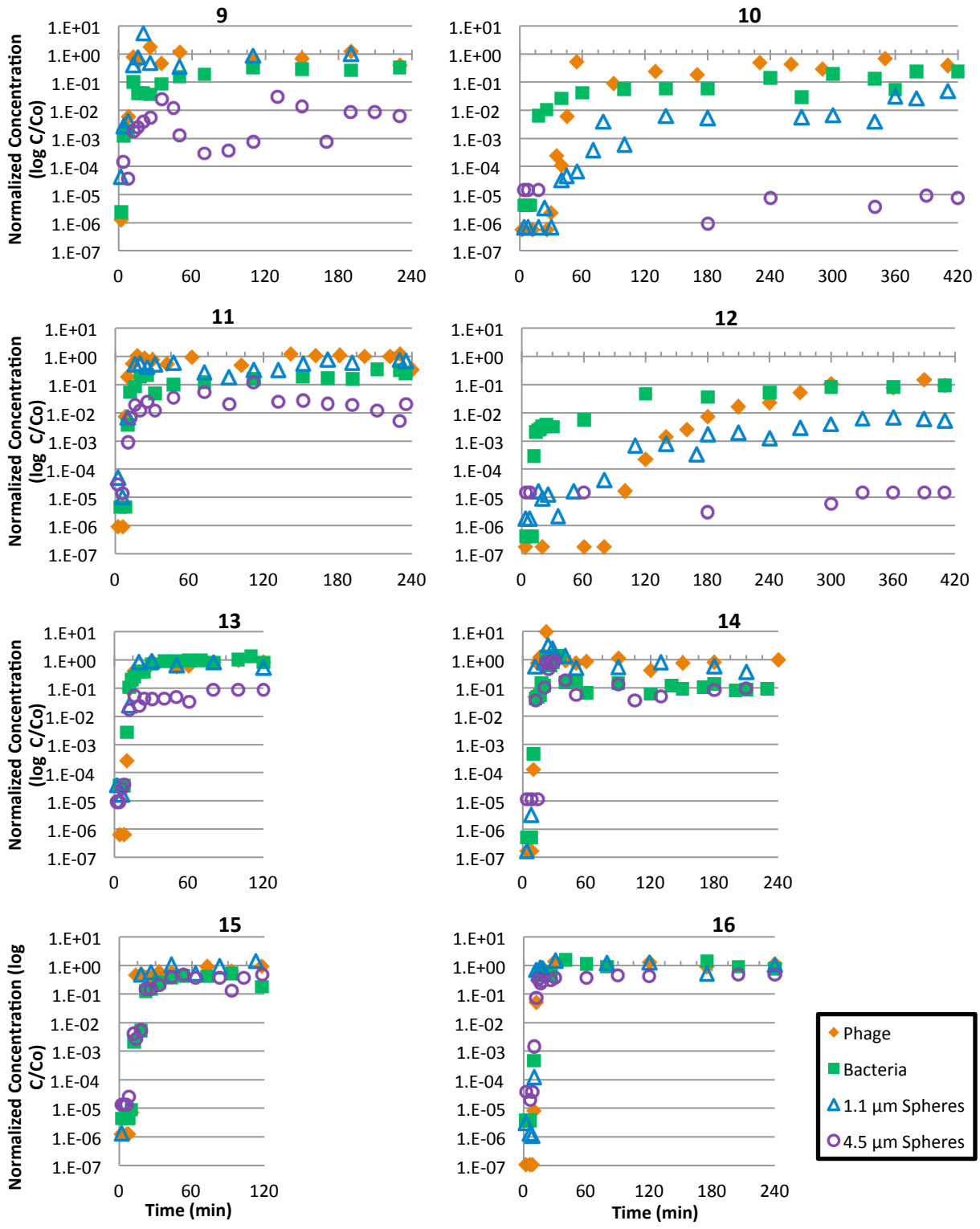


Figure 22: Breakthrough curves for all (bio)colloids from Experiemnt 2 conducted with smaller grain size (9-16).

Table 13: Time to Breakthrough and Time to Plateau for the 16 Trials from Experiment 2.

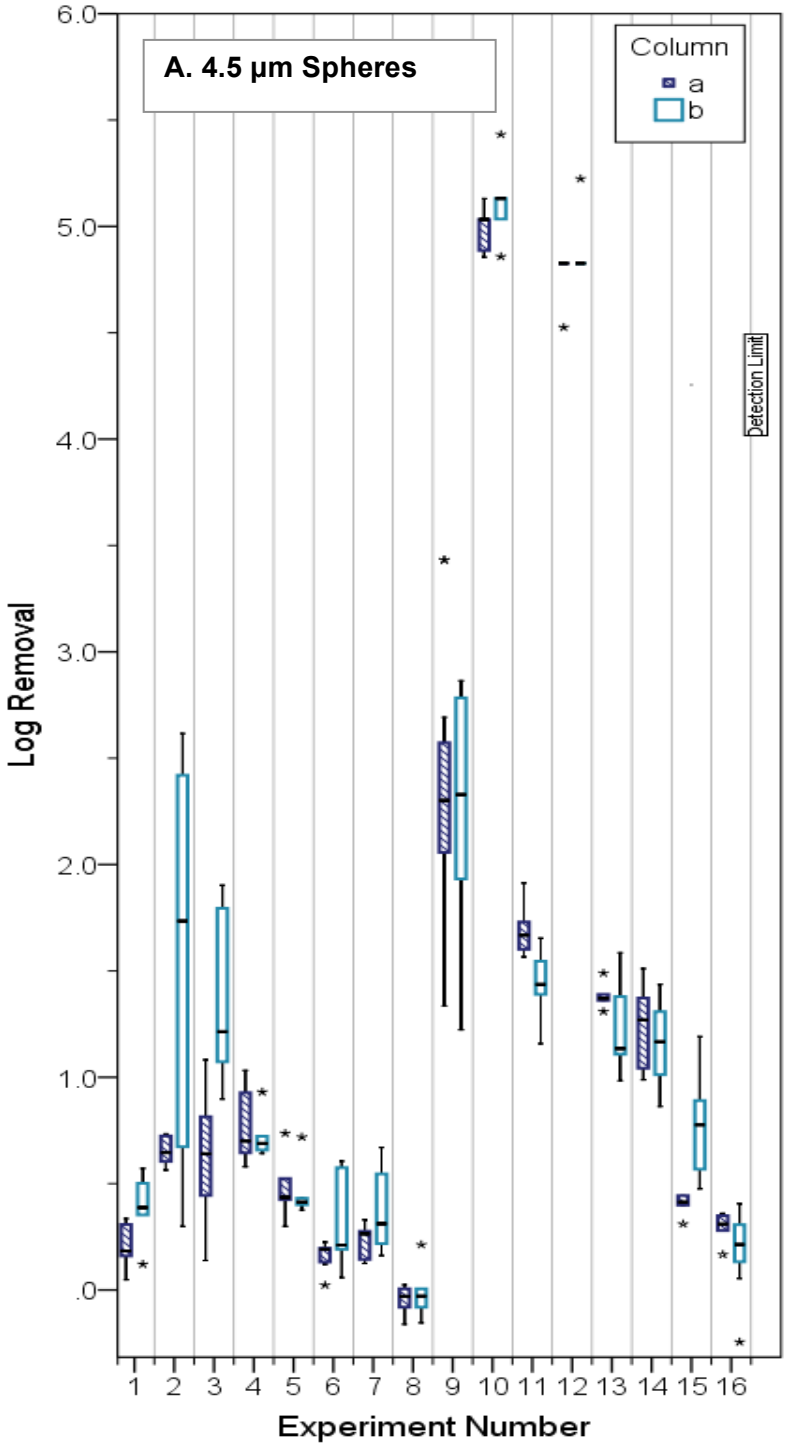
Trial #[a]	~Time to Breakthrough (min)				~Time to Plateau (min)			
	PR772	Bacteria	1.1 µm spheres	4.5 µm spheres	PR772	Bacteria	1.1 µm spheres	4.5 µm spheres
1	5	5	5	5	10	10	10	10
2	5	5	5	5	50	30	84	200
3	5	5	5	5	15	20	15	20
4	10	10	10	10	50	20	40	60
5	5	5	5	5	15	15	15	15
6	5	5	5	5	35	35	15	50
7	5	5	5	5	15	15	15	15
8	5	5	5	10	45	35	15	45
9	5	5	5	5	10	10	10	10
10	25	15	25	na	60	90	140	na
11	5	5	5	5	15	15	15	15
12	100	10	15	na	300	100	120	na
13	5	5	5	5	15	25	20	20
14	5	5	5	5	10	15	10	20
15	5	5	5	5	10	25	15	40
16	5	5	5	5	15	15	15	15

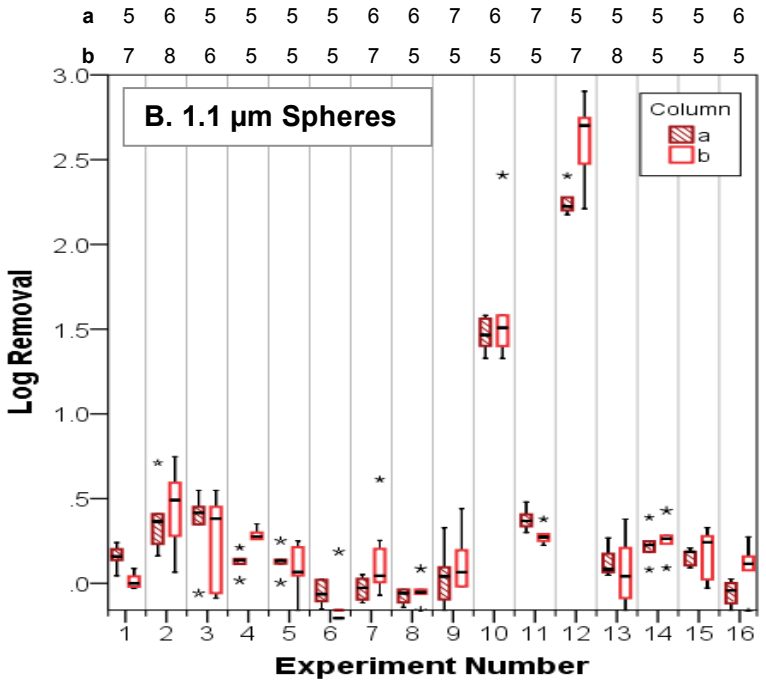
[a] see Table 9 for experimental details

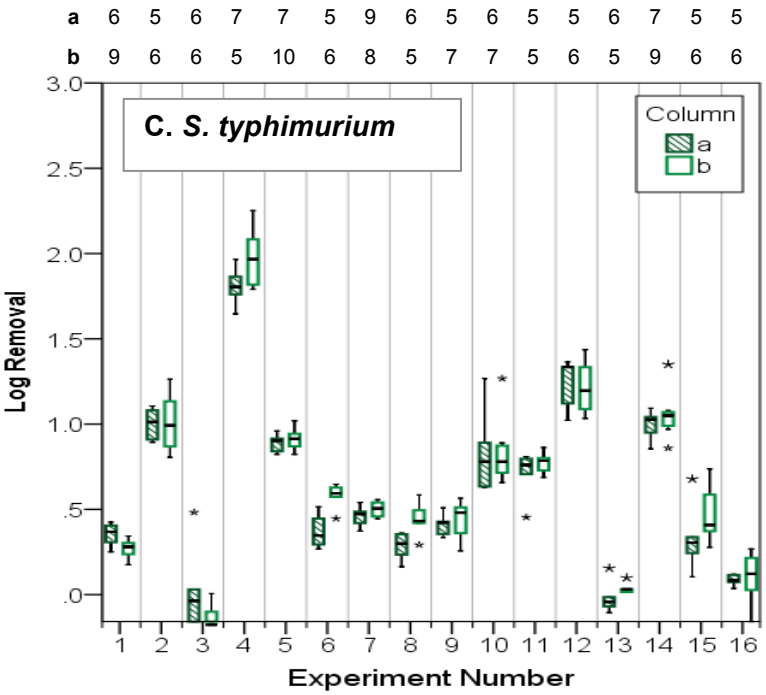
5.4 (Bio)Colloid Removals

Box-and-whisker plots (Figure 23) depict colloid removal (log) during each of the 16 factorial trials. Removal (log) results from both replicate columns, a and b, are presented, to allow for comparison between the reproducibility of the breakthrough results relative to the column apparatus and experimental methods used. The detection limit for each (bio)colloid was dependent on the concentration of colloids added (i.e. if 10^4 colloids was added, only 4-log removal could be detected). As a result, the substantial increase in removal of 4.5 µm spheres in trials 10 and 12 did not allow for accurate enumeration of this colloid from these trials; therefore, the removal is either as much as, or greater than, the concentration of spheres added to those experiments (Table 12).

a	5	5	11	5	5	7	5	5	11	5	5	5	5	7	5	6
b	5	9	5	5	6	6	9	5	6	5	9	5	5	6	5	7




a 5 6 5 5 5 5 6 6 7 6 7 5 5 5 5 6
b 7 8 6 5 5 5 7 5 5 5 5 7 8 5 5 5
B. 1.1 µm Spheres
Column
a
b
Log Removal
3.0
2.5
2.0
1.5
1.0
.5
.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Experiment Number


a 6 5 6 7 7 5 9 6 5 6 5 5 6 7 5 5
b 9 6 6 5 10 6 8 5 7 7 5 6 5 9 6 6
C. S. typhimurium
Column
a
b
Log Removal
3.0
2.5
2.0
1.5
1.0
.5
.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Experiment Number

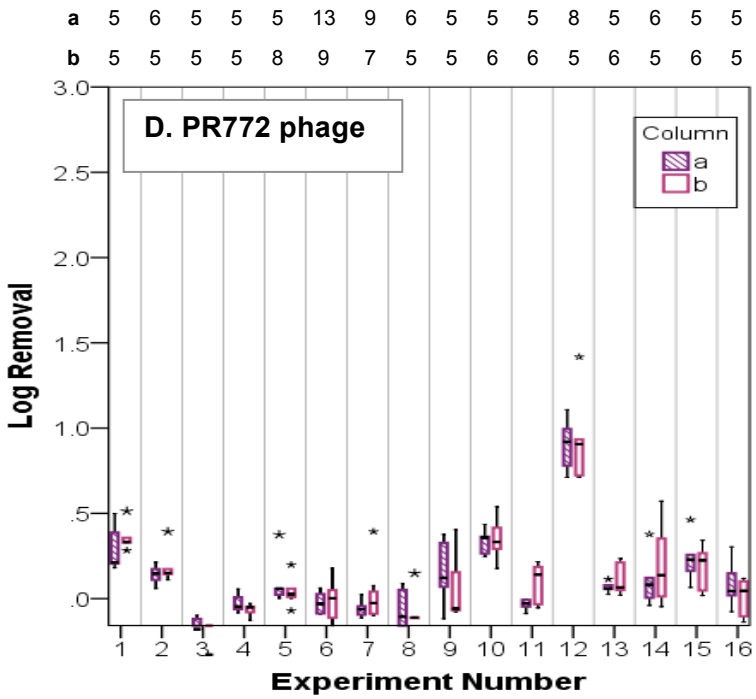


Figure 23: Box-and-whisker plots showing the log removal of each (bio)colloid from all 16 trials of Experiment 2. N-values are shown above each plot for each experiment.

An ANOVA was conducted on the (bio)colloid removal data (log) from the sixteen trials conducted to investigate the concurrent effects of physical (GS, UC) and chemical (IS, NOM) factors affecting pathogen and (bio)colloid removal by filtration (Table 14). Media grain size (GS), IS and NOM all had a significant effect on colloid transport at the conditions investigated ($p < 0.001$). The coefficients of determination (R^2 values) for all (bio)colloids were greater than 0.800 (i.e. 0.805 for PR772 phage, 0.934 for *S. typhimurium* bacteria, 0.994 for 1.1 µm spheres, and 0.935 for 4.5 µm spheres).

Table 14: ANOVA Table of Results for Experiment 2

(Bio)Colloid	Source	df	Mean Square	F_o	Significance[a]
PR772 Bacteriopahge	GS	1	1.266	101.478	.000 [a]
	UC	1	0.000	0.001	.974
	IS	1	1.146	91.918	.000 [a]
	NOM	1	0.432	34.637	.000 [a]
	GS * UC	1	0.788	63.175	.000 [a]
	IS * NOM	1	0.701	56.229	.000 [a]
	Error	168	0.012		
***Salmonella* Bacteria**	GS	1	0.332	21.507	.000 [a]
	UC	1	0.040	2.617	.107
	IS	1	5.404	350.315	.000 [a]
	NOM	1	9.861	639.300	.000 [a]
	GS * UC	1	0.006	0.379	.539
	IS * NOM	1	8.149	528.307	.000 [a]
	Error	184	0.015		
1.1 µm Spheres	GS	1	10.133	402.431	.000 [a]
	UC	1	0.667	26.483	.000 [a]
	IS	1	14.987	595.217	.000 [a]
	NOM	1	9.111	361.873	.000 [a]
	GS * UC	1	0.772	30.666	.000 [a]
	IS * NOM	1	10.810	429.348	.000 [a]
	Error	166	0.025		
4.5 µm Spheres	GS	1	121.483	917.984	.000 [a]
	UC	1	6.623	50.047	.000 [a]
	IS	1	116.258	878.508	.000 [a]
	NOM	1	25.899	195.707	.000 [a]
	GS * UC	1	4.025	30.415	.000 [a]
	IS * NOM	1	46.216	349.233	.000 [a]
	Error	179	0.132		

[a] 0.1% significance detected where $p < 0.001$ ($F_{obs} > F_{0.001}$)

5.5 Discussion

The conditions utilized in trials 10 and 12 (high IS, small GS and the absence of NOM addition) caused substantially more removal of both sizes of spheres; a similar trend was observed for PR772 during trial 12. Overall, removal of PR772 was lower than observed for any of the other (bio)colloids (< 0.5 log) during all of the experiments. The consistent low removal of PR772 indicates that the physical and chemical factors investigated did not have a substantial impact on removal of PR772 under the conditions selected for this study. The significance of the effect of each factor on the removal of PR772 bacteriophage was generally lower than that of other colloids studied, which may be an artifact of the overall low removals of PR772 in all experiments and complicates a comparison of means to identify causative factors. Trends in factors effecting the removal of *S. typhimurium* bacteria in the box-and-whisker plots (Figure 23, C) are difficult to discern visually; however, NOM addition occurred in odd-numbered trials, and it can be seen that greater removal of *S. typhimurium* was observed when NOM was absent.

5.5.1 NOM Effects on (Bio)Colloid Transport

The presence of NOM at 5 mg/L (as Suwanee fulvic acid) resulted in significantly less removal than experiments conducted at the same conditions without NOM addition ($p <= 0.001$), which is consistent with theoretical predictions. NOM was shown to have a significant effect on the removal of all colloids studied; in order from most to least significant: *Salmonella* >> 1.1 μm spheres >> 4.5 μm spheres > PR772 bacteriophage (Table 14). These observations are consistent with those of Metge et al. (2011) who injected (bio)colloids (microspheres and oocysts) and 2.2 mg/L DOC from Russian River into a RBF site in northern California, USA and reported that the presence of NOM showed a greater effect on the transport of 2-3 μm spheres, than on 5 μm spheres and *C. parvum* oocysts. They speculated that the presence of NOM decreased attachment opportunities (i.e. removal) of the colloids studied. The results from this study, and other investigations into the effects of NOM on (bio)colloid removal in saturated porous media environments, suggest that the effects of NOM on the transport of colloids in the size range near the minimum transport efficiency (0.5-3 μm) may be the most significant. Additionally, these results are consistent with what one might conclude regarding colloid transport through porous media when considering the implications of CFT. Accordingly, the transport of bacterial-sized colloids (0.5-3 μm) may be most significantly affected by the presence of NOM.

5.5.2 IS Effects on (Bio)Colloid Transport

Consistent with CFT, (bio)colloid removal was generally greater at high IS conditions investigated in this study (10 mM KCl compared to 0.01 mM KCl) (Figure 22). IS effects on colloid transport in the saturated porous media investigated were significant for all colloids investigated; in the order from most to least significant of: 4.5 μm spheres >> 1.1 μm spheres > *Salmonella* >> PR772 bacteriophage ($p < 0.001$, Table 14). Concepts that are often raised in the literature interpreting the effects of IS on colloid transport involve consideration for the zeta-potential of the surface of the colloids. The removal of microspheres appeared to be greatly affected by IS. This may be the result of relatively highly-negative surface charges of the spheres (having a thick electric double layer) being compressed by the high IS; thereby reducing the energy barrier between the spheres and the media grains to allow more contact opportunities. In contrast, the less-negative surface charges of bacteria (*Salmonella* in this case) and viruses (represented by PR772) were closer to neutral at the conditions investigated, and possibly presented a smaller energy barrier (i.e. thinner electric double layer) to overcome to allow contact opportunities with media grains.

5.5.3 Media GS Effects on (Bio)Colloid Transport

In all cases, experiments conducted with smaller grain size media showed more removal of all colloids investigated (Figure #), in the order of increasing significance of *Salmonella* << PR772 << 1.1 μm spheres < 4.5 μm spheres ($p < 0.001$). The significance of the effect of media grain size on colloid transport is lowest for *Salmonella* (F = 21, Table 14), which may be due to the minimum transport efficiency described by DLVO theory. These results are consistent with CFT, and follow the rational that smaller GS media provides more surface area for attachment of (bio)colloids (see section 2.2).

5.5.4 Media UC Effects on (Bio)Colloid Transport

A consistent trend in the effects of uniformity coefficient on colloid transport is not visible in the results presented (Figure 20 and 21); however, the ANOVA revealed that UC had a significant effect on the removal of microspheres. Uniformity coefficient was shown to significantly affect the transport of 1.1 μm and 4.5 μm spheres in the saturated porous media ($p < 0.001$), but not *Salmonella* ($p = 0.107$) or PR772 bacteriophage ($p = 0.974$) at the conditions investigated (Table 6, UC of 1.7 and 2.2). The fact that the media sieving failed to provide two distinct UC values for investigation may have negatively impacted the investigation of the significance of UC on (bio)colloid removal; as this parameter was not controlled as well as the others (Table #). The porosity data presented in Table 6 show that lower UC media had a slightly higher porosity (0.390) than higher UC media (0.365). This difference in porosity may explain the significant effect that UC had on microsphere removal; as less uniform media may create smaller

porosity regions within a saturated, packed column and allow for greater removal by wedging between grain-to-grain contacts (Li et al. 2006). However, the mechanisms of removal by wedging in low fluid drag regions for (bio)colloid removal has been disputed, as the effects of these mechanisms are not proportional to colloid size and colloid contact with collector grains is primarily influenced by fluid velocity and solution chemistry (Gupta et al. 2009; Johnson et al. 2007).

5.5.5 Interaction Effects Between Factors

Concurrent effects of chemical factors on colloid transport. The results presented in sections 5.3 and 5.4 suggest that IS and NOM concurrently affect colloid transport in saturated porous media environments. It is well understood that both IS and NOM affect the surface chemistry of colloids in aquatic environments, and therefore colloid-collector interactions (see sections 2.1, 2.3 and 2.4.3) and the results from this factorial study highlight that these two factors can interfere with the effects of the other. Accordingly, NOM and IS should be considered concurrently to prevent misinterpretation of results when conducting performance evaluations for (bio)colloid removal in saturated porous media filters.

Concurrent effects of physical factors on colloid transport. Although UC did not consistently show a significant effect on the removal of the suite of (bio)colloids used in this study, the ANOVA revealed that a concurrent effect was present between media grain size and UC. The results indicate that while media grain size shows a large ($F > 400$ for spheres), significant ($p < 0.001$) effect on colloid transport, this effect can be impacted by the media uniformity coefficient. Perhaps the range of uniformity coefficients investigated in this study were not broad enough to highlight this affect to a great extent. Greater differences in UC (while keeping other factors constant) could create greater differences in the resulting porosity of the media in the filter columns used and/or provide greater differences in the surface area of the media grains available for attachment of (bio)colloids; thereby UC could have a substantial effect on the removal of (bio)colloids than was observed in this study. Alternatively, it is possible that differences in UC may not have a substantial effect on (bio)colloid removal in saturated porous media filters, particularly when compared to other factors known to affect (bio)colloid removal in filtration. In a practical sense, UC of subsurface media in RBF cannot be altered effectively and it can be costly for a utility to specify a particular UC for media supplied to engineered filter design. Therefore, although UC should not be ignored as a media characteristic that can possibly effect (bio)colloid removal in filtration, it should be considered in combination with other media characteristics (e.g. GS and surface coatings) when evaluating filter performance for the removal of (bio)colloids.

Chapter 6 Results and Discussion: Experiment 3

The objectives of Experiment 3 were to identify the effects of bacterial exposure to a range of natural source water matrices (GW, AG, URB, WW) on bacterial size, surface characteristics (i.e. EPS characterization), and transport through saturated porous media using three environmentally derived pathogenic bacterial strains. To this end, each bacterial strain was exposed to four water matrices (GW, AG, URB, and WW) and passed through saturated porous media columns at controlled physico-chemical conditions, and the removal (percent) of each bacterial strain was determined. This resulted in 12 column experiments conducted in duplicate (three bacterial strains in four water matrices). Additionally, EPS extraction using DOWEX™ CER was performed on each isolate following exposure, and EPS extracts were characterized for protein and carbohydrate content, to investigate whether a linkage between measured EPS content and bacterial removal (in saturated porous media) could be made with existing methods for EPS analysis. The results from this experimental investigation were coupled with the findings from a critical review of the current state of knowledge on bacterial EPS production and effects on bacterial transport to develop a conceptual model. The conceptual model describes how bacterial metabolic production of EPS production response to environmental conditions may impact transport and removal in natural and engineered porous media filter environments.

6.1 Removal of Pathogens

Results from the investigation into bacterial pathogen removal in saturated granular media following exposure to four different environmental water matrices are presented as box-and-whisker plots in Figure 24. The percent removal of each bacterial pathogen from the replicate columns (designated as 1 and 2 in Experiment 3) showed a high level of reproducibility. The observed removal levels of bacteria were generally consistent with those observed during Experiment 2 (Figure 23) and by others (Tong et al. 2010; Kim et al. 2009; Liu et al. 2007) (Table 15). Bacterial pathogens generally were removed to a greater extent after exposure to higher nutrient/solids containing waters and to a lesser extent after exposure to the groundwater matrix (Appendix D).

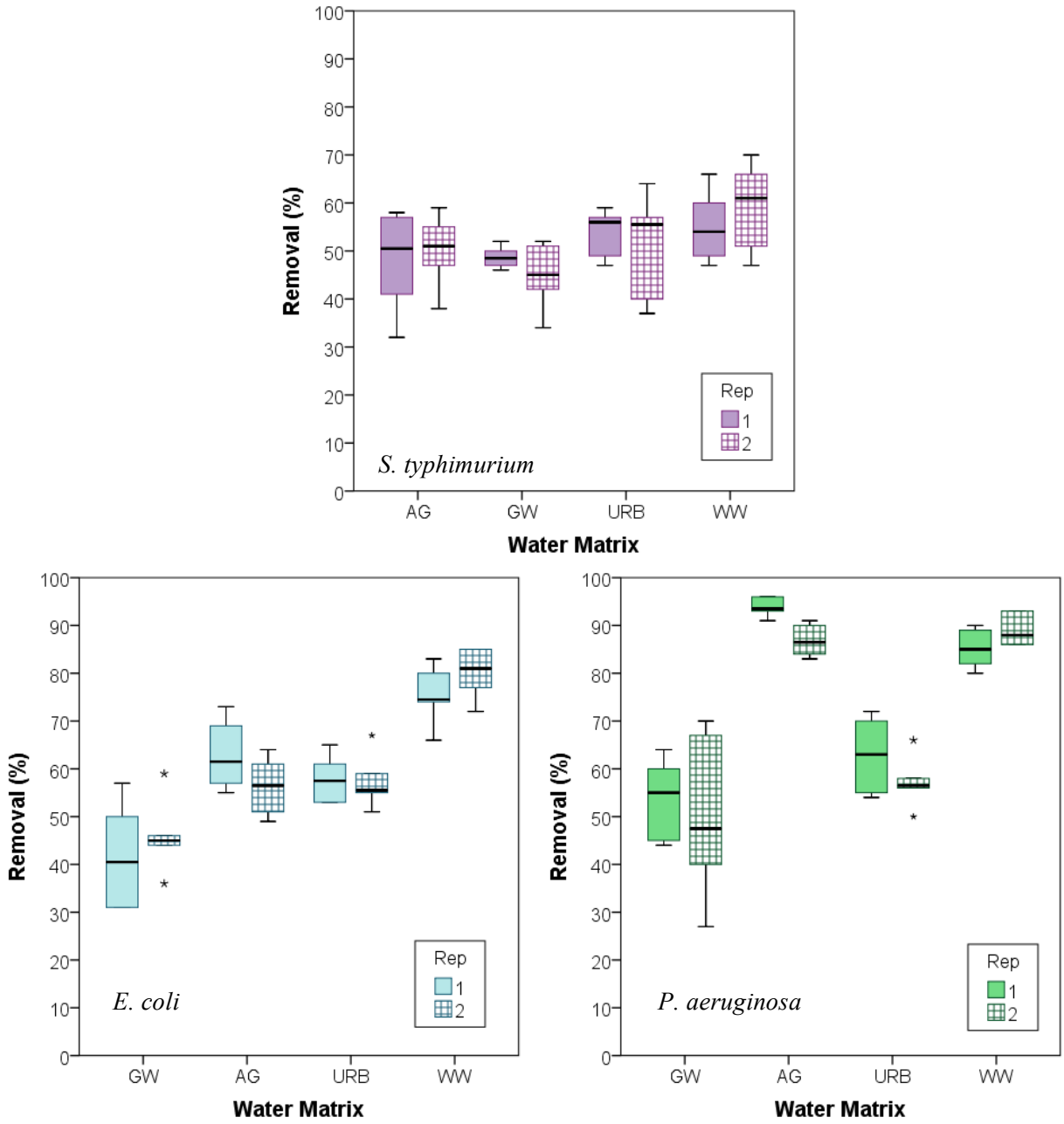


Figure 24. Box-and-whisker plots showing percent removal from replicate columns (1) and (2) for the three bacterial pathogens after exposure to four different water matrices (n=6 for each box-and-whisker plot).

Table 15: Reported Removal of *Pseudomonas* spp. and *E. coli* Strains in Saturated Porous Media

Source	Bacterial Strain	Media Type	Column / Batch	GS (D_{50}, mm)	IS (mM)	Removal (%)
Liu et al. 2007	*Pseudomonas aeruginosa*	glass beads	Column	0.55	1.65	5 - 40
Tong et al. 2010	*Pseudomonas spp.*	quartz sand	Column	0.4 - 0.5	2.5	40 - 50
	E. coli	quartz sand	Column	0.4 - 0.5	2.5	20 - 45
Kim et al. 2009	*E. coli* 0157:H7	quartz sand	Batch	0.275	1	30 - 50

6.2. Bacterial Size Measurements

Size measurements from DLS analysis of each bacterial feed suspension used during Experiment 3 are reported in Table 16. In general, bacteria were slightly larger in size following exposure to water matrices containing higher-nutrient/solids waters (URB and WW matrices).

Table 16: Bacterial Size Measurements (DLS) After ~120 h Exposure to Four Water Matrices

Bacterial Pathogen	Water Matrix	Mean Size (µm)	SD (µm)	Size Measurements (µm)		
				1	2	3
E. coli	GW	1.116	0.031	1.135	1.134	1.080
	AG	1.172	0.001	1.172	1.172	1.173
	URB	1.469	0.010	1.480	1.461	1.466
	WW	1.475	0.145	1.607	1.498	1.319
P. aeruginosa	GW	1.318	0.074	1.307	1.251	1.397
	AG	1.304	0.039	1.337	1.313	1.261
	URB	1.889	0.049	1.840	1.890	1.937
	WW	1.970	0.084	1.879	1.989	2.043
S. typhimurium	GW	1.186	0.010	1.190	1.193	1.175
	AG	1.182	0.024	1.186	1.156	1.204
	URB	1.289	0.006	1.281	1.290	1.292
	WW	1.308	0.080	1.256	1.400	1.269

The ANOVA results, shown in Table 17, indicate that water matrix exposure significantly affected the removal of each bacterial pathogen studied (Table 17). The effect of water matrix exposure on bacterial removal was most significant for *E. coli* and *P. aeruginosa* ($p < 0.001$), and less substantial for *S. typhimurium* ($p = 0.009$). *S. typhimurium* was removed fairly consistently by the porous media regardless of previous water matrix exposure, which explains why the effect of water matrix exposure was not found to be significant at p-value of 0.1%.

The ANOVA indicated that the removals of the *E. coli* and *P. aeruginosa* by porous granular media used in this study were significantly affected by their size ($p < 0.001$, Table 17). These findings are consistent with previous studies investigating bacteria specific factors affecting their removal in saturated porous media (Bolster et al. 2009; Walker et al. 2005a; Gannon et al. 1991). Walker et al. (2005a) studied wild- and mutant-types of *Burkholderia cepacia* and found that nutrient presence altered the size of the cells and that their attachment increased by 60% with nutrient presence. Gannon et al. (1991) studied a suite of indigenous soil bacteria (e.g. *Enterobacter* spp, *Pseudomonas* spp and *Bacillus* spp.) and found that, although the width of the various strains did not vary considerably (0.5 – 0.7 μm), the retention of the bacteria in soil columns was statistically related to cell size. Bacteria shorter than 1.0 μm were removed to a lesser extent (i.e. transported over longer distances) than larger cells, while cell flagellation, cell surface hydrophobicities, net surface charge, and capsule presence were not correlated with bacterial transport in the porous media investigated. These results indicate that the removal of bacterial pathogens (in the size range of 1.0 μm) may be driven primarily by mechanisms described in colloid filtration theory (i.e. diffusion, sedimentation and interception), rather than by physico-chemical mechanisms associated with cell surface properties (e.g. zeta potential, hydrophobicity, EPS); since size has been found to be highly correlated with colloid removal and CFT predicts that the forces that drive colloid-collector contact opportunities are at a minimum for bacterial-sized pathogens. However, many of these transport investigations have been conducted in clean-bed environments, and the concurrent impacts of factors effecting natural environments must be taken into consideration when evaluating filtration performance for pathogen removal.

Overall, the differences in removal between the bacteria after exposure to the four water matrices investigated was < 0.5 log in most cases, similar to that reported by others whom have investigated the transport of bacterial strains with altered EPS surfaces (Section 2.5.6 and Table 15). The differences observed in the *P. aeruginosa* results span > 0.5 log, and this may be due to the fact that *Pseudomons spp.* are recognized as EPS-overproducers and typically hold the genetic coding for all three metabolic pathways for EPS secretion (Wingender et al. 1999). Tsuneda et al. (2004) investigated the removal of 12 strains of bacteria (including *Bacillus subtilis*, *P. aeruginosa*, and other strains isolated from an aerobic

waste water fluidized-bed reactor) and found that EPS coatings altered the "softness" of bacterial cell surfaces, which affected both the removal of bacteria in saturated glass-bead column filters and how ionic strength impacted colloid-collector interactions. Specifically, it was found that in 10 of the 12 strains investigated EPS coatings on cell surfaces increased cell softness and, in 9 of the 12 strains EPS coatings decreased the negative charge density of cellular surfaces (Tsuneda et al. 2004). Tsuneda et al. (2004) also found that cell surface potential, which accounted for EPS polymer presence, had a stronger relationship with cell removal, than zeta-potential measurements.

Table 17: ANOVA Table of Results from Experiment 3.

(Bio)Bolloid	Source	df	Mean Square	F_o	Significance[a]	R Squared
E. coli	Water Matrix	3	2340.188	50.218	.000 [a]	0.774
	Size	3	0.109	19.583	.000 [a]	
	Error	44	46.600			
P. aeruginosa	Water Matrix	3	4455.688	73.835	.000 [a]	0.834
	Size	3	0.386	94.921	.000 [a]	
	Error	44	60.347			
S. typhimurium	Water Matrix	3	246.694	4.342	.009[b]	0.228
	Size	3	0.008	0.347	.793	
	Error	44	56.814			

[a] 0.1% significance detected where $p < 0.001$ ($F_{obs} > F_{0.001}$);

[b] 5% significance detected where $p < 0.05$ ($F_{obs} > F_{0.05}$)

6.3 EPS Characterization

Proteins and carbohydrate enumerated in free- and bound-EPS extracts from each of the bacteria utilized in Experiment 3 are shown in Figure 25 (Appendix D). EPS protein and carbohydrate results are reported in mg / 10^{10} cells and are within the ranges reported by others (Bolster et al. 2009; Gong et al., 2009; Takahashi et al. 2009). Error bars are shown to represent 1 SD of 4 sample measurements – two duplicate measurements taken from two replicate extraction procedures.

The EPS extracts from the *E. coli* strain yielded the highest amounts of proteins and carbohydrates overall, followed by the *P. aeruginosa* and *S. typhimurium* strains. The levels of proteins and carbohydrates that were observed from the *E. coli* extracts of EPS during the present investigation are

consistent with those that have been reported elsewhere (Gong et al, 2009). A clear trend in carbohydrate content in the EPS extracts was difficult to discern. Moreover, the lack of reproducibility associated with EPS quantification makes interpretation of these data difficult (Appendix D). Gong et al. (2009) also had difficulty determining trends in EPS protein and carbohydrate content of bacteria that were exposed to various ionic strengths over time. These investigations underscore that extraction methods for EPS characterization require further development before differences in EPS production by bacterial cultures (essentially different substrates than biofilms) can be reliably quantified and compared.

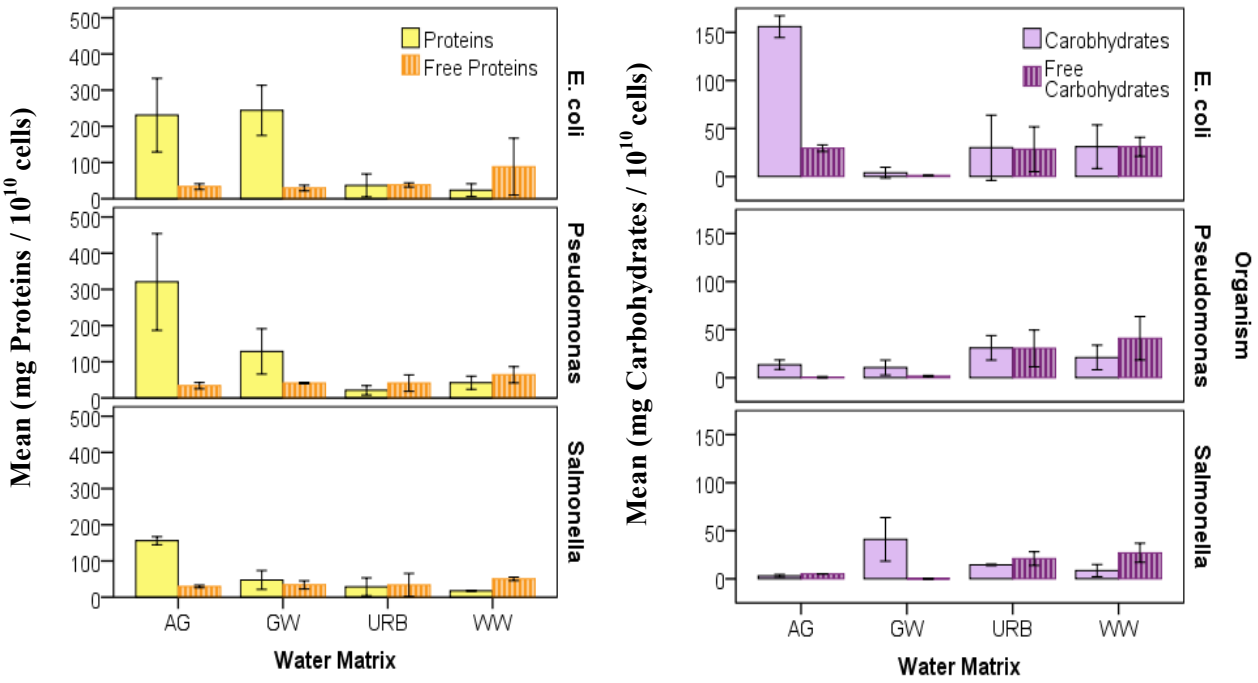


Figure 25: Mean protein and carbohydrate measurements from free- and bound-EPS extracts from the three bacterial pathogens exposed to each water matrix during Experiment 3. Error bars indicate ±1 standard deviation (n=4).

6.3.1. EPS Extract Protein Yield

All bacterial strains exposed to GW and AG water matrices yielded higher amounts of proteins in the bound-EPS extracts. EPS extracts from bacteria exposed to the AG water matrix yielded the highest mean protein concentrations from bound-EPS extracts. Large amounts (> 100 mg/10^{10} cells) of proteins were also enumerated from the bound-EPS extracted from *E. coli* and *P. aeruginosa* after exposure to the GW water matrix. When looking at the protein from free-EPS extracts, greater amounts of proteins were detected in free-EPS extracts after bacterial exposure to the WW matrix (~150 mg/10^{10} cells). Overall, the

lowest mean protein yield was detected in samples of bound- and free-EPS extracted after bacterial exposure to the URB water matrix, for all three bacterial strains. In summary, the protein yield results suggest that bacterial surface EPS production yields greater amounts of proteins after exposure to nutrient-poor waters (e.g. GW).

6.3.2 EPS Extract Carbohydrate Yield

Mean carbohydrate yield was greatest in the bound-EPS extract taken from *E. coli* after exposure to the AG water matrix. Overall, the lowest mean carbohydrate yields (<25 mg/10^{10} cells) were detected after bacteria were exposed to the GW water matrix, although elevated carbohydrate content was detected in the bound-EPS sample after *S. typhimurium* was exposed to the GW water matrix (~40 mg/10^{10} cells). Mean carbohydrate measurements in free-EPS samples were fairly consistent between each bacterial strain (~20 mg/10^{10} cells) after exposure to the URB and WW water matrices; while mean carbohydrate yield in free-EPS extracts after exposure to AG and GW water matrices were much lower for *P. aeruginosa* and *S. typhimurium*. In summary, the carbohydrate yield results suggest that bacteria produce greater amounts of carbohydrate-containing free-EPS after exposure to nutrient-rich waters (e.g. WW).

6.3.3. EPS Extract Protien:Carbohydrate Ratio

The mean ratios of protein and carbohydrate yield from bound- and free-EPS extracts after each bacterial exposure are shown in Table 18. The highest ratio for bound-EPS extracts was detected after exposure to GW for the *E. coli* strain, while substantially higher ratios were detected after exposure to WW for *S. typhimurium* and *P. aeruginosa*. For free-EPS extracts, the highest protein:carbohydrate ratios were detected after bacterial exposure to GW and AG. Many of these values are substantially higher than those reported from studies investigating the composition of EPS mats in sludges and using methods other than CER for EPS extraction (Comte et al. 2006; Liu and Fang, 2002). CER methods for EPS extraction have been sited to preferentially extract the protein component of EPS, which helps to explain the high protein content enumerated in the extraction samples in Experiment 3 (See section 2.5.4). These results highlight that EPS composition, and likely production of free- and bound-EPS, vary between pathogenic bacterial strains, as well as in response to exposure to different natural physico-chemical properties of environmental water matrices.

Table 18. Protein:Carbohydrate Ratio in Bound- and Free-EPS Extracts

Water Matrix	*E. coli*		*S. typhimurium*		*P. aeruginosa*	
	Bound-EPS	Free-EPS	Bound-EPS	Free-EPS	Bound-EPS	Free-EPS
GW	11.5	49.7	4.3	26.9	0.9	104.0
AG	4.4	17.6	3.4	90.4	6.8	10.5
UR	5.7	1.0	3.9	1.4	3.2	1.6
WW	7.2	1.1	9.4	0.8	16.9	1.1

6.4 Conceptual Model: Bacterial EPS Effects on Bacterial Transport

The results from this investigation and literature review on bacterial EPS production response to environmental conditions lends to a conceptual model describing how bacterial EPS may affect bacterial transport in saturated porous media environments (Figure 26). It is understood that bacterial EPS can enhance bacterial attachment in environments favorable for bacterial survival; where either there is a high influx of nutrients required for metabolic activities, few environmental stressors (e.g. bactericides, pH, temperature), or substrates favorable for attachment. Conversely, bacterial attachment may be hindered in unfavorable environments for survival (e.g. non-ideal temperature, pH, lack of substrates for attachment) (see section 2.5.6). These findings can be explained by a conceptual model that includes the ability of bacteria to intentionally alter the composition of the EPS coat and cell shape/size in response to environmental conditions, to favor survival, attachment and proliferation.

Information gathered regarding bacterial EPS production while in favorable environments has been used to contribute to a conceptual model of bacterial EPS production response to environmental conditions. Bacteria that are in, or enter into, favorable conditions for survival and growth may responsively secrete EPS that is higher in carbohydrate content (i.e. “sticky”) and greater in quantity (thereby increasing cell size) (Liu and Li, 2008; Vendevivere and Kirchman, 1993), which would permit greater bacterial attachment and entrapment in these saturated porous media environments (Zolghadr et al. 2010; Evans et al. 1994). This response by the bacteria would allow the bacteria to proliferate and form micro-colonies in such environments (Gancel and Novel, 1994; Allison and Sutherland, 1987) (Figure 26). Conversely, in unfavorable environments, bacteria can respond by producing EPS that is higher in protein content and in smaller quantities, thereby increasing steric repulsion (i.e. hindering attachment) and decreasing cell size to allow for greater transport to possibly more favorable conditions (Prince and Dickenson, 2003; Rijnaarts et al. 1999). This response by bacteria would prevent the cells from lingering in unfavorable

conditions that could threaten survival, and increase the likelihood of survival if the bacteria can be transported to more favorable environments (Figure 26).

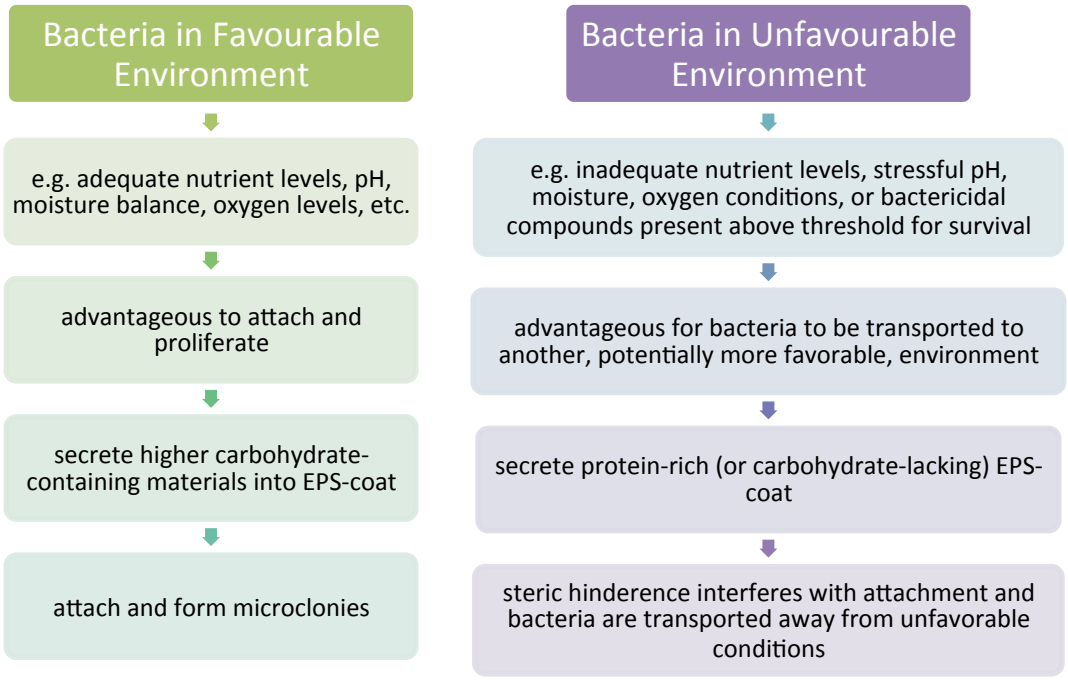


Figure 26. Conceptual model of bacterial and EPS-coat response to favorable and unfavorable conditions for survival and growth.

This conceptual model (Figure 26) is supported by the results presented herein. Higher carbohydrates, or "sticky" compounds, have been extracted from biofilm substrates; while higher protein content has been extracted from pure cultures of *Klebsiella* spp (Brown and Lester, 1980). It is possible that the higher carbohydrate content in the biofilm sample was formed in an effort by the bacteria to create a microcolony within a favorable growth environment, while the higher protein production by the isolated cells was a response to unfavorable conditions that did not allow for symbiotic relationships and microcolony formation. Additionally, Gong et al. (2009) found that EPS extracted from *Salmonella pollorum* SA 1685 using lyophilization, ethanol exposure, and sonication from cells exposed to higher ionic strength conditions (representative of natural groundwater), and for longer periods of time, produced EPS materials with greater carbohydrate content (using the same methods described herein). These findings suggest that bacteria in nutrient rich waters, such as wastewater with higher nutrients than typical ground waters, may alter EPS characteristics to favor attachment and proliferation. Furthermore, the study

described herein revealed that bacterial size increased with higher nutrient concentrations, and was smaller in poor-nutrient water. These findings suggest that bacteria in unfavorable conditions for survival are smaller and therefore may be transported over longer distances in porous media, to possibly more favorable environments – supporting the conceptual model described above.

The applications of this developed conceptual model may be vast, including applications in understanding bacteria that accumulate in hospital fittings (Hall-Stoodley et al. 2004; Habash and Reid, 1999; Gibbons, 1978), RBF (Schwartz et al. 1997), biologically active engineered drinking water filters (Butterfield et al. 2002), or the distribution system (Wingender and Flemming, 2011; LeChevallier et al. 1988). However, there is a need to determine more specifically how to categorize favorable and unfavorable conditions for bacterial attachment to porous media to determine suitable applications. This conceptual model may be limited by the fact that bacterial EPS production and composition is variable between various bacterial strains (see section 2.5.3), and certainly "favorable" and "unfavorable" conditions for survival are different depending on the bacteria of concern (Madigan, 2003; Wingjender, 1999). Additionally, certain experimental results contradict the concepts used to develop this model; where certain bacteria have been shown to produce excess EPS in unfavorable environments (e.g. LeChevallier et al. 1988). It becomes necessary to understand the community of bacteria in a particular system or biofilm, before drawing generalizations and conclusions regarding their overall behavior. In summary, bacterial EPS production tends to respond to a variety of environmental conditions, and compiling current research on this subject can help to formulate a better understanding of general, and specific, trends that are applicable to many areas of research, including pathogen removal in natural and engineered drinking water filters.

Chapter 7 Conclusions

The purpose of the experiments conducted in this research project was to develop a better understanding of factors effecting pathogen and (bio)colloid transport in saturated porous media environments, and to evaluate the use of laboratory bench-scale column investigations for elucidating these effects. The results of the three tiers of experiments conducted will help to better inform regulatory guidance for experimental testing of factors effecting the performance and operation of natural and engineered drinking water filters. Key conclusions of this work are listed below.

1. Laboratory bench-scale investigations using replicate glass columns were proven to be useful tools in investigating factors effecting (bio)colloid transport in saturated porous media for applications in riverbank filtration and engineered filter environments.

2. Column and collector media designs with D/d ratios between 15 and 116 did not have a significant effect on reproducibility and removal of a suite of (bio)colloids in transport investigations using varying ionic strengths and flow velocities representative of natural subsurface environments.

3. Low D/d column designs (<40) in bench-scale investigations, operated at loading rates >1 m/h, interfered with the reproducibility of (bio)colloid transport of colloids ≥4 μm.

4. The effects of physical (GS, UC) and chemical (IS, NOM) factors on (bio)colloid transport were generally consistent with colloid filtration theory.

5. Grain size, ionic strength, and the presence of natural organic matter significantly affected the removal of a suite of (bio)colloids at values representative of natural environmental conditions.

6. The uniformity coefficient values studied did not result in a significant effect on the removal of (bio)colloids PR772 bacteriophage and *Salmonella typhimurium*; however, uniformity coefficient had a significant effect on the removal of 1.1 μm and 4.5 μm polycarbonate fluorescent microspheres.

7. A concurrent effect between media grain size and uniformity coefficient was observed, suggesting that uniformity coefficient is an important factor affecting (bio)colloid transport when grain size is low (<0.4 mm).

8. Ionic strength and natural organic matter both effect the surface chemistry of (bio)colloids and media collectors and a significant interaction effect on the removal of all (bio)colloids investigated was identified between NOM and IS.

9. (Bio)colloid size/type effects on (bio)colloid removal were generally consistent with colloid filtration theory. However, in Experiment 2, PR772 was removed to a lesser extent than *Salmonella typhimurium* bacteria and 1.1 μm spheres; suggesting that mechanisms other than those described in colloid filtration theory impact the transport of (bio)colloids.

10. A literature review on the impacts of bacterial surface EPS on transport and removal in saturated porous media environments revealed several inconsistencies in the current understanding of these effects.

11. Numerous methods for the extraction of bacterial surface EPS have been investigated, and no standardized methods have been developed for various applications. A review of commonly used extraction methods suggests that physical methods are advantageous over chemical methods, as they produce pure extracts that are not contaminated by extraction compounds. Additionally, the use of a combination of physical methods may be advantageous in extracting higher EPS yields, while maintaining cellular integrity, although these methods have yet to be thoroughly trialed.

12. Exposure to various water matrices was shown to significantly affect the size and removal of a suite of environmentally indigenous pathogenic bacterial strains, suggesting that land-use impacts on water quality may affect pathogen transport in subsurface environments. However, these differences (<0.5 log) were less substantial than those observed due to the impacts of physic-chemical factors such as grain size, ionic strength, and the presence of natural organic matter.

13. A conceptual model was developed to describe the interaction between bacterial transport observations and EPS production: some bacteria in unfavourable environments (i.e. with low nutrient concentrations or in the presence of chemical stressors) may alter their surface chemistry/physiology and/or shape to possibly allow for transport to possibly more favourable

conditions. In contrast, bacteria in favourable environments (i.e. high influx or presence of nutrients) may alter their surfaces to allow for attachment and micro-colony (i.e. biofilm) formation.

14. Differences in porous media removal between a suite of pathogenic strains of environmentally isolated bacteria at conditions representative of subsurface filtration were small (<0.5 log), suggesting that nuances between the removal of various strains of bacteria are present at the micro-scale may not be substantial at the macro- or field-scale.

15. Bacterial EPS extract characterization failed to show consistent trends in bacterial EPS production in response to environmental conditions; likely the result of both variation in EPS production and the lack of developed EPSextraction and characterization methods, particularly for relatively dilute bacterial suspensions that would be expected in many drinking water treatment environments.

Chapter 8 Implications and Recommendations

This study contributes to the current state of knowledge on factors effecting pathogen transport in saturated porous media environments. The findings from this project can be used to better inform the use of bench-scale column studies for pathogen and (bio)colloid transport investigations and to improve the design of natural and engineered filter performance demonstrations. Additionally, this work helps to develop a better understanding of colloid transport theory. Several implications and recommendations follow from this research and are discussed.

- Small scale column studies, used either to identify factors of importance for consideration in full-scale filter operation (natural or engineered) or for mechanistic theoretical investigations into pathogen transport in saturated porous media, conducted at D/d ratios <50, should not be universally disregarded because of wall effects concerns. These results should be taken into consideration along with adequate consideration for media depth and hydraulic loading rate. Decisions to employ study designs with lower D/d conditions are supported by this work and the published literature. These experimental designs may be favoured for their use in conservation of materials and more economical research approaches. Based on the literature currently available and the results presented herein, a ratio of 25-30 is supported as a lower limit for (bio)colloid removal studies at the lower loading rates presented in this study in saturated porous media. Further research, using appropriate control measures and demonstrated reproducibility, is necessary to identify practical design recommendations for a variety of pilot- and bench-scale column studies. Additional investigations are warranted to determine individual guidelines for the various applications of column studies.

  Bench-scale column experiments and laboratory experimental outcomes are useful tools for advancing filtration theory and for identifying factors for consideration in field-scale investigations. However, encompassing macro-scale parameters and aquifer characteristics (e.g. preferential pathways and events) on (bio)colloid transport are difficult to mimic at the lab scale. These parameters also require investigation because they may render typically favourable conditions for (bio)colloid attachment vulnerable to (bio)colloid (and therefore pathogen) passage through natural and engineered filters. At present, regulations and best management practices should be based primarily on outcomes demonstrated at the pilot- or field-scale, rather than on parameters selected based on filtration models or simple column tests that may neglect consideration for water quality parameters, such as the presence or influx of natural organic matter.

- Although the transport of select bacterial pathogens was impacted by the exposure to different source waters under controlled conditions, possibly due to the biochemical response of EPS production on the surface of the bacterial cell membranes at the micro-scale, these differences may not be substantial at the micro- and field-scale. The concurrent effects of other factors, such as changing environmental water quality parameters (e.g. NOM and ionic strength) or events (e.g. rainfall or manure spreading), may have a more substantial impact on pathogen transport. The effects of bacterial EPS on (bio)colloid transport, may be more important in environments with profuse biofilm formation (unlike the "clean-bed" environments used in this study) and deserve further investigation. These outcome are especially relevant to porous media filtration processes that are not proceeded by coagulation (e.g. riverbank filtration processes) because coagulation often drives surface charge and therefore attachment processes in these systems.

- Established and standardised methods for EPS extraction and characterization for a range of applications are necessary to improve our understanding of bacterial EPS production and the effects of these compounds in a range of environments.

Bibliography

Abudalo, R.A. Bogatsu, Y.G., Ryan, J.N, Harvey, R.W., and Metge, D.W., Elimelech, M. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and *Cryptosporidium* parvum oocysts in saturated porous media. Environ. Sci. Technol., 39: 6412-6419. 2005.

Abudalo, R.A., Ryan, J.N., Harvey, R.W., Metge, D.W., and Landkamer, L. Influence of organic matter on the transport of *Cryptosporidium parvum* oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. Water Research, 44 (4): 1104-1113. 2010.

Aguilera, A., Souza-Egipsy, V., Martin-Uriz, P.S., and Amils, R. Extraction of extracellular polymeric substances from extreme acidic microbial biofilms. Appl. Microbial. Biotechnol., 78: 1079-1088. 2008.

APHA, American Public Health Association, American Water Works Association, Water Pollution Control Federation, 20th Ed. Standard Methods for the Examination of Water and Wastewater. 1998.

Arbuckle, W.B. and Ho, YF. Adsorber column diameter: particle diameter ratio requirements. Research Journal of the water Pollution Control Federation*, 62* (1): 88-90. 1990.

ASTM Standard D4327-11. Standard test method for anions in water by suppressed ion chromatography. ASTM International, West Conshohocken, PA. 2003.

AWWA, Design of Pilot-Plant Studies. Proc. AWWA Seminar, ISBN-0-89867-285-6. 1982.

Azeredo, J., Henriques, M., Sillankorva, S., and Oliveira, R. Extraction of exopolymers from biofilms: the protective effect of glutaraldehyde. Water, Science and Technology, 47 (5): 175-179. 2003.

Bell, C.H., Arora, B.S., and Camesano, T.A. Ahesion of *Pseudomonas putida* KT2442 is mediated by surface polymers at the nano- and microsclae. Environmental Engineering Science, 22 (5): 629-641. 2005.

Bertelkamp, C., Kramer, F.C., Reungoat, J., Singhal, N., Cornelissen, E.R., Verliefde, A.R.D., van der Hoek, J.P. Organic micropollutant removal during riverbank filtration: Batch studies. Proceedings of the AWWA Water Quality and Technology Conference, Toronto, ON, Canada. 2012.

Bhattacharjee, S., Ko, C.H., and Elimelech, M. DLVO interaction between rough surfaces. Langmuir, 14: 3365-3375. 1998.

Biber, M.V., Gulacar, F.O. and Buffle, J. Seasonal variations in principal Groups of Organic Matter in a Eutrophic Lake Using Pyrolysis/GC/MS, Environmental Science and Technology, 30 (12): 3501. 1996.

Bixby, R.L. and O'Brien, D.J. Influence of fulvic acid on bacteriophage adsorption and complexation in soil. Applied and Environmental Microbiology 38, 840–845. 1979.

Bolster, C.H., Haznedaroglu, B.Z., and Walker, S.L. Diversity in cell properties and transport behavior among 12 different environmental *Escherichia coli* isolates. J. Environ. Qual., 38: 465-472. 2009.

Bolster, C.H., Mills, A.L., Hornberger, G.M., and Herman, J.S. Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. Journal of Contaminant Hydrology, 50: 287-305. 2001.

Bolster, C.H., Mills, A.L., Hornberger, G.M., and Herman, J.S. Spatial distribution of deposited bacteria following miscible displacement experiments in intact cores. Water Resources Research, 35 (6): 1797-1807. 1999.

Bonet, R., Simon-Pujol, M.D., and Congregado, F. Effects of nutrients in exopolysaccharide production and surface properties of *Aeromonas salmonicida*. Applied and Environmental Microbiology, 59 (8): 2437-2441. 1993.

Bradford, S.A. and Bettahar, M. Concentration dependent transport of colloids in saturated porous media. Journal of Contaminant Hydrology, 82: 99-117. 2006.

Bradford, S.A., Bettahar, M., Simunek, J., and van Genuchten, M.T. Straining and attachment of colloids in physically heterogeneous porous media. Vadose Zone Journal, 3 (2): 384-394. 2004.

Bradford, S.A. and Torkzaban, S. Colloid adhesive parameters for chemically heterogeneous porous media. Langmuir, 28 (38): 13643-13651. 2012.

Bradford, S.A., Yates, S.R., Bettahar, M., and Simunek, J. Physical factors affecting the transport and fate of colloids in saturated porous media. Water Resources Research, 38 (12): 1327-1339. 2002.

Brookes, J.D. et al Relative value of surrogate indicators for detecting pathogens in lakes and reservoirs. Environ. Sci. Technol., 39 (22): 8614-8621. 2005.

Brown, M.J. and Lester, J.N. Comparison of bacterial extracellular polymer extraction methods. Applied and Environmental Microbiology, 40 (2); 179-185. 1980.

Brown, D.G., Stencel, J.R., and Jaffé, P.R. Effect of porous media preparation on bacteria transport through laboratory columns. Water Research, 36: 105-114. 2002.

Bunn, R. A., Magelky, R. D., Ryan, J. N., and Elimelech, M. Mobilization of natural colloids from an iron oxide-coated sand aquifer: Effect of pH and ionic strength, Environ. Sci. Technol., 36(3): 314-322. 2002.

Butterfield, P.W., Camper, A.K., Ellis, B.D., and Jones, W.L. Chlorination of model drinking water biofilm: implications for growth and organic carbon removal. Wat. Res., 36: 4391-4405. 2002.

Camesano, T.A. and Logan, B.E. Influence of fluid velocity and cell concentration on the transport of motile and non-motile bacteria in porous media. Environ. Sci. Technol., 32: 1699-1708. 1998.

Caro, A., Got, P., Lesne, J., Binard, S., and Baleaux. Viability and virulence of experimentally stressed nonculturable *Salmonella typhimurium*. Appl. Environ. Microbiol., 65 (7): 3229-3232. 1999.

Carson, C.A., Shear, B.L., Ellersieck, M.R., and Asfaw, A. Identification of fecal *Escherichia coli* from humans and animals by ribotyping. Applied and Environmental Microbiology, 67 (4): 1503-1507. 2001.

Castro, F.D. and Tufenkji, N. Relevance of nontoxigenic strains as surrogates for *Escherichia coli* 0157:H7 in groundwater contamination potential: Role of temperature and cell acclimation time. Environ. Sci. Technol., 41: 4332-4338. 2007.

Chae, G.T., Stimson, J., Emelko, M.B. Blowes, D.W., Ptacek, C.J., and Mesquita, M.M. Statistical assessment of the accuracy and precision of bacteria- and virus-sized microsphere enumerations by epifluorescence microscopy. Water Research, 42 (6-7): 1431-1440. 2008.

Cho, J.C. and Kim, S.J. Viable, but non-culturable, state of a green fluorescence protein-tagged environmental isolate of *Salmonella typhi* in groundwater and pond water. FEMS Microbiology Letters, 170: 257-264. 1999.

Christman, R.F. and Gjessing, E.T. (Editors). Chapter 16: Conditional stability constants of trace metals and naturally occurring humic materials: Application in equilibrium models and verification with field data, In, Aquatic and Terrestrial Humic Materials, Alberts, J.J., and Giesy, J.P. Ann Arbor Sci., Ann Arbor, Mi. 333-348. 1983.

Clancy, J.L. GWUDI Decisions – Still crazy after all these years. Proceedings of the AWWA Water Quality and Technology Conference, Toronto, Ontario, Canada. 2012.

Clark, D.S. Studies on the surface plate method of counting bacteria. Canadian Journal of Microbiology, 17 (7): 943-946. 1971.

Cohen, Y. and Metzner, A.B. Wall effects in laminar flow of fluids through packed beds. The American Institute of Chemical Engineers, 27 (5): 705-715. 1981.

Comte, S., Guilbaud, G., and Baudu, M. Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties Part I. Comparison of the efficiency of eight EPS extraction methods. Enzyme and Microbial Technology, 38: 237-245. 2006.

D'Abzac, P., Bordas, F., Joussein, E., Van Hullebusch, E., Lens, P.N.L., and Guibaud, G. Characterization of the mineral fraction associated to extracellular polymeric substances (EPS) in anaerobic granular sludges. Environ. Sci. technol., 44: 412-418. 2010.

Dade, W.B. et al Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrob. J., 8: 1-16. 1990.

Dai, X. and Hozalski, R.M. Effect of NOM and biofilm on the removal of *Cryptosporidium parvum* oocysts in repid filters. Water Research, 36: 3523-3532. 2002.

De Kerchove, A.J. and Elimelech, M. Bacterial swimming motility enhances cell deposition and surface coverage. Environ. Sci. Technol., 42 (12): 4371-4377. 2008.

Deborde, D.C., Woesner, W.W., Kiley, Q.T., and Ball, P. Rapid transport of viruses in a floodplain aquifer. Wat. Res., 33 (10): 2229-2238. 1999.

Delpha, I., Jung, A.V., Baures, E., Clement, M., and Thomas, O. Impacts of climate change on surface water quality in relation to drinking water production. Environmental International, 35: 1225-1233. 2009.

Derjaguin, B.V. and Landau, L.D. Theory of the stability of strongly charged lyophobic soils and of the adhesion of strongly charged particles in solutions of electrolytes. Acta Physicochim. U.S.S.R., 14: 633. 1941.

Dong, H. et al Simultaneous transport of two bacterial strains in intact cores from Oyster, Virginia: Biological effects and numerical modeling. Applied and Environmental Microbiology, 68 (5): 2120-2132. 2002.

Eckhardt, D.A.V. and Stackelberg, P.E. Relation of groundwater quality to land use on Long Island, New York. Ground Water, 33(6): 1019-1033. 1995.

Edberg, S.C., Rice, E.W., Karlin, R.J., and Allen, M.J. Escherichia coli: The best biological drinking water indicator for public health. Society for Applied Microbiology, 29:106-116. 2000.

Elimelech, M. Kinetics of capture of colloidal particles in packed beds under attractive double layer interactions. Journal of Colloidal and Interface Science, 146 (2) 337-352. 1991.

Elimelech, M., Gregory, J., Jai, X., and Williams, R.A. Particle deposition and aggregation: Measurement, modeling and simulation. Butterworth-Heinemann, Woburn, MA. 1995.

Elimielech, M. and O'Melia, C.R. Effect of electrolyte type on the electrophoretic mobility of polystyrene latex colloids. Colloids Surf. A. 44: 165-178. 1990a.

Elimelech, M. and O'Melia, C.R. Effect of particle size on collision efficiency in the deposition of Brownian particles with electrostatic energy barriers. Langmuir 6: 1153-1163. 1990b.

Emelko, M.B. Transport and fate of colloids and microbes in granular aqueous environments. Water Research, 44: 1027. 2010.

Emelko, M.B. and Huck, P.M. Microspheres as surrogates for *Cryptosporidium* Filtration. Jour. AWWA, 96 (3): 94-105. 2004.

Emelko, M.B., Huck, P.M., and Douglas, I.P. *Cryptosporidium* and microsphere removal during late in-cycle filtration. Jour. AWWA, 95 (5): 173-182. 2003.

Emelko, M.B., Schmidt, P.J., Roberson, J.A. Quantification of Uncertainty in Microbial Data: Reporting and Regulatory Implications. Jour. AWWA, 100:3:94-104, 2008

Emelko, M.B., Silins, U., Bladon, K.D., and Stone, M. Implications of and disturbance on drinking water treatability in a changing climate: Demonstrating the need for "source water supply and protection" strategies. Water Research, 45: 461-472. 2011.

Evans, E., Brown, M.R.W. and Gilbert, P. Iron chelator, expolysaccharide and protease production in *Staphylococcus epidermidis*: a comparative study of the affects of specific growth rate in biofilm and planktonic culture. Microbiology, 140: 153-157. 1994.

Federal Register. The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) Implementation Guidance. USEPA. 5 January. 2006.

Fletcher, M. In Bacterial Adhesion: Ecological and Applied Microbiology. John Wiley and Sons, New York. 1996.

Foppen, J.W.A., Okletey, S., and Schijven, J.F. Effect of goethite coating and humic acid on the transport of bacteriophage PRD1 in columns of saturated sand. Journal of Contaminant Hydrology, 85: 287-301. 2006.

Foppen, J.W.A. and Schijven, J.F. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. Water Research, 40: 401-426. 2006.

Ford, R.M. and Harvey, R.W. Role of chemotaxis in the transport of bacteria through saturated porous media. Advances in Water Resources, 30: 1608-1617. 2007.

Franchi, A. and O'Melia, C.R. Effects of natural organic matter and solution chemistry on the deposition and reentrainment of colloids in porous media. Environ. Sci. Technol., 37: 1122-1129. 2003.

Frank, B.P. and Belfort, G. Polysaccharides and sticky membrane surfaces: critical ionic effects. Journal of Membrane Science, 212: 205-212. 2003.

Frølund, B. Palmgren, R., Keiding, K., and Nielsen, P. Extraction of extracellular polymers from activated sludge using a cation ion exchange resin. Water Res., 30: 1749-1758. 1996.

Gaffney, J.S., Marley, N.A., and Clark, S.B. Humic and fulvic acids and organic colloidal materials in the environment, In Humic and Fulvic acids. ACS Symposium Series, American Chemical Society. Washington, DC. 1996.

Gerba, C.P. Pathogens in the environment. In: Pepper, I.L., Gerba, C.P., Brusseau, M.L. (Eds.), Pollution Science. Academic Press, New York, pp. 279-299. 1996.

Gerba, C, P. and Smith, J.E. Sources of pathogenic microorganisms and their fate during land application of wastes. Journal of Environmental Quality, 34 (10): 42-48. 2005.

Gibbons, R.J. and Qureshi, J.V. Selective binding of blood group-reactive salivary mucins by *Streptocuccus* mutans and other oral organisms. Infect. Immun., 22 (3): 665-671. 1978.

Ginn, T.R., Wood., B.D., Nelson, K.E., Scheibe, T.D., Murphy, E.M., and Clement, T.P. Processes in microbial transport in the natural subsurface. Advances in Water Resources, 25: 1017-1042. 2002.

Gollnitz, W.D., Clancy, J.L., Whitteberry, B.L., and Vogt, J.A. RBF as a microbial treatment process. J. AWWA, 95 (12): 56-66. 2003.

Gómez-Suárez, C., Pasma, Jos, van der Borden, A.J., Wingender, J., Flemming, H.C., Busscher, H.J., and van der Mei, H.C. Influence of extracellular polymeric substances on deposition and redeposition of *Pseudomonas aeruginosa* to surfaces. Microbiology, 148: 1161-1169. 2002

Grasso, D., Subramaniam, K., Butkus, M., Strevett, K., and Bergendahl, J. A review of non-DLVO interactions in environmental colloidal systems. Reviews in Environmental Science and Biotechnology 1: 17-38. 2002.

Gregory, J. Particles in Water: Properties and processes. IWA Publishing, New York. 2006.

Griffin, J.I. Statistics: Methods and Applications. Holt, Rinehart and Winston, Inc., New York.pp. 1962.

Grolimund, D., Elimelech, M., Borkovec, M., Barmettler, K., Kretzschmar, R., and Sticher, H. Transport of in situ mobilized colloidal particles in packed soil columns. *Environ. Sci. Technol., 32* (22): 3562-3569. 1998.

Gupta, V., Johnson, W.P., Shafieian, P., Ryu, H., and Alum, A. Riverbank filtration: Comparison of pilot scale transport with theory. Environ. Sci. Technol., 43 (3): 669-676. 2009.

Habash, M. and Reid, G. Microbial biofilms; their development and significance for medical device-related infections. J. Clin. Pharmacol., 39 (9): 887-898. 1999.

Hall-Stoodley, L., Costerton, J.W., and Stoodley, P. Bacterial biofilms: from the Natural environment to infectious disease. Nature Reviews Microbiology, 2: 95-108. 2004.

Harden, V.P. and Harris, J.O. The isoelectric point of bacterial cells. Bacteriol., 65: 198-202. 1953.

Harvey, R.W. Parameters involved in modeling movement of groundwater. In: Hurst, C.J., E.d. Modeling the environmental fate of microorganisms. Washington, DC: American Society for Microbiology, 89-114. 1991.

Harvey, R.W. and Harms, H. Transport of microorganisms in the terrestrial subsurface: In situ and laboratory methods, In: Hust, C.J., Knudsen, G.R., McInerey, M.J., Stetzenback, L.D., Crawford, R.L., eds. Manual of Environmental Microbiology, 2nd Edition. Washington, ASM Press, pp. 753-776. 2002.

Harvey, R.W., Kinner, N.E., MacDonald, D., Metge, D.W., and Bunn, A. Role of physical heterogeneity in the interpretation of small-scale laboratory and field observations of bacteria, microbial-sized microsphere, and bromide transport through aquifer sediments. Water Resources Research, 29 (8): 2713-2721. 1993.

Havelaar, A.H., van Olphen, M., and Schijven, J.F. Removal and inactivation of viruses by drinking water treatment processes under full scale conditions. Wat. Sci. Techn., 31 (5-6): 55-62. 1995.

Haznedaroglu, B.Z., Kim, H.N., Bradford, S.A., and Walker, S.L. Relative transport behavior of *Escherichia coli* 0157:H7 and *Salmonella enteric* serovar pollorum in packed bed column systems: Influence of solution chemistry and cell concentration. Environ. Sci. Technol., 43 (6): 1838-1844. 2009.

Hirst, C.N. and Jordan, I.A. Distribution of exopolymeric substances in the littoral sediments of an oligotrophic lake. Microb. Ecol., 46: 22-32. 2003.

Hiscock, K.M., and Grischek, T. Attenuation of groundwater pollution by bank filtration. Journal of Hydrology, 266: 139-144. 2002.

Holme, R. Drinking water contamination in Walkerton, Ontario: positive resolution from a tragic event. Wat. Sci. Technol., 47 (3): 1-6. 2003.

Honisch, M., Hellmeier, C., and Weiss, K. Response of surface and subsurface water quality to land use changes. Geoderma, 105: 277-298. 2002.

Ibekew. A M. and Lyon, S.R. Microbiological evaluation of fecal bacterial composition from surface water through aquifer sand material. J. of Water and Health, 6 (3): 411-421. 2008.

IBM Corp. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, IBM Corp. 2011.

Ing, M.J. European Perspective: Bulk and trace organics removal in underground treatment systems. Proceedings of the AWWA Water Quality and Technology Conference, Toronto, Ontario, Canada. 2012.

Jahn, A., and Nielsen, P.H. Extraction of extracellular polymeric substances (EPS) from biofilms using a cation exchange resin. Wat. Sci. Technol., 32 (8): 157-164. 1995.

Jamieson, R.C., Gordon, R.J., Shrples, K.E., Stratton, G.W., and Madani, A. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. Canadian Biosystems Engineering, 44: 1.1-1.9. 2002.

Jewett, D.G., Hilbert, T.A., Logan, B.E., Arnold, R.G., and Bales, R. Bacterial transport in laboratory columns and filters: Influence of ionic strength and pH on collision efficiency. Wat. Res., 29 (7): 1673-1680. 1995.

Johnson, W.P., Li, X., Assemi, S. Deposition and re-entrainment dynamics of microbes and non-biological colloids during non-perturbed transport in porous media in the presence of an energy barrier to deposition. Adv. Water Res., 30 (6-7): 1432-1454. 2007.

Johnson, W.P. and Logan, B.E. Enhanced transport of bacteria in porous media by sediment-phase and aqueous-phase organic matter. Water Research 30, 923-931. 1996.

Jucker, B.A., Harms, H., Hug, S.J., and Zehnder, A.J.B. Adsorption of bacterial surface polysaccharides on mineral oxides is mediated by hydrogen bonds. Colloids and Surfaces B: Bointerfaces, 9: 331-343. 1997.

Jucker, B.A., Zehnder, A.J.B., and Harms, H. Quantification of polymer interactions in bacterial adhesion. Environ. Sci. Technol., 32: 2909-2915. 1998.

Karapanagiotis, N.K., Rudd, T., Sterritt, R.M., and Lester, J.N. Extraction and characterization of extracellular polymers in digested sewage sludge. J. Chem. Tech. Biotechnol., 44: 107-120. 1989.

Kiff, R.J. and Thompson, R. Physical methods for the extraction of bacterial exopolymers from activated sludge biomass. Biotechnology Letters, 1 (4): 183-186. 1979.

Kim, H.N, Bradford, S.A., and Walker, S.L. *Echerichia coli* O157:H7 transport in saturated porous media: Role of solution chemistry and surface macromolecules. Environ. Sci. Technol, 34: 4340-4347. 2009.

Kim, H.N., Walker, S.L., and Bradford, S.A. Macromolecule mediated transport and retention of *Escherichia coli O157: H7* in saturated porous media. Water Research, 44: 1082-1093. 2010.

Kim, J., Nason, J.A., and Lawler, D.F. Influence of surface charge distributions and particle size distributions on particle attachment in granular media filtration. Environ. Sci. Technol., 42: 2557-2562. 2008.

Kim, S.B., Park, S.J., Lee, C.G., Choi, N.C., and Kim, D.J. Bacteria transport through goethite-coated sand: Effects of solution pH and coated sand content. Colloids and Surfaces B: Biointerfaces, 63: 236-242. 2008.

Klock, J.H., Wieland, A., Seifert, R., and Michaelis, W. Extracellular polymeric substances (EPS) from cyanobacterial mats: characterization and isolation method optimization. Mar. Bio., 152: 1077-1085. 2007.

Knappett, P.S.K., Emelko, M.B., Zhuang, J., and McKay, L.D. Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: Effects of ionic strength and grain size. Water Research, 42: 4368-4378. 2008.

Knox, J.H. and Parcher, J.F. Effect of column to particle diameter ratio on the dispersion of unsorbed solutes in chromatography. Analytical Chemistry*, 41* (12): 1599-1606. 1969.

Kuehn, W. and Mueller, U. Riverbank Filtarion: An overview. J. AWWA, 92 (12): 60-69. 2000.

Lang, J.S., Giron, J.J., Hansen, A.T., Trussel, R.R., and Hodges, W.E.Jr. Investigating filter performance as a function of the ratio of filter size to media size. J. AWWA, 85 (10): 122-130. 1992.

LeChevallier, M.W., Cawthon, C.D., and Lee, R.G. Factors promoting survival of bacteria in chlorinated water supplies. Applied and Environmental Microbiology, 54 (3): 649-654. 1988.

LeChevallier, M.W., Seidler, R.J., and Evans, T.M. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Applied and Environmental Microbiology, 40 (5): 922-930. 1980.

Lemarchand, K. and Lebaron, P. Occurrence of *Salmonella* spp. and *Cryptosporidium* spp. In a French coastal watershed: relationship with fecal indicators. FEMS Microbiology Letters, 218: 203-209. 2003.

Li, X,.Lin C.L., Miller, J., and Johnson, W.P. Pore-scale observation of microsphere deposition at grain-grain contacts over assemblage-scale porous media domains using X-ray microtomography. Environ. Sci. Technol., 40 (12) 3762-3768. 2006.

Li, X., Scheibe, T.D., and Johnson, W.P. Apparent decreases in colloid deposition rate coefficients with distance of transport under unfavorable deposition conditions: a general phenomenon. American Chemical Society, 38 (21): 5616-5625. 2004.

Li, X., Zhang, P., Lin, C.L., and Johnson, W.P. Role of hydrodynamic drag on microsphere deposition and re-entrainment in porous media under unfavorable conditions. Environ. Sci. Technol., 39: 4012-4020. 2005.

Liu, H. and Fang, H.P. Extraction of extracellular polymeric substances (EPS) of sludges. Journal of Biotechnology, 95: 249-256. 2002.

Liss, S.N., Droppo, I.G., Flannigan, D.T., and Leppard, G.G. Floc architecture in wastewater and natural riverine systems. Environ. Sci. Technol., 30: 680-686. 1996.

Litton, G.M. and Olson, T.M. 1993. Colloid deposition rates on silica bed media and artifacts related to collector surface preparation methods. Environ. Sci. Technol., 27: 185-193.

Liu, Y. and Li, J. Role of *Pseudomonas aeruginosa* biofilm in the initial adhesion, Growth and detachment of Escherichia coli in porous media. Environ. Sci. Technol., 42: 443-449. 2008.

Liu, Y., Yang, C.H., and Li, J. Influence of extracellular polymeric substances on *Pseudomonas aeruginosa* transport and deposition profiles in porous media. Environ. Sci. technol., 41: 198-205. 2007.

Lowry, O.H., Rosebrough, N.J., Farr, A.L, and Randall, R.J. Protein measurement with the folin phenol reagent. J. Bio. Chem, 193: 265-275. 1951.

Lute, S., Aranha, H., Tremblay, D., Liang, D., Ackermann, HW., Chu, B., Moineau, S., and Brorson, K. Characterization of coliphage PR772 and evaluation of its use for virus filter performance testing. Applied and Environmental Microbiology 70, (8): 4864-4871. 2004.

Macler, B.A. and Merkle, J.C. Current knowledge on groundwater microbial pathogens and their control. Hydrogeol. J. 8: 29-40. 2000.

Madigan, M.T., Martinko, J.M., and Parker, J. Brock biology of microorganisms, 10th Ed. Pearson Education, Inc., Upper Saddle River, NJ. 2003.

Matthness, G. and Pekdeger, A. Survival and transport of pathogenic bacteria and viruses in ground water. Ground Water Wuality, John Wiley and Sons, New York, NY. pp. 472-482. 1985.

May, T.B. and Chakrabarty, A.M. *Pseudomonas aeruginosa*: genes and enzymes of alginate synthesis. Trends in Microbiology, 2 (5): 151-157. 1994.

Mehta, D. and Hawley, M.C. Wall effects in packed columns. I and EC Process Design and Development, 8 (2): 280-282. 1969.

Mesquita, M.M.F., Stimson, J., Chae, G.T., Tufenkji, N., Ptacek, C.J., Blowes, D.W., and Emelko, M.B. Optimal preparation and purification of PRD1-like bacteriophages for use in environmental fate and transport studies. Water Research, 44: 1114-1125. 2010.

Metcalf and Eddy, Inc. Wastewater Engineering: Treatment, Disposal and Reuse, 3rd Edition, McGraw-Hill, New York. 1991.

Metge, D.W., Harvey, R.W., Anders, R., Rosenberry, D.O., Seymour, D., and Jasperse, J. Use of carboxylated microspheres to assess transport potential of *Cryptosporidium parvum* oocysts at the Russian River Water Supply Facility, Sonoma County, California. Geomicrobiology Journal, 24 (3-4): 231-245. 2007.

Metge, D.W., Harvey, R.W., Aiken, G.R., Anders, R., Lincoln, G., Jasperse, and Hill, M.C. Effects of sediment-associated extractable metals, degree of sediment grain sorting, and dissolved organic carbon upon *Cryptosporidium parvum* removal and transport within riverbank filtration sediments, Sonoma County, California. 2010.

Mills, A.L., Herman, J.S., Hornberger, G.M., and DeJesus, T.H. Effect of solution ionic strength and iron coatings on mineral grains on the sorption of bacterial cells to quartz sand. Applied and Environmental Microbiology, 60 (9): 3300-3306. 1994.

Monteith, D.T., et al Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature Letters, 450 (22): 537-541. 2007.

Moore, T.R., Matos, L., and Roulet, N.T. Dynamics and chemistry of dissolved organic carbon in Precambrian Shield catchments and an impounded wetland. Can. J. Fish. Aquat. Sci., 60: 612-623. 2003.

Morgan, J.W., Forster, C.F., and Evison, L.M. A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. Water Res., 6: 743-750. 1990.

Morrow, J.B., Stratton, R., Yang, H.H., Smets, B.F., and Grasso, D. Macro- and nanoscale observations of adhesive behaviour for several *E. coli* strains (O157: H7 and environmental isolates) on mineral surfaces. Environ. Sci. Technol., 39: 6395-6404. 2005.

Murphy, E.M. and Ginn, T.R. Modeling microbial processes in porous media. Hydrogeology Journal, 8: 142-158. 2000.

Neubauer, C.M., Yang, M., and Jennings, H.M. Interparticle potential and sedimentation behavior of cement suspensions: Effects of admixtures. Advn. Cem. Bas. Mat., 8: 17-27. 1998.

Nishikawa, S. and Kuriyama, M. Nucleic acid as a component of mucilage in activated sludge. Water Res., 2: 811-812. 1968.

Nissinen, T.K., Miettinen, I.t., Martikainen, P.J., and Vartiainen, T. Molecular size distribution of natural organic matter in raw and drinking water. Chemosphere, 45: 865-873. 2001.

Omoike, A. and Chorover, J. Spectroscopic study of extracellular polymeric substances from *Bacillus subtilis*: Aqueous chemistry and adsorption effects. Biomacromolecules, 5: 1219-1230. 2004.

Pan, A., Liu, J., Zhang, D., Chen, X., Li, L., Song, W., and Yang, J. A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three-dimensional excitation-emission matrix fluorescence spectroscopy. Water SA, 36 (1): 111-116. 2010.

Passmore, J.M., Rudolph, D.L., Mesquita, M.M.F., Cey, E.E., and Emelko, M.B. The utility of microspheres as surrogates for the transport of *E. coli* RS2g in partially saturated agricultural soil. Water Research 44, (4): 1235-1245. 2010.

Pavoni, J.L., Tenney, M.W. and Echelberger, W.F. Bacterial exocellular polymers and biological flocculation. J. Water Pollut. Control Fed., 44: 414-431. 1972.

Public Health Agency of Canada (PHAC). C-EnterNet 2006 Annual Report: National Integrated Enteric Pathogen Surveillance Program. Retrieved July 9th, 2009 from http://www.phac-aspc.gc.ca/c-enternet/index.html. 2007.

Pieper, A.P., Ryan, J.N., Harvey, R.W., Amy, G.L., Illangasekare, T.H., and Metge, D.W. Transport and recovery of bacteriophage PRD1 in a sand and gravel aquifer: Effect of sewage-derived organic matter. Environ. Sci. Technol., 31: 1163-1170. 1997.

Prince, J.L. and Dickinson, R.B. Kinetics and forces of adhesion for a pair of capsular/unenxapsulated *Staphylococcus* mutant strains. Langmuir, 19: 154-159. 2003.

Quintero , E.J. and Weiner, R.M. Evidence for the adhesive function of the exopolysaccharide of *Hyphomonas* strain MHS-3 in its attachment to surfaces. Applied and Environmental Microbiology, 61 (5): 1897-1903. 1995.

Ray, C. Worldwide potential of riverbank filtration. Clean Techn. Environ. Policy, 10: 223-225. 2008.

Ray, C., Melin, G., and Linsky, R.B. Riverbank Filtration: Improving Source-Water Quality. Springer, Netherlands, 50-59. 2003.

Redman, J.A., Walker, S.L., and Elimelech, M. Bacterial adhesion and transport in porous media: Role of the secondary energy minimum. Environ. Sci. Technol., 38: 1777-1785. 2004.

Rijnaarts, H.H.M., Norde, W., Lyklema, J., and Zehnder, A.J.B. DLVO and steric contributions to bacterial deposition in media of different ionic strengths. Colloids and Surfaces B: Biointerfaces, 14: 179-195. 1999.

Rudd, T, Sterritt, R.M., and Lester, J.N. Extraction of extracellular polymers from activated sludge. Biotechnology Letters, 5 (5): 327-332. 1983.

Ryan, J.N. and Elimelech, M. Review: Colloid mobilization and transport in groundwater. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 107: 1-56. 1996.

Ryan, J.N. and Gschwend, P.M. Effect of iron diagenesis on the transport of colloidal clay in an unconfined sand aquifer. Geochemica et Cosmochemica Acta, 56: 1507-1521. 1992.

Sachse, A., Henrion, R., Gelbrecht, J., and Steinberg, C.E.W. Classification of dissolved organic carbon (DOC) in river systems: Influence of catchment characteristics and autochthonous processes. Organic Geochemistry 36: 923-935. 2005.

Sadeghi, G., Schijven, J.F., Behrends, T., Hassanizadeh, S.M., Gerritse, J., and Kleingeld, P.J. Systematic study of effects of pH and ionic strength on attachment of phage PRD1. Ground Water, 49 (1): 12-19. 2011.

Saiers, J.E., and Ryan, J.N. Colloid deposition on non-ideal porous media: The influence of collector shape and roughness on the single-collector efficiency. Geophys. Res. Lett., 32: L21406. 2005.

Scanlon, B.R., Reedy, R.C., Stonestrom, D.A., Pridic, D.E., and Dennehy, K.F. Impact of land use and land cover change on groundwater recharge and quality in the southwestern US. Global Change Biology, 11: 1577-1593. 2005.

Schijven, J.F., Berger, P., and Miettinen, I. Removal of pathogens, surrogates, indicators, and toxins using riverbank filtration. In Riverbank filtration: Improving source water quality. Eds. C. Ray, G. Melin, and R.B. Linsky, Dordrecht: Kluwer Academic Publishers, pp 73-116. 2002.

Schijven, J.F. and Hassanizadeh, S.M. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. Critical reviews in Environmental Science and Technology, 30 (1): 49-127. 2000.

Schijven, J.F., de Bruin, H.A.M., Hassanizadeh, S.M., and de Roda Husman, A. M. Bacteriophages and clostridium spores as indicator organisms for removal of pathogens by passage through saturated dune sand. Water Research, 37: 2186-2194. 2003.

Scholl, M.A. and Harvey, R.W. Laboratory investigations on the ole of sediment surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer. Environ. Sci. Technol., 26 (7): 1410-1417. 1992.

Schwartz, C.E. and Smith, J.M. Flow distribution in packed beds. *Industrial and Engineering Chemistry, 45* (6): 1209-1218. 1953.

Schwartz, T., Hoffmann, S. and Obst, U. Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. Wat. Res., 32 (9): 2787-2797. 1998.

Sen, T.K. Processes in pathogenic biocolloidal contaminants transport in saturated and unsaturated porous media: A review. Water Air Soil Pollut., 216: 239-256. 2011.

Shankararaman, C. and Wiesner, M.R. Slip flow through porous media with permeable boundaries: Implications for the dimensional scaling of packed beds. Water Environment Research, 65 (6): 744-749. 1993.

Shellenberger, K. and Logan, B.E. Effect of molecular scale roughness of glass beads on colloidal and bacterial deposition. Environ. Sci. Technol., 36: 184-189. 2002.

Sheng, G.P., Yu, H.Q., and Li, X.Y. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. Biotechnology Advances, 28: 882-894. 2010.

Silliman, S.E. Particle transport through two-dimensional, saturated porous media: influence of physical structure of the medium. Journal of Hydrology, 167: 79-98. 1995.

Stevik, T.K., Aa, K, Ausland, G., and Hanssen, J.F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research, 38, 1355-1367. 2004.

Stimson, J., Chae, G., Ptacek, C.J., Emelko, M.B., Mesquita, M.M., Hirata, R.A., and Blowes, D.W. Basic oxygen furnace slag as a treatment material for pathogens: Contribution of inactivation and attachment in virus attenuation. Water Research, 44 (4): 1150-1157. 2010.

Stinton, L.W., Braithwaite, R.R., Hall, C.H., Pang, L., Close, M.E., and Noonan, M.J. Tracing the movement of irrigated effluent into an alluvial gravel aquifer. Water, Air, and Soil Pollution, 166: 287-301. 2005.

Tabachnick, B.G and Fidell, L.S. Using multivariate statistics (2nd Ed.). Boston: Pearson.2007.

Takahashi, E., Ledauphin, J., Goux, D., and Orvain, F. Optimising extraction of extracellular polymeric substances (EPS) from benthic diatoms; comparison of the efficiency of six EPS extraction methods. Marine and Freshwater Research, 60: 1201-1210. 2009.

Tchobanoglous, G., and Schroeder, E.D. Water Quality Characteristics, Modeling, modification. Addison- Wesley Publishing Co., Reading, Massachusetts. 1985.

Tezuka, Y. A zoologea bacterium with gelatinous mucopolysaccharide matrix. J. Water Pollut. Control Fed., 45: 531-536. 1973.

Theng, B.K.G. Interactions between montmorillonite and fulvic acid. Geoderma, 15: 243-251. 1976.

Thurman, E. M. Developments in biochemistry: Organic geochemistry of natural waters. Springer Science and Business. Pp. 5-10. 1985.

Tobiason, J.E. and Vaigneswaran, B. Evaluation of a modified model for deep bed filtration. *Water Research, 28* (2): 335-342. 1994.

Tobiason, J.E. Chemical effects on the deposition of non-Brownian particles. Colloids and Surfaces, 39: 53-77. 1989.

Tong, M., Ma, H., and Johnson, W.P. Funneling of flow into grain-to-grain contacts drives colloid-colloid aggregation in the presence of an energy barrier. Environ. Sci. Technol., 42: 2826-2832. 2008.

Tong, M., Long, G., Jiang, X., and Kim, H.N. Contribution of extracellular polymeric substances on representative Gram negative and Gram positive bacterial deposition in porous media. Environ. Sci. Technol., 44: 2393-2399. 2010.

Tong, M., Zhu, P., Jiang, X., and Kim, Hyunjung. Influence of natural organic matter on the deposition kinetics of extracellular polymeric substances (EPS) on silica. Colloids and Surfaces B: Biointerfaces, 87: 151-158. 2011.

Toran, L. and Palumbo, A.V. Colloid transport through fractured and unfractured laboratory sand columns. Journal of Contaminant Hydrology, 9: 289-303. 1992.

Tsuneda, S., Aikawa, H., Hayashi, H., and Hirata, A. Significance of cell electrokinetic properties determined by soft-particle analysis in bacterial adhesion onto a solid surface. Journal of Colloid and Interface Science, 279: 410-417. 2004.

Tufenkji, N. and Elimelech, M. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. Environ. Sci. Technol., 38: 529-536. 2004.

Tufenkji, N. Modeling microbial transport in porous media: Traditional approaches and recent developments. Advances in Water Resources, 20: 1455-1469. 2007.

Tufenkji, N., Miller, G.F., Ryan, J.N., Harvey, R.W., and Elimelech, M. Transport of *Cryptosporidium* oocysts in porous media: Role of straining and physicochemical filtration. Environ. Sci. Technol., 38: 5932-5938. 2004.

Tufenkji, N., Redman, J.A., and Elimelech, M. Interpreting deposition patterns of microbial particles in laboratory-scale column experiments. Environ. Sci. Technol., 37: 616-623. 2003.

Tufenkji, N., Ryan, J.N., and Elimelech, M. The promise of riverbank filtration. Environ. Sci. Technol., 36: A422-A428. 2002.

Unc, A. and Gross, M.J. Transport of bacteria from manure and protection of water resources. Applied Soil Ecology, 25: 1-18. 2004.

Underwood, G.J.C., Patterson, D.M., and Parkes, R.J. The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnol. Oceanogr., 40 (70): 1243-1253. 1995.

Vandevivere, P. and Kirchman, D.L. Attachment stimulates exopolysaccharide synthesis by a bacterium. Applied and environmental microbiology, 59 (10): 3280-3286. 1993.

Verwey, E.J. and Overbeek, J.Th.G. Theory of the stability of lyophobic colloids: The interaction of soil particles having an electric double layer. Elsevier, Amsterdam. 1948.

Vu, B., Chen, M., Crawford, R.J., and Ivanova, E.P. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules, 14: 2535-2554. 2009.

Wagai, R., Mayer, L.M., and Kitayama, K. Extent and nature of organic coverage of soil mineral surfaces assessed by a gas sorption approach. Geoderma, 149: 152-160. 2009.

Wagner, M., Ivleva, N.P., Haisch, C., Niessner, R., and Horn, H. Combined use of confocal laser scanning microscopy (CLSM) and Raman microscopy (RM): Investigations on EPS – Matrix. Water Research, 43: 63-76. 2009.

Walker, L.W., Redman, J.A., and Elimelech, M. Role of cell surface lipopolysaccharides in *Escherichia coli* K12 adhesion and transport. Langmuir, 20: 7736-7746. 2004.

Walker, S.L., Hill, J.E., Redman, J.A., and Elimelech, M. Influence of growth phase on adhesion kinetics of *Escherichia coli* D21g. Applied and Environmental Microbiology, 71 (6): 3093-3099. 2005.

Water Pollution Research Laboratory (WPRL). Report of the Director. Her Majesty's Stationery Office, London. 1971.

Weiss, W.J. et al Riverbank filtration for control of microorgnaisms: Results from field monitoring. Water Research, 39: 1990-2001. 2005.

Wijesinghe, R.U., Feng, Y., Wood, C.W., Stoeckel, D.M., and Shaw, J.N. Population dynamics and genetic variability of *Escherichia coli* in a mixed land-use watershed. J. Water and Health, 7 (3):484-496. 2009.

Wilkes, G. et al Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. Water Research, 43 (8): 2209-2223. 2009.

Wingender, J. and Flemming, H.C. Biofilms in drinking water and their role as reservoir for pathogens. International Journal of Hygiene and Environmental Health, 214 (6): 417-423. 2011.

Wingender, J., Neu, T.R., and Flemming, H.C. Microbial extracellular polymeric substances: Characterization, Structure and Function. Springer: New York. Pp. 85-239. 1999.

Wuertz, S., Spaeth, R., Hinderberger, A., Griebe, T., Flemming, H.C., and Wilderer, P.A. A new method for extraction of extracellular polymeric substances from biofilms and activated sludge suitable for direct quantification of sorbed metals. Water Sci. Technol., 43: 25-31. 2001.

Yang, H.H., Vinopal, R.T., Grasso, D., and Smets, B.F. High diversity among environmental *Escherichia coli* isolates from a bovine feedlot. Applied and Environmental Microbiology, 70 (3): 1528-1536. 2004.

Yang, X., Flynn, R., von der Kammer, F., and Hofmann, T. Quantifying the influence of humic acid adsorption on colloidal microsphere deposition onto iron-oxide-coated sand. Environmental Pollution, 158: 3498-3506. 2010.

Yao, K.M., Habibian, M.T., and O'Melia, C.R. Water and Waste Water Filtration: Concepts and Applications. Environ. Sci. Technol., 5 (11): 1105-1112. 1971.

Yee, N., Fein, J.B., and Daughney, C.J. Experimental study of the pH, ionic strength, and reversibility behavior of bacteria-mineral adsorption. Geochemica et Cosmochimica Acta, 64 (4): 609-617. 2000.

Zablotowicz, R.M., Locke, M.A., Hoagland, R.E., Knight, S.S., and Cash, B. Fluorescent *Pseudomonas* isolates from Mississippi Delta Oxbow Lakes: In vitro herbicide Biotransformations. Environmental Toxicology, 16 (1): 9-19. 2001.

Zhang, X., Bishop, P.L., and Kinkle, B.K. Comparison of extraction methods for quantifying extracellular polymers in biofilms. Wat. Sci. Technol., 39 (7); 211-218. 1999.

Zolghadr., B., Klingl, A., Koerdt, A., Driessen, A.J.M., Rachel, R., and Albers, S.V. Appendage-mediated surface adherence of *Solfolobus solfataricus*. J. Bacteriology, 192 (1): 104-110. 2010.

Appendix A – Materials & Methods

Table A. 1. Media Grain Size Distribution Analysis - Raw Data

Experiment	Code	Sieve #	Sieve Size (mm)	Weight Retained (g)	% Retained	Cumulative % Retained	Cumulative % Passing
		8	2.38	2.25	0.02	2.26	97.74
		10	2.00	16.84	0.17	19.16	80.84
		12	1.68	26.92	0.27	46.17	53.83
		14	1.40	27.72	0.28	73.99	26.01
		16	1.19	11.90	0.12	85.93	14.07
1	**Lg**	18	1.00	7.78	0.08	93.74	6.26
		20	0.85	2.99	0.03	96.74	3.26
		25	0.71	1.40	0.01	98.14	1.86
		pan		1.85	0.02	100.00	0.00
		Total:		99.65			
		Initial dried weight:		99.99			
		16	1.19	7.53	0.08	7.53	92.47
		18	1.00	6.84	0.07	14.37	85.63
		20	0.85	8.15	0.08	22.52	77.48
		25	0.71	13.34	0.13	35.86	64.14
		30	0.60	18.07	0.18	53.93	46.07
1 & 2	**+/-**	40	0.43	34.59	0.35	88.52	11.48
		50	0.30	9.21	0.09	97.73	2.27
		70	0.21	1.98	0.02	99.71	0.29
		pan		0.29	0.00	100.00	0.00
		Total:		100.00			
		Initial dried weight:		100.05			
		12	1.68	0.56	0.01	0.56	99.44
		14	1.40	4.32	0.04	4.85	95.15
		16	1.19	8.93	0.09	13.71	86.29
		20	0.85	42.62	0.42	56.03	43.97
		25	0.71	17.42	0.17	73.32	26.68
2 & 3	**+/+**	30	0.60	10.73	0.11	83.98	16.02
		40	0.43	10.81	0.11	94.71	5.29
		50	0.30	4.15	0.04	98.83	1.17
		pan		1.18	0.01	100.00	0.00
		Total:		100.72			
		Initial dried weight:		100.03			

		40	0.43	0.27	0.00	0.18	99.82
		50	0.30	16.24	0.11	11.06	88.94
		60	0.25	24.55	0.16	27.51	72.49
		70	0.21	47.55	0.32	59.38	40.62
		80	0.18	13.84	0.09	68.65	31.35
		100	0.15	29.16	0.20	88.19	11.81
2	**-/-**	170	0.09	16.55	0.11	99.28	0.72
		200	0.07	0.64	0.00	99.71	0.29
		230	0.06	0.27	0.00	99.89	0.11
		pan		0.16	0.00	100.00	0.00
		Total:		149.23			
		Initial dried weight:		149.80			
		40	0.43	1.23	0.01	0.82	99.18
		50	0.30	15.10	0.10	10.90	89.10
		60	0.25	22.44	0.15	25.89	74.11
		70	0.21	35.54	0.24	49.62	50.38
		80	0.18	11.88	0.08	57.56	42.44
		100	0.15	27.68	0.18	76.04	23.96
2	**-/+**	170	0.09	28.30	0.19	94.94	5.06
		200	0.07	3.50	0.02	97.28	2.72
		230	0.06	2.24	0.01	98.77	1.23
		pan		1.84	0.01	100.00	0.00
		Total:		149.75			
		Initial dried weight:		148.90			

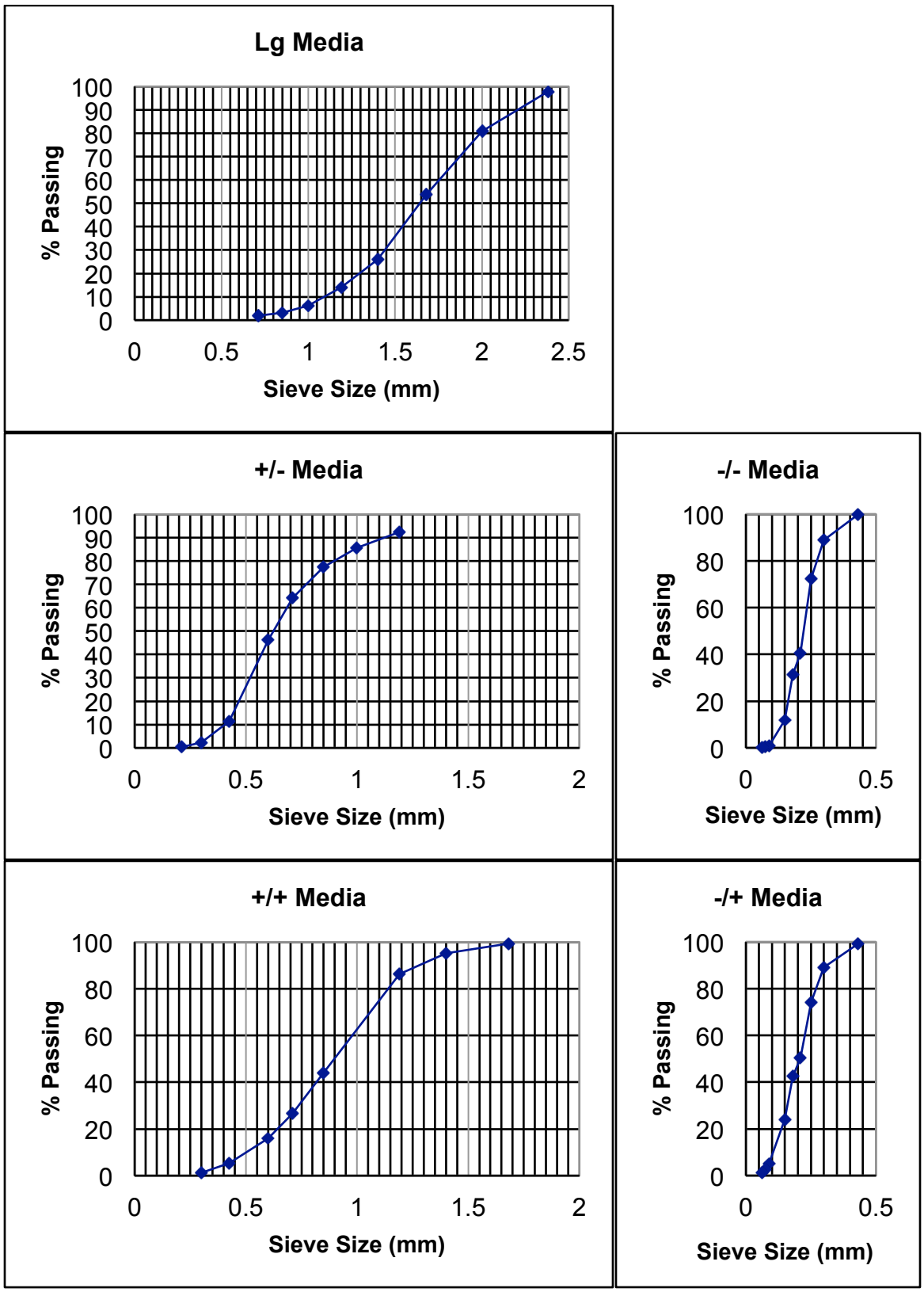


Figure A.1. Grain Size distribution plots for all media used in this study

Table A. 2. Flow Rate Conversions for Experiments 1, 2 & 3

Experiment 1 (Trials a and b), 2 and 3		
Column X (small)		
Pump Rate (v)	1.2	mL/min
Influent Flow Rate (Q)	0.00173	m^3/day
Column Diameter (Ø)	16	mm
Surface Area of Column	0.0002	m^2
Linear Velocity	8.60	m/day
GW Velocity	22.0	m/day
Loading Rate	0.919	m/h
Experiment 1 (Trial c)		
Column X (small)		
Pump Rate (v)	6.5	mL/min
Influent Flow Rate (Q)	0.00936	m^3/day
Column Diameter (Ø)	16	mm
Surface Area of Column	0.0002	m^2
Linear Velocity	46.6	m/day
GW Velocity	119	m/day
Loading Rate	4.98	m/h
Experiment 1 (Trials a and b)		
Column Y (large)		
Pump Rate (v)	11.5	mL/min
Influent Flow Rate (Q)	0.01656	m^3/day
Column Diameter (Ø)	50	mm
Surface Area of Column	0.00196	m^2
Linear Velocity	8.44	m/day
GW Velocity	21.6	m/day
Loading Rate	0.902	m/h
Experiment 1 (Trial c)		
Column Y (large)		
Pump Rate (v)	65	mL/min
Influent Flow Rate (Q)	0.0936	m^3/day
Column Diameter (Ø)	50	mm
Surface Area of Column	0.00196	m^2
Linear Velocity	47.6	m/day
GW Velocity	122	m/day
Loading Rate	5.10	m/h

1000	mm in a m
1000	mL in a L
1000	L in a m^3
0.39	~media porosity

Table A. 3. Pore Volume (PV) Calculations for Experiments 1, 2 & 3

Experiment 1 (Trials a and b), 2 and 3		
Column X (small)		
Column Diameter ()	16	mm
Column Length (L)	150	mm
Volume in Column	3.01E-05	m^3
~Volume in Tubing	3.01E-06	m^3
Total Volume	3.32E-05	m^3
~Porosity	0.4	
PV (L)	0.0133	L
PV (mL)	13.3	mL
Flow Rate	1.2	mL/min
Time to center of advective flux	11.1	min

1000	mm in a m
1000	L in a m^3
1000	L in a mL

Experiment 1 (Trial c)		
Column X (small)		
Column Diameter ()	16	mm
Column Length (L)	150	mm
Volume in Column	3.01E-05	m^3
~Volume in Tubing	3.01E-06	m^3
Total Volume	3.32E-05	m^3
~Porosity	0.4	
PV (L)	0.0133	L
PV (mL)	13.3	mL
Flow Rate	6.5	mL/min
Time to center of advective flux	2.04	min

Experiment 1 (Trials a and b)		
Column Y (large)		
Column Diameter ()	50	mm
Column Length (L)	150	mm
Volume in Column	2.94E-04	m^3
~Volume in Tubing	1.92E-05	m^3
Total Volume	3.14E-04	m^3
~Porosity	0.4	
PV (L)	0.125	L
PV (mL)	125	mL
Flow Rate	11.5	mL/min
Time to center of advective flux	10.9	min

Experiment 1 (Trial c)		
Column Y (large)		
Column Diameter ()	50	mm
Column Length (L)	150	mm
Volume in Column	2.94E-04	m^3
~Volume in Tubing	1.92E-05	m^3
Total Volume	3.14E-04	m^3
~Porosity	0.4	
PV (L)	0.125	L
PV (mL)	125	mL
Flow Rate	65	mL/min
Time to center of advective flux	1.93	min

Appendix B - Experiment 1

Table B. 1. Bromide Breakthrough Curve Raw Data for Both Flow Rates in Experiment 1

Time	Column X		Column Y		Feed
(min)	Conductivity	C/C_o	Conductivity	C/C_o	Conductivity
			Flow Rate: 5 m/h		
0					350
0					348
0					352
Mean					**350**
0.3	32	0.09	29	0.08	
0.5	67	0.19	39	0.11	
0.8	48	0.14	50	0.14	
1.0	100	0.28	74	0.21	
1.3	108	0.31	114	0.32	
1.5	187	0.53	162	0.46	
1.8	261	0.75	202	0.58	
2.0	277	0.79	258	0.74	
2.3	283	0.81	270	0.77	
2.5	280	0.80	275	0.79	
2.8	284	0.81	286	0.82	
3.0	286	0.82	288	0.82	
3.3	282	0.81	288	0.82	
3.5	285	0.81	290	0.83	
3.8	284	0.81	287	0.82	
4.0	282	0.81	282	0.81	
4.3	286	0.82	290	0.83	
4.5	284	0.81	291	0.83	
4.8	284	0.81	297	0.85	
5.0	283	0.81	291	0.83	
			Flow Rate: 1 m/h		
0					263
0					271
0					272
Mean					**269**
1	4	0.01	3.4	0.01	
2	3.4	0.01	3.5	0.01	
3	4.9	0.02	3.1	0.01	
4	3.7	0.01	2.6	0.01	
5	4	0.01	2.9	0.01	
6	5.2	0.02	2.6	0.01	

7	4.5	0.02	2.8	0.01
8	6.6	0.02	6.5	0.02
9	19.5	0.07	19.7	0.07
10	54	0.20	53.2	0.20
11	111.2	0.41	103.1	0.38
12	173.5	0.65	167.7	0.62
13	266	0.99	222	0.83
14	299	1.11	226	0.84
15	318	1.18	318	1.18
16	335	1.25	330	1.23
17	330	1.23	339	1.26
18	344	1.28	324	1.21
19	343	1.28	345	1.28
20	338	1.26	349	1.30
21	349	1.30	349	1.30
22	347	1.29	349	1.30
23	344	1.28		0.00
24	346	1.29	348	1.30
25			349	1.30
26	346	1.29	355	1.32
27	365	1.36	354	1.32
28	344	1.28	356	1.33
29	349	1.30	353	1.31
30	345	1.28	356	1.33

Table B 3. PR772 and *E. coli* Breakthrough Curve Raw Data – Trial (a) in Experiment 1

Time (min)	PR772 Phage				*E. coli* RS2g			
	Column X		Column Y		Column X		Column Y	
	PFU/mL	C/C_o	PFU/mL	C/C_o	CFU/mL	C/C_o	CFU/mL	C/C_o
Feed	3.61E+04		3.61E+04		5.80E+04		5.80E+04	
2	5.00E+00	1.39E-04	5.00E+00	1.39E-04	1.00E+01	1.72E-04	1.00E+01	1.72E-04
4	5.00E+00	1.39E-04			1.00E+01	1.72E-04	1.00E+01	1.72E-04
6	5.00E+00	1.39E-04	5.00E+00	1.39E-04				
8	5.00E+00	1.39E-04	5.00E+00	1.39E-04	1.00E+01	1.72E-04	1.00E+01	1.72E-04
10	5.00E+01	1.39E-03	5.00E+00	1.39E-04	1.90E+02	3.28E-03	6.00E+01	1.03E-03
12	2.05E+03	5.89E-02	6.50E+02	1.80E-02	5.80E+03	1.13E-01	2.78E+03	4.79E-02
14	1.04E+04	2.83E-01	6.80E+03	1.87E-01	4.20E+04	6.98E-01	2.43E+04	4.16E-01
16	2.35E+04	6.02E-01	5.30E+04	1.33E+00	7.40E+04	1.39E+00	9.70E+04	1.59E+00
18	2.15E+04	6.37E-01	5.10E+04	1.16E+00	8.90E+04	1.58E+00	7.30E+04	1.46E+00
20	2.90E+04	8.45E-01	5.60E+04	1.30E+00	8.70E+04	1.21E+00	1.20E+05	1.98E+00
30	3.55E+04	1.37E+00	7.35E+04	1.77E+00	9.40E+04	1.78E+00	1.18E+05	1.90E+00
40	5.05E+04	1.53E+00	4.75E+04	1.25E+00	1.23E+05	2.34E+00	1.13E+05	1.80E+00
50	6.60E+04	1.67E+00	4.55E+04	1.28E+00	1.22E+05	2.09E+00	1.03E+05	1.66E+00

Table B. 4. 1.0 µm and 4.5 µm Sphere Breakthrough Curve Raw Data – Trial (a) in Experiment 1

Time (min)	1.0 µm Spheres				4.5 µm Spheres			
	Column X		Column Y		Column X		Column Y	
	MS/mL	C/C_o	MS/mL	C/C_o	MS/mL	C/C_o	MS/mL	C/C_o
Feed	2.50E+04		2.54E+04		5.78E+03		5.78E+03	
2	1.15E+01	4.60E-04	5.00E-01	2.00E-05	1.00E+00	1.73E-04	1.00E+00	1.73E-04
4	1.25E+01	5.00E-04			5.00E-01	8.65E-05		
6							1.00E+00	1.73E-04
8	1.00E+00	4.00E-05	5.00E-01	2.00E-05	1.00E+00	1.73E-04	1.00E+00	1.73E-04
10	1.14E+02	4.56E-03			4.60E+01	7.96E-03	3.00E+00	5.19E-04
12	1.88E+03	6.76E-02	2.90E+02	1.62E-02	6.90E+02	9.69E-02	7.65E+01	1.32E-02
14	5.16E+03	1.65E-01	3.43E+03	1.69E-01	1.21E+03	1.92E-01	3.40E+02	9.82E-02
16	2.42E+04	8.32E-01	2.69E+04	1.20E+00	1.20E+03	1.70E-01	1.60E+03	2.34E-01
18	1.97E+04	8.32E-01	3.34E+04	1.42E+00	7.50E+02	2.21E-01	1.60E+03	2.21E-01
20	1.94E+04	8.37E-01	3.96E+04	1.38E+00	4.00E+03	7.66E-01	5.65E+03	9.78E-01
30	2.54E+04	1.10E+00	4.29E+04	1.74E+00	3.10E+03	3.81E-01	3.00E+03	1.15E+00
40	1.63E+04	9.06E-01	2.78E+04	1.12E+00	1.60E+03	4.15E-01	4.50E+03	6.83E-01
50	2.21E+04	1.16E+00	3.49E+04	1.35E+00	1.10E+03	5.45E-01	5.60E+03	1.06E+00

Table B. 5. PR772 and *E. coli* Breakthrough Curve Raw Data – Trial (b) in Experiment 1

Time (min)	PR772 Phage				*E. coli* RS2g			
	Column X		Column Y		Column X		Column Y	
	PFU/mL	C/C_o	PFU/mL	C/C_o	CFU/mL	C/C_o	CFU/mL	C/C_o
Feed	4.21E+04		4.21E+04		1.26E+04		1.26E+04	
4	1.00E+00	2.38E-05	1.00E+00	2.38E-05	1.00E+00	7.94E-05	1.00E+00	7.94E-05
8	1.00E+00	2.38E-05	1.00E+00	2.38E-05	1.00E+00	7.94E-05	1.00E+00	7.94E-05
10	5.00E+00	1.19E-04	9.00E+01	1.96E-03	1.00E+00	7.94E-05	6.00E+01	4.76E-03
12	2.55E+03	6.53E-02	1.09E+04	2.61E-01	4.50E+02	3.57E-02	2.28E+03	2.03E-01
17	5.30E+04	9.56E-01	7.35E+04	1.72E+00	9.10E+03	7.34E-01	8.30E+03	5.79E-01
22	3.70E+04	8.97E-01	3.75E+04	9.38E-01	1.18E+04	8.85E-01	1.12E+04	8.61E-01
27	3.30E+04	7.84E-01	4.00E+04	9.50E-01	1.14E+04	8.93E-01	9.20E+03	7.22E-01
32	3.70E+04	8.79E-01	3.25E+04	7.72E-01	1.08E+04	8.10E-01	9.60E+03	7.54E-01
37	4.05E+04	9.62E-01	2.60E+04	6.18E-01	1.39E+04	1.06E+00	1.16E+04	9.37E-01
42	3.15E+04	7.48E-01	3.45E+04	8.19E-01	1.23E+04	9.72E-01	1.08E+04	8.81E-01

Table B. 6. 1.0 µm and 4.5 µm Sphere Breakthrough Curve Raw Data – Trial (b) in Experiment 1

Time (min)	1.0 µm Spheres				4.5 µm Spheres			
	Column X		Column Y		Column X		Column Y	
	MS/mL	C/C_o	MS/mL	C/C_o	MS/mL	C/C_o	MS/mL	C/C_o
Feed	3.64E+03		3.64E+03		6.83E+03		6.83E+03	
2	1.00E+01	2.75E-03			3.00E+00	4.39E-04		
4			2.00E-01	5.49E-05			4.60E+00	6.73E-04
6	7.00E+00	1.92E-03			3.00E+00	4.39E-04		
8	7.00E+00	1.92E-03			1.00E+00	1.46E-04		
10	1.10E+01	3.02E-03	2.30E+01	6.32E-03	1.00E+00	1.46E-04	2.50E+01	3.66E-03
12	4.26E+02	1.17E-01	1.26E+03	3.46E-01	1.49E+02	2.18E-02	8.60E+02	1.26E-01
17	4.50E+03	1.24E+00	5.27E+03	1.45E+00	2.30E+03	3.37E-01	9.75E+02	1.43E-01
22	5.35E+03	1.47E+00	7.55E+03	2.07E+00	6.50E+02	9.52E-02	2.50E+03	3.66E-01
27	4.80E+03	1.32E+00	7.20E+03	1.98E+00	1.50E+03	2.20E-01	1.45E+03	2.12E-01
32	5.80E+03	1.59E+00	9.00E+03	2.47E+00	2.65E+03	3.88E-01	1.30E+03	1.90E-01
37	5.95E+03	1.63E+00	1.10E+04	3.02E+00	2.35E+03	3.44E-01		
42	5.50E+03	1.51E+00	1.46E+04	4.00E+00	2.25E+03	3.29E-01		

Table B. 7. PR772 and *E. coli* Breakthrough Curve Raw Data – Trial (c) in Experiment 1

Time (min)	PR772 Phage				*E. coli* RS2g			
	Column X		Column Y		Column X		Column Y	
	PFU/mL	C/C_o	PFU/mL	C/C_o	CFU/mL	C/C_o	CFU/mL	C/C_o
Feed	2.79E+04		2.79E+04		4.62+04		4.62E+04	
0.3	1.00E+00	3.58E-05	1.00E+00	3.58E-05	1.00E+00	2.16E-05	1.00E+00	2.16E-05
0.8	1.00E+00	3.58E-05	1.00E+00	3.58E-05	1.00E+00	2.16E-05	1.00E+00	2.16E-05
1.0	1.00E+00	3.58E-05	1.00E+00	3.58E-05	1.00E+00	2.16E-05	1.00E+00	2.16E-05
1.3	1.00E+00	3.58E-05	1.00E+00	3.58E-05	1.00E+00	2.16E-05	1.00E+01	2.16E-04
1.5	2.00E+01	7.17E-04	1.00E+00	3.58E-05	1.00E+02	2.16E-03	1.00E+01	2.16E-04
1.8	1.80E+03	6.45E-02	1.45E+02	5.20E-03	2.60E+03	5.63E-02	3.10E+03	6.71E-02
	1.60E+03	5.73E-02			3.00E+03	6.49E-02	1.20E+03	2.60E-02
2.0	8.10E+03	2.90E-01	2.00E+03	7.17E-02	9.80E+03	2.12E-01	2.80E+03	6.06E-02
	7.95E+03	2.85E-01	1.30E+03	4.66E-02	1.21E+04	2.62E-01	2.00E+03	4.33E-02
2.3	2.05E+04	7.35E-01	4.50E+03	1.61E-01	1.88E+04	4.07E-01	6.30E+03	1.36E-01
	1.25E+04	4.48E-01	5.10E+03	1.83E-01	1.71E+04	3.70E-01	6.30E+03	1.36E-01
2.5	7.00E+03	2.51E-01	1.65E+04	5.91E-01	1.78E+04	3.85E-01	1.07E+04	2.32E-01
	2.35E+04	8.42E-01	2.15E+04	7.71E-01	1.90E+04	4.11E-01	1.25E+04	2.71E-01
3.0	2.25E+04	8.06E-01	2.60E+04	9.32E-01	2.40E+04	5.19E-01	2.60E+04	5.63E-01
	2.45E+04	8.78E-01	2.00E+04	7.17E-01	2.20E+04	4.76E-01	2.20E+04	4.76E-01
3.5	3.90E+04	1.40E+00	2.25E+04	8.06E-01	3.40E+04	7.36E-01	3.60E+04	7.79E-01
	3.40E+04	1.22E+00	1.35E+04	4.84E-01	1.70E+04	3.68E-01	3.20E+04	6.93E-01
4.5	3.60E+04	1.29E+00	3.85E+04	1.38E+00	3.10E+04	6.71E-01	2.80E+04	6.06E-01
	3.05E+04	1.09E+00	2.45E+04	8.78E-01	3.70E+04	8.01E-01	3.90E+04	8.44E-01

Table B. 8. 1.0 µm and 4.5 µm Sphere Breakthrough Curve Raw Data – Trial (c) in Experiment 1

Time (min)	1.0 µm Spheres				4.5 µm Spheres			
	Column X		Column Y		Column X		Column Y	
	MS/mL	C/C_o	MS/mL	C/C_o	MS/mL	C/C_o	MS/mL	C/C_o
Feed	1.35E+04		1.35E+04		5.11E+03		5.11E+03	
0.5	1.00E+00	7.39E-05	1.05E+01	7.75E-04			1.00E+00	1.96E-04
0.8	1.00E+00	7.39E-05			1.00E+00	1.96E-04		
1.0	6.00E+00	4.43E-04	2.00E+00	1.48E-04	1.00E+00	1.96E-04	1.00E+00	1.96E-04
1.3	2.00E+00	1.48E-04	1.00E+00	7.39E-05	1.00E+00	1.96E-04	1.00E+00	1.96E-04
1.5	1.00E+02	7.39E-03	1.00E+00	7.39E-05	1.00E+00	1.96E-04	1.00E+00	1.96E-04
1.8	1.12E+03	8.27E-02	1.32E+02	9.71E-03	1.00E+02	1.96E-02	3.00E+00	5.87E-04
	3.30E+02	2.44E-02			7.00E+01	1.37E-02		
2.0	3.98E+03	2.94E-01	1.19E+03	8.75E-02	3.30E+02	6.46E-02	6.50E+01	1.27E-02
	5.60E+03	4.13E-01	8.15E+02	6.02E-02	3.10E+02	6.07E-02	2.50E+01	4.89E-03
2.3	8.45E+03	6.24E-01	3.05E+03	2.25E-01	3.00E+02	5.87E-02	9.50E+01	1.86E-02
	1.08E+04	7.98E-01	6.75E+03	4.99E-01	1.00E+02	1.96E-02		
2.5	9.50E+03	7.02E-01	5.50E+03	4.06E-01	4.80E+02	9.39E-02	8.00E+01	1.57E-02
	8.70E+03	6.43E-01	6.85E+03	5.06E-01	4.80E+02	9.39E-02	3.50E+02	6.85E-02
3.0	9.20E+03	6.79E-01	1.08E+04	7.98E-01	3.65E+02	7.14E-02	1.00E+02	1.96E-02
	1.50E+04	1.11E+00	1.21E+04	8.90E-01	5.30E+02	1.04E-01	2.40E+02	4.70E-02
3.5	1.08E+04	7.94E-01	1.21E+04	8.90E-01	6.65E+02	1.30E-01	5.60E+02	1.10E-01
	1.18E+04	8.71E-01	1.10E+04	8.12E-01	6.75E+02	1.32E-01	9.50E+01	1.86E-02
4.5	2.06E+04	1.52E+00	1.31E+04	9.64E-01	7.00E+02	1.37E-01	9.50E+01	1.86E-02
	1.94E+04	1.43E+00	7.70E+03	5.69E-01	5.25E+02	1.03E-01	2.75E+02	5.38E-02

Table B. 9. (Bio)Colloid Percent Removal in Experiment 1 Raw Data

Trial	IS (mM KCl)	Flow (m/h)	ES (mm)	D/d	Column	PR772	*E. coli*	1.1 µm MS	4.5 µm MS
a	0.01	1	1.1	15	X	34.9	3.1	12.5	46.4
a	0.01	1	1.1	15	X	44.6	3.9	28.6	53.3
a	0.01	1	1.1	15	X	40.4	5.5	20.7	
a	0.01	1	1.1	15	X	32.1	1.6	29.9	44.6
a	0.01	1	1.1	15	X	19.7	-4.7	18.5	
a	0.01	1	1.1	15	X	11.4		8.0	
a	0.01	1	1.1	15	X	1.7		-6.5	55.0
a	0.01	1	1.1	15	X	-9.4		-5.1	67.1
a	0.01	1	1.1	15	X			19.9	
a	0.01	1	1.1	15	X			0.7	
a	0.01	1	1.1	45	Y	25.2	5.5	2.7	48.1
a	0.01	1	1.1	45	Y	34.9	13.4	7.6	22.1
a	0.01	1	1.1	45	Y	40.4	7.1	21.6	41.2
a	0.01	1	1.1	45	Y	25.2	19.7	-0.9	51.5
a	0.01	1	1.1	45	Y	34.9	11.0	28.3	49.8
a	0.01	1	1.1	45	Y	41.8	18.9	-6.9	
a	0.01	1	1.1	45	Y	40.4		22.5	
a	0.01	1	1.1	45	Y	37.7		20.5	
a	0.01	1	1.1	45	Y	15.5		-0.7	
a	0.01	1	1.1	45	Y			-1.8	
b	0.01	1	0.43	37	X	12.1	4.1	18.3	65.6
b	0.01	1	0.43	37	X	3.8	11.0	24.5	67.1
b	0.01	1	0.43	37	X	25.2	15.2	21.0	67.1
b	0.01	1	0.43	37	X	8.6	15.9	25.8	64.1
b	0.01	1	0.43	37	X	-1.0	7.6	20.3	67.8
b	0.01	1	0.43	37	X	10.9	2.8		67.8

b	0.01	1	0.43	37	X		-4.1		
b	0.01	1	0.43	116	Y	22.8	20.0	27.6	78.8
b	0.01	1	0.43	116	Y	38.2	17.2	-3.7	81.0
b	0.01	1	0.43	116	Y	18.1	25.5	1.1	64.9
b	0.01	1	0.43	116	Y	14.5	21.4	28.2	79.5
b	0.01	1	0.43	116	Y	10.9	-4.8	0.4	77.3
b	0.01	1	0.43	116	Y		0.0		
b	0.01	1	0.43	116	Y		-2.1		
c	10	5	0.43	37	X	26.5	48.1	55.6	87.0
c	10	5	0.43	37	X	55.2	52.4	68.2	86.8
c	10	5	0.43	37	X	26.5	26.4	65.1	86.3
c	10	5	0.43	37	X	15.8	63.2	39.2	89.7
c	10	5	0.43	37	X	19.4	32.9	42.8	87.2
c	10	5	0.43	37	X	12.2	19.9	62.7	86.3
c	10	5	0.43	116	Y	6.8	43.7	68.0	98.0
c	10	5	0.43	116	Y	28.3	52.4	64.3	95.3
c	10	5	0.43	116	Y	19.4	22.1	64.3	98.9
c	10	5	0.43	116	Y	51.6	30.7	67.5	98.1
c	10	5	0.43	116	Y	12.2	39.4	61.4	98.1
c	10	5	0.43	116	Y		15.6		94.6
c	10	5	0.43	116	Y		28.6		94.9
c	10	5	0.43	116	Y		35.1		97.0

Appendix C – Experiment 2

Table C. 1. Bromide Breakthrough Curve Raw Data for Trials 1 & 2 of Experiment 2

	Trial									
	1					2				
Time	Column A		Column B		Feed	Column A		Column B		Feed
(min)	$[Br^-]$	C/C_o	$[Br^-]$	C/C_o	$[Br^-]$	$[Br^-]$	C/C_o	$[Br^-]$	C/C_o	$[Br^-]$
0					80.3					83.7
0					81.2					90.1
0					80.2					85.3
Mean					80.5					86.4
3	0.1	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
7	0.0	0.0	0.1	0.0		0.0	0.0	0.0	0.0	
10						0.0	0.0	0.0	0.0	
12						2.0	0.0	0.1	0.0	
13	34.5	0.4	43.9	0.5		13.4	0.2	3.3	0.0	
14	57.6	0.7	64.8	0.8		35.3	0.4	19.1	0.2	
15	70.9	0.9	74.2	0.9						
16	75.4	0.9	77.6	1.0		79.0	0.9	62.8	0.7	
18	78.9	1.0	79.3	1.0						
19						84.8	1.0	86.0	1.0	
21	79.0	1.0	82.0	1.0						
25	78.9	1.0	79.7	1.0						
27						86.2	1.0	87.7	1.0	
30*										
31										
34						87.1	1.0	89.5	1.0	
37	79.3	1.0	81.4	1.0						
41						92.8	1.1	89.8	1.0	
43						63.0	0.7	64.0	0.7	
44	53.5	0.7	63.2	0.8						
46	10.1	0.1	16.9	0.2						
47	4.2	0.1	6.4	0.1		1.3	0.0	1.0	0.0	
48	2.2	0.0	2.3	0.0						
50	0.9	0.0	0.7	0.0		0.3	0.0	0.4	0.0	
53	0.3	0.0	0.3	0.0						
54						0.0	0.0	0.0	0.0	
57	0.1	0.0	0.1	0.0						
58						0.0	0.0	0.0	0.0	
61	0.1	0.0	0.1	0.0		0.0	0.0	0.0	0.0	

*The dashed line indicates where the Br^--feed solution was switched to a Br^--free feed solution.

Table C. 2. Bromide Breakthrough Curve Raw Data for Trials 1 & 2 of Experiment 2

	Trial									
	3					4				
Time	Column A		Column B		Feed	Column A		Column B		Feed
(min)	$[Br^-]$	C/C_o	$[Br^-]$	C/C_o	$[Br^-]$	$[Br^-]$	C/C_o	$[Br^-]$	C/C_o	$[Br^-]$
0					84.1					83.7
0					79.5					90.1
0										85.3
Mean					81.8					86.4
3	5.0	0.1	3.0	0.0		1.0	0.0	1.0	0.0	
7	5.0	0.1	2.0	0.0		1.0	0.0	1.0	0.0	
10	0.0	0.0	0.0	0.0		1.0	0.0	1.0	0.0	
12	5.2	0.1	1.4	0.0		2.0	0.0	1.0	0.0	
13	20.7	0.3	12.9	0.2		13.4	0.2	3.3	0.0	
14	44.4	0.5	38.2	0.5		35.3	0.4	19.1	0.2	
16	75.8	0.9	68.0	0.8		79.0	0.9	62.8	0.7	
19	83.5	1.0	83.2	1.0		84.8	1.0	86.0	1.0	
27	83.1	1.0	83.0	1.0		86.2	1.0	87.7	1.0	
30*										
31										
34	84.9	1.0	81.8	1.0		87.1	1.0	89.5	1.0	
41	78.9	1.0	55.9	0.7		92.8	1.1	89.8	1.0	
43	38.7	0.5	9.5	0.1		63.0	0.7	64.0	0.7	
44	19.9	0.2	3.2	0.0						
45	8.4	0.1	0.9	0.0						
47	1.5	0.0	0.2	0.0		1.3	0.0	1.0	0.0	
50	0.4	0.0	0.1	0.0		1.0	0.0	1.0	0.0	
54	0.2	0.0	3.0	0.0		1.0	0.0	1.0	0.0	
58	0.0	0.0	0.0	0.0		1.0	0.0	1.0	0.0	
61	0.0	0.0	1.0	0.0		1.0	0.0	1.0	0.0	

*Dashed line indicates where the Br^--feed solution was switched to a Br^--free feed solution.

Table C. 3. (Bio)Colloid Feed Concentrations for the 16 Trials in Experiment 2

Trial	*S. typhimurium*	PR772	1.1 µm MS	4.5 µm MS
1	5.85E+06	9.78E+06	4.09E+06	5.37E+04
2	4.22E+06	9.40E+06	2.82E+05	8.68E+04
3	3.65E+05	3.31E+06	5.66E+06	8.40E+04
4	2.04E+07	6.37E+06	9.74E+04	5.92E+04
5	1.15E+07	3.32E+06	4.90E+06	5.18E+04
6	5.73E+06	8.14E+06	3.78E+06	6.24E+04
7	1.84E+07	3.34E+06	3.49E+06	3.93E+04
8	4.77E+06	9.01E+06	4.56E+06	9.80E+04
9	4.26E+06	3.93E+06	4.77E+06	5.41E+04
10	2.41E+06	1.24E+06	3.02E+06	1.35E+05
11	2.19E+06	5.52E+06	7.66E+06	6.99E+04
12	2.38E+06	5.76E+06	5.69E+05	6.71E+04
13	2.90E+05	7.74E+06	1.15E+07	1.08E+05
14	2.06E+06	5.97E+06	5.48E+06	8.64E+04
15	2.24E+06	4.07E+06	7.60E+06	7.77E+04
16	2.71E+05	9.30E+06	4.65E+06	4.88E+04
Max	2.04E+07	9.78E+06	1.15E+07	1.35E+05
Min	2.71E+05	1.24E+06	9.74E+04	3.93E+04
Mean	5.46E+06	6.01E+06	4.51E+06	7.39E+04
SD	6.12E+06	2.66E+06	2.92E+06	2.51E+04

Table C. 4. *S. typhimurium* and PR772 Breakthrough Raw Data from the 16 Trials of Experiment 2

Time	*S. typhimurium*				PR772			
	[Bacteria A]		[Bacteria B]		[PR772 A]		[PR772 B]	
(min)	(CFU/mL)	C/Co	(CFU/mL)	C/Co	(PFU/mL)	C/Co	Conc.	C/Co
				Trial #1				
4	1.00E+01	1.71E-06	1.00E+01	1.70E-06	5.00E+00	5.10E-07	5.00E+00	5.10E-07
8	1.05E+06	1.80E-01	7.60E+02	1.30E-04	5.00E+00	5.10E-07	3.65E+02	3.70E-05
10	2.91E+06	4.97E-01	2.69E+05	4.60E-02	2.42E+06	2.47E-01	2.15E+05	2.20E-02
12	3.00E+06	5.13E-01	1.17E+06	2.00E-01	1.40E+06	1.43E-01	1.90E+06	1.94E-01
16	4.29E+06	7.33E-01	5.25E+06	8.97E-01	4.80E+06	4.91E-01		
20	5.16E+06	8.82E-01	5.05E+06	8.63E-01	6.55E+06	6.70E-01	4.80E+06	4.91E-01
26	4.16E+06	7.11E-01	3.23E+06	5.52E-01	4.65E+06	4.75E-01	3.60E+06	3.68E-01
35	3.16E+06	5.40E-01	3.58E+06	6.12E-01	4.70E+06	4.80E-01	2.70E+06	2.76E-01
50	3.16E+06	5.40E-01	3.39E+06	5.79E-01	8.25E+06	8.43E-01	6.45E+06	6.59E-01
70	2.93E+06	5.01E-01	2.94E+06	5.03E-01	5.10E+06	5.21E-01	3.10E+06	3.17E-01
110	2.50E+06	4.27E-01	2.65E+06	4.53E-01	4.30E+06	4.40E-01	4.00E+06	4.09E-01
				Trial #2				
10	2.46E+03	4.29E-04	1.62E+03	2.83E-04	3.60E+02	4.42E-05	4.55E+02	5.59E-05
12	7.30E+04	1.27E-02	3.80E+02	6.63E-05	1.20E+04	1.47E-03	2.00E+04	2.46E-03
14	3.00E+05	5.24E-02	1.40E+05	2.44E-02	5.80E+04	7.13E-03	7.05E+04	8.66E-03
18	5.80E+05	1.01E-01	2.47E+05	4.31E-02	2.80E+05	3.44E-02	3.65E+05	4.49E-02
22	6.90E+05	1.20E-01	3.10E+05	5.41E-02	6.50E+05	7.99E-02	1.13E+06	1.38E-01
26	7.60E+05	1.33E-01	3.50E+05	6.11E-02	1.25E+06	1.54E-01	2.80E+06	3.44E-01
28	1.94E+05	3.39E-02			2.30E+06	2.83E-01		
30			6.50E+05	1.13E-01			5.00E+06	6.14E-01
35	1.29E+06	2.25E-01	5.70E+05	9.95E-02	5.40E+06	6.64E-01	5.35E+06	6.57E-01
40	1.35E+06	2.36E-01	7.50E+05	1.31E-01	7.40E+06	9.09E-01	7.15E+06	8.79E-01
45	1.42E+06	2.48E-01	7.90E+05	1.38E-01	9.55E+06	1.17E+00	8.75E+06	1.08E+00
50	1.53E+06	2.67E-01	8.70E+05	1.52E-01	8.10E+06	9.95E-01	8.70E+06	1.07E+00
55	1.50E+06	2.62E-01	9.50E+05	1.66E-01	7.05E+06	8.66E-01	7.05E+06	8.66E-01
90	2.05E+06	3.58E-01	1.41E+06	2.46E-01	7.25E+06	8.91E-01	7.65E+06	9.40E-01

120	2.91E+06	5.08E-01	1.57E+06	2.74E-01	1.31E+07	1.60E+00	9.95E+06	1.22E+00
180	3.09E+06	5.39E-01	1.75E+06	3.05E-01	1.06E+07	1.30E+00	1.00E+07	1.23E+00
240	3.92E+06	6.84E-01	2.58E+06	4.50E-01	1.30E+07	1.60E+00	1.16E+07	1.43E+00
Trial #3								
4	1.00E+01	2.74E-05	1.00E+01	2.74E-05	5.00E+00	1.51E-06	5.00E+00	1.51E-06
8	1.00E+01	2.74E-05	1.00E+01	2.74E-05	5.00E+00	1.51E-06	5.00E+00	1.51E-06
10	2.70E+02	7.40E-04	1.00E+01	2.74E-05	1.95E+03	5.89E-04	5.00E+00	1.51E-06
12	6.60E+03	1.81E-02	1.00E+01	2.74E-05	2.10E+04	6.35E-03	5.00E+00	1.51E-06
16	8.60E+04	2.36E-01	1.00E+01	2.74E-05	2.29E+06	6.92E-01	5.00E+02	1.51E-04
20	1.29E+05	3.53E-01	4.80E+03	1.32E-02	5.10E+06	1.54E+00	5.00E+03	1.51E-03
26	8.70E+04	2.38E-01	7.00E+04	1.92E-01	4.15E+06	1.25E+00	2.55E+06	7.71E-01
30	1.06E+05	2.90E-01	8.90E+04	2.44E-01	5.00E+06	1.51E+00		
40	4.30E+05	1.18E+00	2.32E+05	6.36E-01	5.20E+06	1.57E+00	3.35E+06	1.01E+00
50	2.23E+05	6.11E-01	1.41E+05	3.86E-01	4.35E+06	1.31E+00	6.80E+06	2.06E+00
60	3.40E+05	9.32E-01	5.90E+05	1.62E+00	5.80E+06	1.75E+00	8.55E+06	2.58E+00
70	3.50E+05	9.59E-01	4.60E+05	1.26E+00	4.55E+06	1.38E+00		
90	5.30E+05	1.45E+00	6.40E+05	1.75E+00	7.45E+06	2.25E+00	7.05E+06	2.13E+00
110	4.50E+05	1.23E+00	6.90E+05	1.89E+00	7.15E+06	2.16E+00	1.26E+07	3.81E+00
120	1.20E+05	3.29E-01	3.60E+05	9.86E-01	6.55E+06	1.98E+00		
Trial #4								
7					1.00E+00	1.57E-07	1.00E+00	1.57E-07
9	2.18E+03	1.07E-04	3.15E+03	1.55E-04				
13	7.20E+04	3.53E-03	1.04E+05	5.10E-03				
20	1.19E+05	5.84E-03	2.35E+05	1.15E-02				
30	2.76E+05	1.35E-02	3.20E+05	1.57E-02				
40	2.63E+05	1.29E-02	5.90E+05	2.90E-02				
50	2.20E+05	1.08E-02	1.59E+05	7.78E-03	3.62E+06	5.69E-01	3.12E+06	4.90E-01
60	1.35E+05	6.63E-03	5.30E+05	2.60E-02				
70	1.45E+05	7.12E-03	2.70E+05	1.33E-02				
80	2.29E+05	1.12E-02	4.80E+05	2.36E-02				
90	3.10E+05	1.52E-02	4.20E+05	2.06E-02				
150	3.15E+05	1.55E-02	5.50E+05	2.70E-02	5.45E+06	8.56E-01	7.20E+06	1.13E+00
210	2.50E+05	1.23E-02	4.10E+05	2.01E-02	7.15E+06	1.12E+00	5.60E+06	8.80E-01

240	1.14E+05	5.60E-03	1.31E+05	6.43E-03	7.10E+06	1.12E+00	7.35E+06	1.15E+00
300	1.68E+05	8.25E-03	3.20E+05	1.57E-02	8.55E+06	1.34E+00	7.70E+06	1.21E+00
360	3.30E+05	1.62E-02	2.50E+05	1.23E-02	6.80E+06	1.07E+00	7.10E+06	1.12E+00
420	2.20E+05	1.08E-02	3.80E+05	1.87E-02	7.65E+06	1.20E+00	6.25E+06	9.82E-01
470	3.10E+05	1.52E-02	4.60E+05	2.26E-02				
Trial #5								
4	1.00E+01	8.71E-07	1.00E+01	8.71E-07	5.00E+00	1.51E-06	5.00E-01	1.51E-07
8	1.00E+01	8.71E-07	2.50E+02	2.18E-05	5.00E+00	1.51E-06	5.00E+01	1.51E-05
10	1.43E+04	0.001244831			4.25E+03	0.001280603		
12	3.83E+04	0.003334059			4.25E+05	0.128060264	1.26E+06	0.378154426
16	1.31E+06	0.114036997	1.81E+06	0.157562568	1.45E+06	0.436911488	2.55E+06	0.768361582
20	1.73E+06	0.150598477	1.56E+06	0.135799782	2.37E+06	0.712617702	2.67E+06	0.803013183
24	1.54E+06	0.13405876	1.67E+06	0.145375408	3.20E+06	0.964218456	2.60E+06	0.783427495
28	1.32E+06	0.114907508	1.53E+06	0.133188248	3.10E+06	0.934086629	3.20E+06	0.964218456
35	1.69E+06	0.147116431	1.62E+06	0.141022851	3.90E+06	1.175141243	3.95E+06	1.190207156
45	1.41E+06	0.122742111	1.73E+06	0.150598477	2.10E+06	0.632768362	2.20E+06	0.662900188
55	1.55E+06	0.134929271	1.44E+06	0.125353645	2.90E+06	0.873822976	3.45E+06	1.039548023
75	1.39E+06	0.121001088	1.36E+06	0.118389554	2.90E+06	0.873822976	4.30E+06	1.29566855
95	1.27E+06	0.110554951	1.26E+06	0.10968444	3.30E+06	0.994350282	3.45E+06	1.039548023
115	1.10E+06	0.095756257	1.44E+06	0.125353645	3.15E+06	0.949152542	2.20E+06	0.662900188
Trial #6								
10	2.46E+03	4.29E-04	1.62E+03	2.83E-04	3.60E+02	4.42E-05	4.55E+02	5.59E-05
12	7.30E+04	1.27E-02	3.80E+02	6.63E-05	1.20E+04	1.47E-03	2.00E+04	2.46E-03
14	3.00E+05	5.24E-02	1.40E+05	2.44E-02	5.80E+04	7.13E-03	7.05E+04	8.66E-03
18	5.80E+05	1.01E-01	2.47E+05	4.31E-02	2.80E+05	3.44E-02	3.65E+05	4.49E-02
22	6.90E+05	1.20E-01	3.10E+05	5.41E-02	6.50E+05	7.99E-02	1.13E+06	1.38E-01
26	7.60E+05	1.33E-01	3.50E+05	6.11E-02	1.25E+06	1.54E-01	2.80E+06	3.44E-01
28	1.94E+05	3.39E-02			2.30E+06	2.83E-01		
30			6.50E+05	1.13E-01			5.00E+06	6.14E-01
35	1.29E+06	2.25E-01	5.70E+05	9.95E-02	5.40E+06	6.64E-01	5.35E+06	6.57E-01
40	1.35E+06	2.36E-01	7.50E+05	1.31E-01	7.40E+06	9.09E-01	7.15E+06	8.79E-01
45	1.42E+06	2.48E-01	7.90E+05	1.38E-01	9.55E+06	1.17E+00	8.75E+06	1.08E+00
50	1.53E+06	2.67E-01	8.70E+05	1.52E-01	8.10E+06	9.95E-01	8.70E+06	1.07E+00

55	1.50E+06	2.62E-01	9.50E+05	1.66E-01	7.05E+06	8.66E-01	7.05E+06	8.66E-01
90	2.05E+06	3.58E-01	1.41E+06	2.46E-01	7.25E+06	8.91E-01	7.65E+06	9.40E-01
120	2.91E+06	5.08E-01	1.57E+06	2.74E-01	1.31E+07	1.60E+00	9.95E+06	1.22E+00
180	3.09E+06	5.39E-01	1.75E+06	3.05E-01	1.06E+07	1.30E+00	1.00E+07	1.23E+00
240	3.92E+06	6.84E-01	2.58E+06	4.50E-01	1.30E+07	1.60E+00	1.16E+07	1.43E+00
Trial #7								
4	7.00E+01	3.80E-06	8.00E+01	4.35E-06	5.00E+00	1.50E-06	5.00E+00	1.50E-06
8	1.20E+02	6.52E-06	2.00E+01	1.09E-06	5.00E+00	1.50E-06	5.00E+00	1.50E-06
10	9.90E+04	5.38E-03	1.11E+04	6.03E-04	2.85E+04	8.53E-03	8.45E+02	2.53E-04
12	2.73E+06	1.48E-01	9.60E+05	5.22E-02	7.60E+05	2.27E-01	2.65E+05	7.93E-02
16	7.50E+06	4.08E-01	7.90E+06	4.29E-01	3.15E+06	9.43E-01	1.85E+06	5.52E-01
20	6.20E+06	3.37E-01	5.40E+06	2.93E-01	3.70E+06	1.11E+00	4.20E+06	1.26E+00
24	7.80E+06	4.24E-01	6.00E+06	3.26E-01	4.15E+06	1.24E+00	4.15E+06	1.24E+00
28	7.00E+06	3.80E-01	6.50E+06	3.53E-01	4.15E+06	1.24E+00	3.30E+06	9.88E-01
35	6.00E+06	3.26E-01	5.50E+06	2.99E-01	4.05E+06	1.21E+00	4.05E+06	1.21E+00
45	5.30E+06	2.88E-01	6.60E+06	3.59E-01	3.85E+06	1.15E+00	3.55E+06	1.06E+00
55	6.60E+06	3.59E-01	6.30E+06	3.42E-01	4.35E+06	1.30E+00	1.35E+06	4.04E-01
Trial #8								
4	1.00E+00	0.00E+00	1.00E+00	2.10E-07	1.00E+00	0.00E+00	1.00E+00	1.11E-07
6	1.00E+00	0.00E+00	1.00E+00	2.10E-07	1.00E+00	0.00E+00	1.00E+00	1.11E-07
8	1.00E+00	0.00E+00	1.00E+00	2.10E-07	1.00E+00	0.00E+00	1.00E+00	1.11E-07
10	4.69E+03	0.00E+00	1.17E+03	2.45E-04	3.90E+02	0.00E+00	1.55E+02	1.72E-05
12					8.15E+03	0.00E+00	5.35E+03	5.94E-04
14	5.39E+05	1.10E-01	3.08E+05	6.00E-02	2.35E+04	0.00E+00	1.01E+04	1.12E-03
16					1.50E+05	2.00E-02	1.00E+05	1.00E-02
18	6.10E+05	1.30E-01	5.36E+05	1.10E-01				
22	4.40E+05	9.00E-02	3.20E+05	7.00E-02	1.20E+05	1.00E-02	9.50E+04	1.00E-02
26	6.70E+05	1.40E-01	4.60E+05	1.00E-01	6.00E+05	7.00E-02	2.05E+05	2.00E-02
30	6.70E+05	1.40E-01	4.60E+05	1.00E-01	2.70E+06	3.00E-01	1.35E+06	1.50E-01
35					6.75E+06	7.50E-01	4.10E+06	4.60E-01
40	2.11E+06	4.40E-01	1.24E+06	2.60E-01	8.00E+06	8.90E-01	4.40E+06	4.90E-01
45					7.35E+06	8.20E-01	6.40E+06	7.10E-01
50	2.08E+06	4.40E-01	1.51E+06	3.20E-01	1.26E+07	1.39E+00	1.17E+07	1.29E+00

70	2.16E+06	4.50E-01	1.78E+06	3.70E-01	1.45E+07	1.60E+00	1.40E+07	1.55E+00
90	2.65E+06	5.60E-01	1.81E+06	3.80E-01	1.48E+07	1.64E+00	1.17E+07	1.29E+00
110	3.27E+06	6.90E-01	2.45E+06	5.10E-01	1.05E+07	1.17E+00	1.18E+07	1.30E+00
120	2.78E+06	5.80E-01	2.94E+06	6.20E-01				
Trial #9								
2	1.00E+01	2.35E-06	1.00E+01	2.35E-06	5.00E+00	1.27E-06		
4	5.19E+03	1.22E-03	1.00E+01	2.35E-06			5.00E+00	1.27E-06
6			1.00E+01	2.35E-06				
8	7.80E+03	1.83E-03	1.00E+01	2.35E-06	2.32E+04	5.90E-03	5.00E+00	1.27E-06
10			2.41E+03	5.65E-04			1.70E+03	4.32E-04
12	4.40E+05	1.03E-01	1.71E+05	4.01E-02	3.20E+06	8.14E-01	2.02E+05	5.14E-02
16	1.70E+05	3.99E-02	8.90E+04	2.09E-02	2.15E+06	5.47E-01	7.40E+05	1.88E-01
20	1.70E+05	3.99E-02	5.10E+05	1.20E-01			1.70E+06	4.32E-01
26	1.60E+05	3.75E-02	4.30E+05	1.01E-01	6.90E+06	1.76E+00	4.75E+06	1.21E+00
35	3.60E+05	8.45E-02	2.83E+05	6.64E-02	1.85E+06	4.71E-01	3.20E+06	8.14E-01
50	6.60E+05	1.55E-01	8.20E+05	1.92E-01	4.55E+06	1.16E+00	6.35E+06	1.62E+00
70	8.10E+05	1.90E-01	1.06E+06	2.49E-01			6.35E+06	1.62E+00
110	1.41E+06	3.31E-01	1.17E+06	2.74E-01	2.15E+06	5.47E-01	6.35E+06	1.62E+00
150	1.23E+06	2.89E-01	1.09E+06	2.56E-01	2.75E+06	7.00E-01	5.15E+06	1.31E+00
190	1.16E+06	2.72E-01	1.32E+06	3.10E-01	4.70E+06	1.20E+00	2.98E+06	7.58E-01
230	1.40E+06	3.28E-01	1.59E+06	3.73E-01	1.55E+06	3.94E-01	1.65E+06	4.20E-01
Trial #10								
4	1.00E+01	4.15E-06	1.00E+01	4.15E-06	5.00E+00	5.56E-07		
6	1.00E+01	4.15E-06	1.00E+01	4.15E-06				
8	1.00E+01	4.15E-06	1.00E+01	4.15E-06				
10	1.00E+01	4.15E-06	1.00E+01	4.15E-06				
14					5.00E+00	5.56E-07		
18	1.49E+04	6.19E-03	5.60E+03	2.33E-03				
26	2.51E+04	1.04E-02	1.71E+04	7.10E-03				
28					5.00E+00	5.56E-07		
35					2.00E+01	2.22E-06		
40	6.30E+04	2.62E-02	3.97E+04	1.65E-02	2.15E+03	2.39E-04		
45					1.00E+03	1.11E-04	6.00E+02	6.67E-05

50					5.35E+04	5.94E-03	7.50E+03	8.33E-04
60	1.02E+05	4.24E-02	6.70E+04	2.78E-02	4.75E+06	5.28E-01	3.15E+05	3.50E-02
100	1.36E+05	5.65E-02	1.13E+05	4.69E-02	7.90E+05	8.78E-02	1.31E+06	1.45E-01
140	1.40E+05	5.81E-02	1.23E+05	5.11E-02	2.25E+06	2.50E-01	1.65E+06	1.83E-01
180	1.42E+05	5.90E-02	2.42E+05	1.00E-01	1.65E+06	1.83E-01	4.80E+05	5.33E-02
240	3.37E+05	1.40E-01	3.35E+05	1.39E-01	4.55E+06	5.06E-01		
270	7.00E+04	2.91E-02	3.10E+05	1.29E-01	3.85E+06	4.28E-01	3.90E+06	4.33E-01
300	4.70E+05	1.95E-01	4.00E+05	1.66E-01	2.60E+06	2.89E-01	3.30E+06	3.67E-01
340	3.10E+05	1.29E-01	4.50E+05	1.87E-01				
360	1.30E+05	5.40E-02	1.30E+05	5.40E-02	6.00E+06	6.67E-01	4.90E+06	5.44E-01
380	5.60E+05	2.33E-01	5.30E+05	2.20E-01				
420	5.70E+05	2.37E-01	4.80E+05	1.99E-01	3.45E+06	3.83E-01	3.95E+06	4.39E-01
Trial #11								
4	1.00E+01	4.57E-06	1.00E+01	4.57E-06	5.00E+00	9.06E-07	5.00E+00	9.06E-07
8	1.00E+01	4.57E-06	1.00E+01	4.57E-06	5.00E+00	9.06E-07	5.00E+00	9.06E-07
10	8.20E+03	3.75E-03	7.90E+02	3.61E-04	3.85E+04	6.98E-03		
12	1.16E+05	5.30E-02	6.20E+04	2.83E-02	1.03E+06	1.86E-01	6.20E+05	1.12E-01
16	1.84E+05	8.41E-02	1.94E+05	8.86E-02	3.05E+06	5.53E-01		
20	4.30E+05	1.96E-01	2.90E+05	1.32E-01	5.90E+06	1.07E+00	3.60E+06	6.52E-01
26	4.90E+05	2.24E-01	3.96E+05	1.81E-01	4.90E+06	8.88E-01	4.25E+06	7.70E-01
32	1.07E+05	4.89E-02	1.75E+05	8.00E-02	4.15E+06	7.52E-01		
47	2.17E+05	9.91E-02	2.59E+05	1.18E-01	3.00E+06	5.44E-01	3.35E+06	6.07E-01
72	2.64E+05	1.21E-01	4.50E+05	2.06E-01	5.15E+06	9.33E-01		
112	3.40E+05	1.55E-01	3.59E+05	1.64E-01	2.65E+06	4.80E-01	3.75E+06	6.80E-01
152	4.30E+05	1.96E-01	3.46E+05	1.58E-01	6.75E+06	1.22E+00	6.25E+06	1.13E+00
172	3.80E+05	1.74E-01	3.00E+05	1.37E-01	5.90E+06	1.07E+00		
192	3.50E+05	1.60E-01	4.09E+05	1.87E-01	6.15E+06	1.11E+00	5.95E+06	1.08E+00
212	7.70E+05	3.52E-01	8.10E+05	3.70E-01	5.60E+06	1.01E+00		
230	7.00E+05	3.20E-01	7.00E+05	3.20E-01	5.55E+06	1.01E+00	7.45E+06	1.35E+00
235	5.50E+05	2.51E-01	7.70E+05	3.52E-01	6.75E+06	1.22E+00	5.30E+06	9.60E-01
Trial #12								
4	1.00E+00	4.21E-07	1.00E+00	4.21E-07	1.00E+00	1.74E-07	1.00E+00	1.74E-07
8	1.00E+00	4.21E-07						

10	1.00E+00	4.21E-07	1.00E+00	4.21E-07				
12	2.00E+01	8.42E-06	6.90E+02	2.91E-04				
14	1.00E+03	4.21E-04	4.90E+03	2.06E-03				
16	4.80E+03	2.02E-03	5.80E+03	2.44E-03				
18	7.80E+03	3.28E-03	6.40E+03	2.69E-03				
20	8.30E+03	3.49E-03	8.00E+03	3.37E-03	1.00E+00	1.74E-07	1.00E+00	1.74E-07
24	7.90E+03	3.33E-03	8.80E+03	3.71E-03				
30	8.70E+03	3.66E-03	7.60E+03	3.20E-03				
60	1.00E+04	4.21E-03	1.30E+04	5.47E-03	1.00E+00	1.74E-07	1.00E+00	1.74E-07
80					1.00E+00	1.74E-07	1.00E+00	1.74E-07
100					5.00E+00	8.68E-07	9.50E+01	1.65E-05
120	4.00E+04	1.68E-02	1.10E+05	4.63E-02	9.40E+02	1.63E-04	1.30E+03	2.26E-04
140					7.35E+03	1.28E-03	8.30E+03	1.44E-03
160					1.69E+04	2.93E-03	1.50E+04	2.60E-03
180	1.03E+05	4.34E-02	8.70E+04	3.66E-02	4.20E+04	7.29E-03	4.00E+04	6.95E-03
210					7.70E+04	1.34E-02	9.35E+04	1.62E-02
240	1.10E+05	4.63E-02	1.20E+05	5.05E-02	1.60E+05	2.78E-02	1.33E+05	2.30E-02
270					2.20E+05	3.82E-02	3.10E+05	5.38E-02
300	1.80E+05	7.58E-02	1.94E+05	8.17E-02	7.15E+05	1.24E-01	6.05E+05	1.05E-01
360	1.10E+05	4.63E-02	1.90E+05	8.00E-02	6.70E+05	1.16E-01	4.50E+05	7.81E-02
390					1.12E+06	1.95E-01	8.40E+05	1.46E-01
410	2.26E+05	9.52E-02	2.20E+05	9.26E-02	1.09E+06	1.89E-01	5.60E+05	9.73E-02
Trial #13								
4	1.00E+01	3.45E-05	1.00E+01	3.45E-05	5.00E+00	6.46E-07	5.00E+00	6.46E-07
8	1.00E+01	3.45E-05	1.00E+01	3.45E-05	5.00E+00	6.46E-07	5.00E+00	6.46E-07
10	1.43E+03	4.93E-03	7.90E+02	2.72E-03	4.75E+03	6.14E-04	2.10E+03	2.71E-04
12	3.00E+04	1.03E-01	3.10E+04	1.07E-01	7.95E+05	1.03E-01	7.15E+05	9.24E-02
14	6.30E+04	2.17E-01	5.90E+04	2.03E-01	2.10E+06	2.71E-01		
16	1.04E+05	3.59E-01	7.20E+04	2.48E-01	3.60E+06	4.65E-01	3.10E+06	4.00E-01
20	1.13E+05	3.90E-01	1.11E+05	3.83E-01	3.35E+06	4.33E-01		
24	1.46E+05	5.03E-01	1.10E+05	3.79E-01	5.00E+06	6.46E-01	3.25E+06	4.20E-01
30	1.57E+05	5.41E-01	2.09E+05	7.21E-01	5.05E+06	6.52E-01	6.90E+06	8.91E-01
40	3.70E+05	1.28E+00	2.70E+05	9.31E-01	8.05E+06	1.04E+00		

50	1.54E+05	5.31E-01	2.70E+05	9.31E-01	4.70E+06	6.07E-01	4.50E+06	5.81E-01
60	3.70E+05	1.28E+00	2.80E+05	9.66E-01	6.00E+06	7.75E-01	4.75E+06	6.14E-01
70	3.30E+05	1.14E+00	2.80E+05	9.66E-01	6.80E+06	8.78E-01		
80	3.40E+05	1.17E+00	2.31E+05	7.97E-01	6.45E+06	8.33E-01	6.65E+06	8.59E-01
100	3.00E+05	1.03E+00	3.10E+05	1.07E+00	7.30E+06	9.43E-01	7.40E+06	9.56E-01
110	3.10E+05	1.07E+00	3.90E+05	1.34E+00	5.95E+06	7.69E-01		
120	2.03E+05	7.00E-01	2.39E+05	8.24E-01	6.75E+06	8.72E-01	6.65E+06	8.59E-01
Trial #14								
4	1.00E+00	4.95E-07	1.00E+00	4.95E-07	1.00E+00	1.68E-07	1.00E+00	1.68E-07
6	1.00E+00	4.95E-07	1.00E+00	4.95E-07	1.00E+00	1.68E-07	1.00E+00	1.68E-07
8	1.00E+00	4.95E-07	1.00E+00	4.95E-07	1.00E+00	1.68E-07	1.00E+00	1.68E-07
10	1.69E+04	8.37E-03	9.00E+02	4.46E-04	2.40E+04	4.02E-03	7.50E+02	1.26E-04
12	7.70E+04	3.81E-02	8.60E+04	4.26E-02	7.50E+05	1.26E-01	3.00E+05	5.03E-02
14	8.00E+04	3.96E-02	1.01E+05	5.00E-02	1.60E+06	2.68E-01	4.45E+06	7.45E-01
16	7.60E+04	3.76E-02	1.13E+05	5.59E-02	6.75E+06	1.13E+00	7.20E+06	1.21E+00
18	1.33E+05	6.58E-02	3.00E+05	1.49E-01	7.10E+06	1.19E+00	7.70E+06	1.29E+00
20	2.72E+05	1.35E-01	2.36E+05	1.17E-01	8.75E+06	1.47E+00	8.05E+06	1.35E+00
22	2.93E+06	1.45E+00	2.57E+06	1.27E+00	4.96E+07	8.31E+00	5.93E+07	9.93E+00
24	1.09E+06	5.40E-01	1.50E+06	7.43E-01				
26	1.62E+06	8.02E-01	1.24E+06	6.14E-01				
28	1.93E+06	9.55E-01	1.63E+06	8.07E-01	4.13E+07	6.91E+00		
30	1.81E+06	8.96E-01	2.74E+06	1.36E+00				
35	1.37E+06	6.78E-01	2.88E+06	1.43E+00				
40	4.80E+05	2.38E-01	3.10E+05	1.53E-01	5.60E+06	9.38E-01	5.50E+06	9.21E-01
50	2.20E+05	1.09E-01	3.60E+05	1.78E-01	6.10E+06	1.02E+00	4.50E+06	7.54E-01
60	2.80E+05	1.39E-01	1.40E+05	6.93E-02	3.30E+06	5.53E-01	5.10E+06	8.54E-01
90	1.72E+05	8.51E-02	3.01E+05	1.49E-01	4.35E+06	7.29E-01	6.55E+06	1.10E+00
120	2.80E+05	1.39E-01	1.22E+05	6.04E-02	1.60E+06	2.68E-01	2.50E+06	4.19E-01
140	1.92E+05	9.50E-02	2.41E+05	1.19E-01				
150	2.17E+05	1.07E-01	1.86E+05	9.21E-02	2.65E+06	4.44E-01	4.50E+06	7.54E-01
170	2.07E+05	1.02E-01	2.13E+05	1.05E-01				
180	9.00E+04	4.46E-02	2.82E+05	1.40E-01	5.80E+06	9.72E-01	4.80E+06	8.04E-01
200	1.81E+05	8.96E-02	1.63E+05	8.07E-02				

210	1.79E+05	8.86E-02	1.81E+05	8.96E-02				
230	1.69E+05	8.37E-02	1.90E+05	9.41E-02				
240					6.65E+06	1.11E+00	5.90E+06	9.88E-01
Trial #15								
2	1.00E+01	4.47E-06	1.00E+01	4.47E-06	5.00E+00	1.23E-06	5.00E+00	1.23E-06
6	1.00E+01	4.47E-06	1.00E+01	4.47E-06	5.00E+00	1.23E-06	5.00E+00	1.23E-06
8	1.00E+01	4.47E-06	1.00E+01	4.47E-06	5.00E+00	1.23E-06	5.00E+00	1.23E-06
10	9.00E+01	4.02E-05	2.00E+01	8.94E-06	3.20E+03	7.87E-04	3.00E+01	7.38E-06
12	5.10E+03	2.28E-03	4.80E+03	2.14E-03				
14					1.89E+06	4.65E-01	1.90E+06	4.67E-01
18	2.70E+04	1.21E-02	1.20E+04	5.36E-03	2.00E+06	4.92E-01	2.00E+06	4.92E-01
22	2.60E+05	1.16E-01	2.80E+05	1.25E-01	1.20E+06	2.95E-01	1.55E+06	3.81E-01
26	3.70E+05	1.65E-01	3.30E+05	1.47E-01	1.85E+06	4.55E-01	1.55E+06	3.81E-01
33	8.00E+05	3.57E-01	5.80E+05	2.59E-01	5.10E+06	1.25E+00	2.35E+06	5.78E-01
43	1.11E+06	4.96E-01	8.30E+05	3.71E-01	1.40E+06	3.44E-01	2.20E+06	5.41E-01
53	1.03E+06	4.60E-01	9.50E+05	4.24E-01	2.80E+06	6.89E-01	1.85E+06	4.55E-01
73	1.28E+06	5.72E-01	9.20E+05	4.11E-01	2.25E+06	5.53E-01	3.65E+06	8.98E-01
93	1.76E+06	7.86E-01	1.18E+06	5.27E-01	2.40E+06	5.90E-01	2.50E+06	6.15E-01
118	4.70E+05	2.10E-01	4.10E+05	1.83E-01	3.50E+06	8.61E-01	3.90E+06	9.59E-01
Trial #16								
2	1.00E+00	0.00E+00	1.00E+00	3.83E-06	1.00E+00	0.00E+00	1.00E+00	1.09E-07
4	1.00E+00	0.00E+00	1.00E+00	3.83E-06				
6	4.00E+00	0.00E+00	1.00E+00	3.83E-06	1.00E+00	0.00E+00	1.00E+00	1.09E-07
8					1.00E+00	0.00E+00	1.00E+00	1.09E-07
10	2.60E+03	1.00E-02	1.20E+02	4.60E-04	7.45E+04	1.00E-02	7.50E+01	8.20E-06
12					2.40E+06	2.60E-01	4.80E+05	5.00E-02
14	1.55E+05	5.90E-01	1.25E+05	4.80E-01	4.50E+06	4.90E-01	3.50E+06	3.80E-01
16					2.35E+06	2.60E-01	6.95E+06	7.60E-01
18	1.03E+05	3.90E-01	1.56E+05	6.00E-01	7.50E+06	8.20E-01	7.25E+06	7.90E-01
26	1.11E+05	4.30E-01	1.21E+05	4.60E-01				
30					1.18E+07	1.29E+00	1.26E+07	1.37E+00
40	2.33E+05	8.90E-01	3.96E+05	1.52E+00				
60	3.09E+05	1.18E+00	2.89E+05	1.11E+00				

80	2.88E+05	1.10E+00	2.34E+05	9.00E-01	7.35E+06	8.00E-01		
100	2.02E+05	7.70E-01						
120	1.94E+05	7.40E-01			1.09E+07	1.19E+00	1.16E+07	1.27E+00
175	4.30E+05	1.65E+00	3.70E+05	1.42E+00	6.50E+06	7.10E-01	8.25E+06	9.00E-01
205	1.40E+05	5.40E-01	2.40E+05	9.20E-01				
240	2.46E+05	9.40E-01	2.00E+05	7.70E-01	4.55E+06	5.00E-01	1.07E+07	1.16E+00

Table C. 4. 1.1 µm and 4.5 µm MS Breakthrough Raw Data from the 16 Trials of Experiment 2

Time (min)	1.1 MS				4.5 MS			
	[1.1 MS A]		[1.1 MS B]		[4.5 MS A]		[4.5 MS B]	
	(MS/mL)	C/Co	(MS/mL)	C/Co	(MS/mL)	C/Co	(MS/mL)	C/Co
Trial #1								
2	2.80E+01	7.00E-06	7.30E+01	2.00E-05	1.00E+00	2.00E-05	2.00E+00	3.73E-05
6					1.87E+02	3.50E-03	1.30E+01	2.42E-04
8	1.45E+06	3.54E-01	3.00E+03	7.00E-04			6.63E+03	1.24E-01
10	3.31E+06	8.09E-01	6.36E+05	1.55E-01	2.00E+04	3.73E-01	1.70E+03	3.17E-02
12	2.96E+06	7.23E-01	2.32E+06	5.67E-01				
16	3.19E+06	7.79E-01	3.54E+06	8.65E-01	2.71E+04	5.05E-01		
20	5.39E+06	1.32E+00			3.03E+04	5.65E-01	1.69E+04	3.15E-01
26	2.29E+06	5.59E-01	1.34E+06	3.27E-01	2.61E+04	4.85E-01		
35	3.54E+06	8.65E-01			3.18E+04	5.92E-01	2.20E+04	4.10E-01
50	6.71E+06	1.64E+00	2.98E+06	7.28E-01	2.88E+04	5.36E-01	1.44E+04	2.68E-01
70	4.21E+06	1.03E+00	2.57E+06	6.28E-01	5.02E+04	9.35E-01		
110	3.92E+06	9.58E-01	2.35E+06	5.74E-01	2.64E+04	4.92E-01	2.38E+04	4.43E-01
Trial #2								
2	4.70E+01	1.24E-05	4.80E+01	1.27E-05				
4	4.30E+01	1.14E-05	3.40E+01	8.99E-06	1.00E+00	1.60E-05		
6	9.60E+01	2.54E-05	3.80E+01	1.00E-05				
8	4.26E+02	1.13E-04	1.34E+02	3.54E-05				
10	1.46E+03	3.86E-04	1.46E+02	3.86E-05			1.30E+01	2.08E-04
14	8.38E+05	2.21E-01	1.68E+06	4.44E-01	7.80E+02	1.25E-02	1.00E+02	1.60E-03
18	3.09E+06	8.17E-01	1.56E+06	4.12E-01				
22	1.52E+06	4.02E-01	7.02E+06	1.86E+00	2.17E+03	3.48E-02	9.60E+02	1.54E-02
26	7.40E+05	1.96E-01	1.59E+06	4.20E-01	6.70E+03	1.07E-01		
30	2.12E+06	5.60E-01	9.00E+05	2.38E-01	8.72E+03	1.40E-01	2.98E+03	4.77E-02
35					1.30E+04	2.08E-01		
40	3.76E+06	9.94E-01	5.30E+06	1.40E+00	1.04E+04	1.67E-01	7.80E+03	1.25E-01
50	6.30E+06	1.66E+00	8.50E+06	2.25E+00	2.42E+04	3.88E-01	1.26E+04	2.02E-01

70	3.18E+06	8.40E-01	2.46E+06	6.50E-01				
90	5.78E+06	1.53E+00	6.32E+06	1.67E+00	3.00E+04	4.81E-01	1.66E+04	2.66E-01
110					1.95E+04	3.13E-01	1.55E+04	2.48E-01
120	5.34E+06	1.41E+00	5.50E+06	1.45E+00				
140					4.72E+04	7.56E-01	3.72E+04	5.96E-01
160	4.38E+06	1.16E+00						
180			6.06E+06	1.60E+00	5.92E+04	9.48E-01	4.02E+04	6.44E-01
200	3.60E+06	9.51E-01						
210					4.50E+04	7.21E-01	3.96E+04	6.34E-01
220	3.56E+06	9.41E-01						
240	4.82E+06	1.27E+00	6.50E+06	1.72E+00	3.98E+04	6.38E-01	5.46E+04	8.75E-01
Trial #3								
2	2.60E+01	4.59E-06			1.00E+00	1.19E-05		
4	4.00E+00	7.07E-07	3.20E+01	5.65E-06	4.00E+00	4.76E-05	4.00E+00	4.76E-05
6	2.30E+01	4.06E-06	1.50E+01	2.65E-06	1.00E+00	1.19E-05	1.00E+00	1.19E-05
8	1.80E+02	3.18E-05	3.00E+01	5.30E-06	1.00E+00	1.19E-05		
10	4.00E+03	7.07E-04	2.40E+03	4.24E-04	2.00E+00	2.38E-05		
12	3.07E+05	5.42E-02	1.65E+04	2.92E-03	8.08E+02	9.62E-03		
16	4.15E+06	7.33E-01	6.30E+05	1.11E-01	1.90E+02	2.26E-03		
18					1.50E+03	1.79E-02		
20	5.29E+06	9.35E-01	7.80E+05	1.38E-01	8.80E+03	1.05E-01	1.35E+03	1.61E-02
26	5.37E+06	9.49E-01	6.59E+05	1.16E-01	1.24E+04	1.48E-01	1.05E+03	1.25E-02
30	6.11E+06	1.08E+00			2.45E+04	2.92E-01		
40	6.78E+06	1.20E+00	2.54E+06	4.49E-01	3.56E+04	4.24E-01		
50	5.91E+06	1.04E+00	1.60E+06	2.83E-01	1.92E+04	2.28E-01		
60	5.07E+06	8.96E-01	6.47E+06	1.14E+00	1.69E+04	2.01E-01	1.06E+04	1.27E-01
70	3.65E+06	6.45E-01			6.10E+04	7.26E-01		
80					6.95E+03	8.27E-02		
90	8.57E+06	1.51E+00	2.00E+06	3.53E-01	3.45E+04	4.11E-01	7.10E+03	8.45E-02
100					1.34E+04	1.60E-01		
110	8.09E+06	1.43E+00	2.17E+06	3.83E-01				
120	7.40E+06	1.31E+00			2.64E+04	3.14E-01		
Trial #4								

3	5.00E+00	5.14E-05	2.00E+00	2.05E-05			1.00E+00	1.69E-05
7							1.00E+00	1.69E-05
11	3.07E+03	3.16E-02	2.47E+03	2.54E-02	8.74E+02	1.48E-02	5.46E+02	9.23E-03
15	7.98E+03	8.20E-02	1.34E+04	1.37E-01	1.75E+03	2.95E-02	1.65E+03	2.79E-02
30	4.68E+04	4.81E-01	4.12E+04	4.23E-01	3.18E+04	5.37E-01	3.20E+03	5.41E-02
35			2.44E+04	2.51E-01	3.24E+03	5.48E-02	3.40E+03	5.75E-02
40	2.48E+04	2.55E-01			4.00E+03	6.76E-02		
50	5.26E+04	5.40E-01	3.98E+04	4.09E-01	4.00E+03	6.76E-02	3.40E+03	5.75E-02
60	3.56E+04	3.66E-01	8.02E+04	8.24E-01	2.00E+04	3.38E-01	7.00E+03	1.18E-01
65	8.32E+04	8.55E-01					2.80E+03	4.73E-02
70	3.84E+04	3.94E-01	2.13E+04	2.19E-01	3.42E+04	5.78E-01	3.80E+03	6.42E-02
75			1.11E+04	1.14E-01	1.22E+03	2.06E-02	1.61E+03	2.72E-02
80	4.16E+04	4.27E-01	3.06E+04	3.14E-01	1.72E+04	2.91E-01	3.06E+04	5.17E-01
90	5.72E+04	5.88E-01	3.38E+04	3.47E-01	5.40E+03	9.13E-02	5.10E+03	8.62E-02
150	6.08E+04	6.25E-01	3.01E+04	3.09E-01	7.60E+03	1.28E-01	2.90E+03	4.90E-02
210	7.12E+04	7.31E-01	2.13E+04	2.19E-01	5.20E+03	8.79E-02	4.00E+03	6.76E-02
Trial #5								
2	1.15E+02	2.35E-05	2.84E+02	5.80E-05	1.72E+02	3.32E-03	4.10E+01	7.91E-04
6	1.39E+02	2.84E-05	2.65E+02	5.41E-05	1.59E+02	3.07E-03	1.90E+01	3.67E-04
10					2.74E+02	5.29E-03		
12	5.98E+05	1.22E-01	2.40E+06	4.89E-01	3.90E+03	7.52E-02	1.33E+04	2.57E-01
16	4.13E+06	8.43E-01			1.23E+04	2.37E-01		
20	4.35E+06	8.88E-01	1.85E+06	3.78E-01	6.80E+03	1.31E-01	1.00E+04	1.93E-01
24	1.90E+06	3.88E-01	4.40E+06	8.98E-01	1.06E+04	2.05E-01		
28	3.15E+06	6.43E-01			1.30E+04	2.51E-01	1.92E+04	3.70E-01
35	5.05E+06	1.03E+00			1.35E+04	2.60E-01		
45					9.00E+03	1.74E-01	2.06E+04	3.97E-01
55	2.75E+06	5.61E-01	2.75E+06	5.61E-01	1.90E+04	3.67E-01		
75	3.55E+06	7.24E-01	7.15E+06	1.46E+00	9.50E+03	1.83E-01	2.18E+04	4.21E-01
95	3.60E+06	7.35E-01	3.00E+06	6.12E-01	1.95E+04	3.76E-01	1.97E+04	3.80E-01
115	3.75E+06	7.65E-01			2.60E+04	5.02E-01	9.90E+03	1.91E-01
Trial #6								
2	4.70E+01	1.24E-05	4.80E+01	1.27E-05				

4	4.30E+01	1.14E-05	3.40E+01	8.99E-06	1.00E+00	1.60E-05		
6	9.60E+01	2.54E-05	3.80E+01	1.00E-05				
8	4.26E+02	1.13E-04	1.34E+02	3.54E-05				
10	1.46E+03	3.86E-04	1.46E+02	3.86E-05			1.30E+01	2.08E-04
14	8.38E+05	2.21E-01	1.68E+06	4.44E-01	7.80E+02	1.25E-02	1.00E+02	1.60E-03
18	3.09E+06	8.17E-01	1.56E+06	4.12E-01				
22	1.52E+06	4.02E-01	7.02E+06	1.86E+00	2.17E+03	3.48E-02	9.60E+02	1.54E-02
26	7.40E+05	1.96E-01	1.59E+06	4.20E-01	6.70E+03	1.07E-01		
30	2.12E+06	5.60E-01	9.00E+05	2.38E-01	8.72E+03	1.40E-01	2.98E+03	4.77E-02
35					1.30E+04	2.08E-01		
40	3.76E+06	9.94E-01	5.30E+06	1.40E+00	1.04E+04	1.67E-01	7.80E+03	1.25E-01
50	6.30E+06	1.66E+00	8.50E+06	2.25E+00	2.42E+04	3.88E-01	1.26E+04	2.02E-01
70	3.18E+06	8.40E-01	2.46E+06	6.50E-01				
90	5.78E+06	1.53E+00	6.32E+06	1.67E+00	3.00E+04	4.81E-01	1.66E+04	2.66E-01
110					1.95E+04	3.13E-01	1.55E+04	2.48E-01
120	5.34E+06	1.41E+00	5.50E+06	1.45E+00				
140					4.72E+04	7.56E-01	3.72E+04	5.96E-01
160	4.38E+06	1.16E+00						
180			6.06E+06	1.60E+00	5.92E+04	9.48E-01	4.02E+04	6.44E-01
200	3.60E+06	9.51E-01						
210					4.50E+04	7.21E-01	3.96E+04	6.34E-01
220	3.56E+06	9.41E-01						
240	4.82E+06	1.27E+00	6.50E+06	1.72E+00	3.98E+04	6.38E-01	5.46E+04	8.75E-01
Trial #7								
4	8.00E+01	2.29E-05	2.30E+02	6.59E-05	1.80E+01	4.58E-04	2.70E+01	6.86E-04
8	2.02E+02	5.79E-05	1.70E+02	4.87E-05	4.00E+00	1.02E-04		
10	6.82E+04	1.95E-02						
12			3.10E+05	8.88E-02	3.66E+03	9.31E-02	2.10E+03	5.34E-02
16	1.55E+06	4.44E-01	4.00E+06	1.15E+00	2.38E+04	6.05E-01		
20	2.00E+06	5.73E-01	4.55E+06	1.30E+00	1.92E+04	4.88E-01	1.45E+04	3.69E-01
24	3.15E+06	9.02E-01	4.35E+06	1.25E+00	2.71E+04	6.89E-01		
28	8.50E+05	2.43E-01	3.10E+06	8.88E-01	2.01E+04	5.11E-01	2.08E+04	5.29E-01
35	3.70E+06	1.06E+00	3.45E+06	9.88E-01	8.40E+03	2.14E-01	1.84E+04	4.68E-01

45	2.45E+06	7.02E-01			1.63E+04	4.14E-01	2.82E+04	7.17E-01
55	3.20E+06	9.16E-01	3.28E+06	9.38E-01	1.12E+04	2.85E-01	2.14E+04	5.44E-01
Trial #8								
4			2.00E+00	4.39E-07				
8	1.00E+00	2.19E-07						
10	9.47E+03	2.08E-03	3.85E+03	8.44E-04	5.80E+01	5.92E-04	2.10E+01	2.14E-04
14	1.90E+06	4.17E-01	1.29E+06	2.83E-01	5.00E+03	5.10E-02	4.00E+03	4.08E-02
16					1.01E+04	1.03E-01		
18	3.67E+06	8.05E-01	1.55E+05	3.40E-02	1.20E+04	1.22E-01	8.10E+03	8.27E-02
22	2.62E+06	5.75E-01	3.64E+06	7.98E-01	1.10E+04	1.12E-01	1.90E+04	1.94E-01
26	3.74E+06	8.20E-01	3.21E+06	7.04E-01	2.10E+04	2.14E-01	2.50E+04	2.55E-01
35	5.15E+06	1.13E+00	5.91E+06	1.30E+00	6.00E+04	6.12E-01	5.30E+04	5.41E-01
50	5.26E+06	1.15E+00	4.96E+06	1.09E+00	1.05E+05	1.07E+00	9.27E+04	9.46E-01
70	4.96E+06	1.09E+00	6.32E+06	1.39E+00	1.18E+05	1.21E+00	1.42E+05	1.45E+00
90					9.68E+04	9.88E-01	1.52E+05	1.55E+00
110	6.60E+06	1.45E+00	2.88E+06	6.32E-01	1.40E+05	1.43E+00	1.78E+05	1.82E+00
Trial #9								
4	1.74E+04	2.72E-03	5.98E+03	9.34E-04	1.00E+01	1.85E-04	8.00E+00	1.48E-04
8	2.60E+04	4.06E-03	1.68E+02	2.62E-05	2.00E+00	3.70E-05	2.00E+00	3.70E-05
12	2.64E+06	4.12E-01	9.18E+05	1.43E-01	6.32E+02	1.17E-02	9.80E+01	1.81E-03
16	4.86E+06	7.59E-01	5.18E+06	8.09E-01	8.90E+01	1.64E-03	1.33E+02	2.46E-03
20	3.55E+07	5.55E+00	4.92E+06	7.68E-01	3.38E+02	6.24E-03	2.11E+02	3.90E-03
26	3.12E+06	4.87E-01			3.24E+03	5.99E-02	3.00E+02	5.54E-03
35			4.30E+06	6.71E-01			1.36E+03	2.52E-02
45							6.34E+02	1.17E-02
50	2.32E+06	3.62E-01	6.44E+06	1.01E+00	1.90E+02	3.51E-03	7.00E+01	1.29E-03
70			6.98E+06	1.09E+00	7.40E+01	1.37E-03	1.60E+01	2.96E-04
80			6.39E+06	9.98E-01				
90			2.42E+06	3.78E-01			2.00E+01	3.70E-04
100			6.09E+06	9.51E-01				
110	5.50E+06	8.59E-01	4.56E+06	7.12E-01	3.00E+01	5.54E-04	4.00E+01	7.39E-04
130							1.62E+03	2.98E-02
150			9.19E+06	1.43E+00	3.50E+03	6.47E-02	7.30E+02	1.35E-02

170							4.00E+01	7.39E-04
190	6.68E+06	1.04E+00	5.28E+06	8.24E-01			4.85E+02	8.96E-03
210							4.65E+02	8.59E-03
230			5.02E+06	7.84E-01	1.74E+03	3.21E-02	3.30E+02	6.10E-03
Trial #10								
4	2.00E+00	6.63E-07	2.00E+00	6.63E-07	2.00E+00	1.48E-05	2.00E+00	1.48E-05
8	2.00E+00	6.63E-07	2.00E+00	6.63E-07	2.00E+00	1.48E-05	2.00E+00	1.48E-05
18	2.00E+00	6.63E-07	4.00E+00	1.33E-06	2.00E+00	1.48E-05	2.00E+00	1.48E-05
24	1.00E+01	3.31E-06						
26			1.74E+02	5.76E-05				
30	2.00E+00	6.63E-07						
40	9.80E+01	3.25E-05	1.20E+02	3.98E-05				
45	1.36E+02	4.51E-05						
55	2.00E+02	6.63E-05	1.65E+02	5.47E-05				
70	1.12E+03	3.71E-04						
80	1.18E+04	3.91E-03						
100	1.80E+03	5.96E-04	4.20E+03	1.39E-03				
140	1.88E+04	6.23E-03	1.68E+04	5.57E-03				
180	1.58E+04	5.23E-03	1.92E+04	6.36E-03	1.25E-01	9.24E-07		
240			1.52E+05	5.03E-02	1.00E+00	7.39E-06	1.00E+00	7.39E-06
270	1.68E+04	5.57E-03						
300	1.96E+04	6.49E-03	3.96E+04	1.31E-02				
340	1.18E+04	3.90E-03	5.78E+04	1.91E-02	5.00E-01	3.69E-06		
360	9.38E+04	3.11E-02	1.52E+04	5.04E-03				
380	7.92E+04	2.62E-02	8.28E+04	2.74E-02				
390					1.25E+00	9.24E-06	1.25E+00	9.24E-06
410	1.42E+05	4.70E-02						
420			1.14E+05	3.78E-02	1.00E+00	7.39E-06	1.75E+00	1.29E-05
Trial #11								
2	3.80E+02	4.96E-05	9.38E+02	1.23E-04	2.00E+00	2.86E-05	1.00E+00	1.43E-05
6	7.70E+01	1.01E-05			1.00E+00	1.43E-05		
10	5.15E+04	6.73E-03	3.67E+03	4.79E-04	6.40E+01	9.16E-04	1.80E+01	2.58E-04
12			8.55E+05	1.12E-01	4.66E+02	6.67E-03		

16	3.92E+06	5.12E-01			1.35E+03	1.93E-02	1.71E+03	2.45E-02
20	4.30E+06	5.62E-01			8.20E+02	1.17E-02		
26	3.20E+06	4.18E-01			1.72E+03	2.46E-02	1.99E+03	2.85E-02
32	4.08E+06	5.33E-01	5.13E+06	6.70E-01	8.50E+02	1.22E-02	1.55E+03	2.22E-02
47	4.56E+06	5.96E-01	3.61E+06	4.72E-01	2.35E+03	3.36E-02	2.80E+03	4.01E-02
72	2.08E+06	2.72E-01	3.03E+06	3.96E-01	3.95E+03	5.65E-02	2.30E+03	3.29E-02
92	1.46E+06	1.91E-01			1.40E+03	2.00E-02		
112	2.56E+06	3.34E-01	3.44E+06	4.49E-01	8.25E+03	1.18E-01	4.85E+03	6.94E-02
132	2.59E+06	3.38E-01			1.75E+03	2.50E-02		
152	4.22E+06	5.51E-01	3.28E+06	4.28E-01	1.90E+03	2.72E-02	3.15E+03	4.51E-02
172	5.94E+06	7.76E-01	2.98E+06	3.89E-01	1.50E+03	2.15E-02		
192	4.64E+06	6.06E-01	2.54E+06	3.32E-01	1.30E+03	1.86E-02	2.85E+03	4.08E-02
212					8.55E+02	1.22E-02		
230	5.76E+06	7.52E-01	3.84E+06	5.02E-01	3.50E+02	5.01E-03	2.56E+03	3.66E-02
235	5.30E+06	6.92E-01	4.42E+06	5.77E-01	1.40E+03	2.00E-02		
Trial #12								
4	1.00E+00	1.76E-06	1.00E+00	1.76E-06	1.00E+00	1.49E-05	1.00E+00	1.49E-05
8	1.00E+00	1.76E-06	1.00E+00	1.76E-06	1.00E+00	1.49E-05	1.00E+00	1.49E-05
16	9.00E+00	1.58E-05	8.00E+00	1.41E-05				
20	5.00E+00	8.79E-06	4.00E+00	7.04E-06				
26	7.00E+00	1.23E-05	1.60E+01	2.81E-05				
35	1.20E+00	2.11E-06	4.40E+00	7.74E-06				
50	9.60E+00	1.69E-05	1.20E+00	2.11E-06				
60					1.00E+00	1.49E-05	1.00E+00	1.49E-05
80	2.30E+01	4.05E-05	3.23E+01	5.69E-05				
110	3.96E+02	6.96E-04	7.20E+01	1.27E-04				
140	4.53E+02	7.97E-04	4.38E+02	7.70E-04				
170	1.90E+02	3.34E-04			1.00E+00	1.49E-05		
180	9.94E+02	1.75E-03	7.10E+02	1.25E-03			2.00E-01	2.98E-06
210	1.13E+03	1.99E-03	1.13E+03	1.99E-03				
240	6.90E+02	1.21E-03	9.30E+02	1.64E-03				
270	1.64E+03	2.88E-03	1.12E+03	1.97E-03				
300	2.24E+03	3.94E-03	6.21E+03	1.09E-02	1.00E+00	1.49E-05	4.00E-01	5.97E-06

330	3.58E+03	6.30E-03			2.00E+00	2.98E-05	1.00E+00	1.49E-05
340			2.84E+03	4.99E-03				
360	3.80E+03	6.68E-03	3.50E+03	6.16E-03	1.00E+00	1.49E-05	1.00E+00	1.49E-05
390	3.40E+03	5.98E-03	1.27E+03	2.23E-03	1.00E+00	1.49E-05	1.00E+00	1.49E-05
410	3.00E+03	5.28E-03	9.40E+03	1.65E-02	1.00E+00	1.49E-05	1.00E+00	1.49E-05
Trial #13								
2	6.00E+00	5.22E-07	4.13E+02	3.59E-05	1.00E+00	9.27E-06	7.00E+00	6.49E-05
4	4.10E+01	3.57E-06			1.00E+00	9.27E-06		
6	1.36E+02	1.18E-05	1.89E+02	1.64E-05	3.00E+00	2.78E-05	4.00E+00	3.71E-05
8	2.01E+02	1.75E-05			4.00E+00	3.71E-05		
10	9.10E+03	7.91E-04						
12	1.51E+06	1.31E-01	2.80E+05	2.43E-02	1.82E+03	1.69E-02	1.23E+03	1.14E-02
16	4.07E+06	3.54E-01			5.63E+03	5.22E-02		
20	4.46E+06	3.88E-01	9.89E+06	8.60E-01	2.60E+03	2.41E-02	2.80E+03	2.60E-02
24	7.20E+06	6.26E-01			4.60E+03	4.27E-02		
30	7.01E+06	6.10E-01	1.03E+07	8.93E-01	4.40E+03	4.08E-02	4.50E+03	4.17E-02
40	1.02E+07	8.87E-01			4.70E+03	4.36E-02		
50	4.80E+06	4.17E-01	7.70E+06	6.70E-01	5.30E+03	4.91E-02	8.40E+03	7.79E-02
60	1.19E+07	1.03E+00			3.50E+03	3.25E-02		
80	1.07E+07	9.30E-01	9.50E+06	8.26E-01	9.30E+03	8.62E-02	1.12E+04	1.04E-01
100	2.18E+07	1.90E+00			9.40E+03	8.72E-02		
120	1.66E+07	1.44E+00	6.20E+06	5.39E-01	9.50E+03	8.81E-02	7.90E+03	7.33E-02
Trial #14								
4	1.00E+00	1.62E-07	1.00E+00	1.62E-07	1.00E+00	1.14E-05	1.00E+00	1.14E-05
8	2.00E+01	3.24E-06	4.00E+01	6.48E-06	1.00E+00	1.14E-05	1.00E+00	1.14E-05
12	3.51E+06	5.69E-01	1.74E+06	2.82E-01	3.20E+03	3.66E-02	2.40E+03	2.74E-02
14					1.00E+00	1.14E-05	5.32E+02	6.08E-03
20	4.87E+06	7.89E-01	5.16E+06	8.36E-01	8.80E+03	1.01E-01	5.30E+03	6.06E-02
22					7.44E+04	8.50E-01	6.46E+04	7.38E-01
24	2.00E+07	3.24E+00	5.45E+07	8.83E+00	4.00E+04	4.57E-01	1.60E+05	1.83E+00
26					6.30E+04	7.20E-01	3.84E+04	4.39E-01
28	1.52E+07	2.47E+00	4.20E+07	6.81E+00	7.62E+04	8.71E-01	3.74E+04	4.27E-01
30					7.68E+04	8.78E-01	7.04E+04	8.05E-01

40	8.35E+06	1.35E+00	4.61E+06	7.47E-01	1.58E+04	1.81E-01	8.20E+03	9.37E-02
50	3.22E+06	5.22E-01	3.66E+06	5.93E-01	4.80E+03	5.49E-02	1.03E+04	1.18E-01
90	3.36E+06	5.45E-01	4.02E+06	6.52E-01	1.20E+04	1.37E-01	9.30E+03	1.06E-01
105					3.20E+03	3.66E-02	1.40E+03	1.60E-02
130	5.00E+06	8.10E-01	5.12E+06	8.30E-01	4.30E+03	4.91E-02	2.70E+03	3.09E-02
180	3.60E+06	5.83E-01	2.52E+06	4.08E-01	7.40E+03	8.46E-02	9.00E+03	1.03E-01
210	2.30E+06	3.73E-01	3.48E+06	5.64E-01	8.50E+03	9.71E-02	4.70E+03	5.37E-02
Trial #15								
2			1.00E+01	1.32E-06	1.00E+00	1.29E-05	1.00E+00	1.29E-05
4	1.00E+00	1.32E-07			1.00E+00	1.29E-05		
6	8.00E+00	1.05E-06			1.00E+00	1.29E-05		
8					2.00E+00	2.58E-05	4.00E+00	5.15E-05
10	1.63E+04	2.14E-03						
12					3.25E+02	4.18E-03		
14	3.47E+06	4.57E-01			2.14E+02	2.76E-03		
18	1.84E+06	2.42E-01	3.57E+06	4.70E-01	4.20E+02	5.41E-03	8.50E+02	1.09E-02
22	5.70E+06	7.50E-01			1.17E+04	1.51E-01		
26	7.95E+06	1.05E+00	4.35E+06	5.72E-01	1.20E+04	1.55E-01	5.00E+03	6.44E-02
33	4.70E+06	6.18E-01			1.55E+04	2.00E-01		
43	6.15E+06	8.09E-01	8.10E+06	1.07E+00	3.10E+04	3.99E-01	1.30E+04	1.67E-01
53	5.95E+06	7.83E-01			3.80E+04	4.89E-01		
63	4.95E+06	6.51E-01	4.00E+06	5.26E-01	3.00E+04	3.86E-01		
73							1.00E+04	1.29E-01
83	4.95E+06	6.51E-01	7.20E+06	9.47E-01	2.80E+04	3.61E-01		
93	4.19E+07	5.51E+00			1.00E+04	1.29E-01	2.60E+04	3.35E-01
103					2.80E+04	3.61E-01		
108	9.70E+06	1.28E+00						
113	1.02E+07	1.34E+00	1.10E+07	1.44E+00				
118					3.80E+04	4.89E-01	2.10E+04	2.70E-01
Trial #16								
2	3.10E+01	5.69E-06	1.70E+01	3.12E-06			2.00E+00	3.89E-05
6	2.70E+01	4.95E-06	7.00E+00	1.28E-06	2.00E+00	3.89E-05	1.00E+00	1.95E-05
8	4.60E+01	8.44E-06	6.00E+00	1.10E-06			2.00E+00	3.89E-05

10	1.16E+05	2.13E-02	6.60E+02	1.21E-04	1.42E+03	2.76E-02	7.50E+01	1.46E-03
12	3.01E+06	5.52E-01	3.78E+06	6.94E-01	7.20E+03	1.40E-01	3.65E+03	7.10E-02
14	2.54E+06	4.66E-01	2.90E+06	5.32E-01	9.00E+03	1.75E-01	1.67E+04	3.24E-01
16	3.88E+06	7.12E-01	4.56E+06	8.37E-01	1.27E+04	2.46E-01	1.17E+04	2.28E-01
18	2.72E+06	4.99E-01	4.18E+06	7.67E-01	9.20E+03	1.79E-01	1.48E+04	2.88E-01
26					1.17E+04	2.27E-01	1.60E+04	3.11E-01
30	6.22E+06	1.14E+00	7.98E+06	1.46E+00	2.04E+04	3.97E-01	1.94E+04	3.78E-01
60					2.02E+04	3.93E-01	1.86E+04	3.62E-01
80	7.14E+06	1.31E+00	6.54E+06	1.20E+00				
90					9.02E+04	1.76E+00	2.30E+04	4.48E-01
120	8.60E+06	1.58E+00	6.84E+06	1.26E+00	3.14E+04	6.11E-01	2.24E+04	4.36E-01
175	5.13E+06	9.42E-01	2.83E+06	5.20E-01				
205					3.14E+04	6.11E-01	2.52E+04	4.91E-01
240	5.43E+06	9.97E-01	5.57E+06	1.02E+00	3.16E+04	6.15E-01	2.52E+04	4.91E-01

Table C. 6. (Bio)Colloid Removal (Log) During the 16 Trials of Experiment 2

Trial	Column	ES (mm)	UC*	IS (mM KCl)	NOM (mg/L)	PR772	*S. typhimurium*	1.1 µm MS	4.5 µm MS
						Removal (Log)			
1	a	0.4	1	100	5	0.18	0.37	0.14	0.31
1	a	0.4	1	10	5	0.50	0.41	0.20	0.18
1	a	0.4	1	10	5	0.39	0.31	0.24	0.16
1	a	0.4	1	10	5	0.21	0.43	0.16	0.05
1	a	0.4	1	10	5	0.21	0.25	0.04	0.34
1	b	0.4	1	10	5	0.28	0.26	0.06	0.50
1	b	0.4	1	10	5	0.36	0.21	-0.21	0.39
1	b	0.4	1	10	5	0.33	0.24	-0.01	0.57
1	b	0.4	1	10	5	0.33	0.30	0.02	0.35
1	b	0.4	1	10	5	0.51	0.34	0.00	0.12
1	b	0.4	1	10	5		0.30	-0.03	
1	b	0.4	1	10	5		0.33	0.09	
1	b	0.4	1	10	5		0.17		
1	b	0.4	1	10	5		0.28		
2	a	0.4	1	10	0	0.17	1.08	0.40	0.60
2	a	0.4	1	10	0	0.15	1.01	0.71	0.65
2	a	0.4	1	10	0	0.14	0.91	0.23	0.72
2	a	0.4	1	10	0	0.11	1.11	0.41	0.56
2	a	0.4	1	10	0	0.21	0.89	0.33	0.73
2	a	0.4	1	10	0	0.06		0.16	
2	b	0.4	1	10	0	0.39	0.87	0.62	2.42
2	b	0.4	1	10	0	0.17	1.26	0.42	1.70
2	b	0.4	1	10	0	0.15	1.07	0.75	0.67
2	b	0.4	1	10	0	0.14	1.13	0.15	0.57
2	b	0.4	1	10	0	0.11	0.81	0.56	2.62
2	b	0.4	1	10	0		0.92	0.07	2.34

2	b	0.4	1	10	0			0.41	0.30
2	b	0.4	1	10	0			0.56	2.58
2	b	0.4	1	10	0				1.73
3	a	0.4	0	10	5	-0.19	0.03	0.35	0.83
3	a	0.4	0	10	5	-0.10	0.02	0.55	0.54
3	a	0.4	0	10	5	-0.18	-0.16	-0.06	0.37
3	a	0.4	0	10	5	-0.20	-0.09	0.45	0.64
3	a	0.4	0	10	5	-0.12	0.48	0.42	0.70
3	a	0.4	0	10	5		-0.17		0.14
3	a	0.4	0	10	5				1.08
3	a	0.4	0	10	5				0.39
3	a	0.4	0	10	5				0.80
3	a	0.4	0	10	5				0.50
3	a	0.4	0	10	5				0.92
3	b	0.4	0	10	5	-0.31	-0.21	0.35	1.79
3	b	0.4	0	10	5	-0.41	-0.10	0.55	1.90
3	b	0.4	0	10	5	-0.33	-0.24	-0.06	0.90
3	b	0.4	0	10	5	-0.58	-0.28	0.45	1.07
3	b	0.4	0	10	5		0.01	0.42	1.21
3	b	0.4	0	10	5		-0.15	-0.09	
3	b	0.4	0	10	5	-0.18			
4	a	0.4	0	10	0	0.06	1.79	0.14	1.03
4	a	0.4	0	10	0	-0.06	1.97	0.21	0.58
4	a	0.4	0	10	0	-0.08	1.82	0.14	0.70
4	a	0.4	0	10	0	-0.05	1.80	0.02	0.65
4	a	0.4	0	10	0	0.01	1.91	0.11	0.93
4	a	0.4	0	10	0		1.73		
4	a	0.4	0	10	0		1.65		
4	b	0.4	0	10	0	-0.05	2.25	0.26	0.64
4	b	0.4	0	10	0	-0.05	2.08	0.35	0.66
4	b	0.4	0	10	0	-0.13	1.79	0.30	0.69

4	b	0.4	0	10	0	-0.03	1.97	0.28	0.93
4	b	0.4	0	10	0	-0.08	1.82	-0.31	0.72
5	a	0.4	1	0.01	5	0.06	0.85	0.25	0.44
5	a	0.4	1	0.01	5	0.06	0.82	0.14	0.74
5	a	0.4	1	0.01	5	0.00	0.90	0.13	0.42
5	a	0.4	1	0.01	5	0.02	0.93	0.12	0.30
5	a	0.4	1	0.01	5	0.38	0.96	0.00	0.52
5	a	0.4	1	0.01	5		0.90		
5	a	0.4	1	0.01	5		0.83		
5	b	0.4	1	0.01	5	0.02	0.94	0.05	0.43
5	b	0.4	1	0.01	5	0.03	0.82	0.25	0.40
5	b	0.4	1	0.01	5	-0.07	0.87	-0.16	0.38
5	b	0.4	1	0.01	5	0.20	0.94	0.21	0.42
5	b	0.4	1	0.01	5	0.06	0.83	0.07	0.72
5	b	0.4	1	0.01	5	0.06	0.91		0.41
5	b	0.4	1	0.01	5	0.00	0.87		
5	b	0.4	1	0.01	5	0.02	0.92		
5	b	0.4	1	0.01	5		0.96		
5	b	0.4	1	0.01	5		1.02		
6	a	0.4	1	0.01	0	-0.03	0.446401	-0.15	0.22
6	a	0.4	1	0.01	0	-0.03	0.294262	-0.06	0.19
6	a	0.4	1	0.01	0	0.06	0.268196	0.02	0.20
6	a	0.4	1	0.01	0	0.03	0.515117	0.03	0.12
6	a	0.4	1	0.01	0	-0.09	0.346535	-0.11	0.02
6	a	0.4	1	0.01	0	-0.09			0.14
6	a	0.4	1	0.01	0				0.20
6	a	0.4	1	0.01	0				
6	a	0.4	1	0.01	0				
6	b	0.4	1	0.01	0	0.18	0.65	0.19	0.58
6	b	0.4	1	0.01	0	0.04	0.63	-0.22	0.61
6	b	0.4	1	0.01	0	-0.07	0.61	-0.16	0.22

6	b	0.4	1	0.01	0	0.00	0.57	-0.21	0.19
6	b	0.4	1	0.01	0	0.06	0.58	-0.24	0.20
6	b	0.4	1	0.01	0	0.05	0.45		0.06
6	b	0.4	1	0.01	0	-0.21			
6	b	0.4	1	0.01	0	-0.11			
6	b	0.4	1	0.01	0	-0.20			
7	a	0.4	0	0.01	5	0.03	0.39	-0.06	0.28
7	a	0.4	0	0.01	5	-0.04	0.47	-0.12	0.33
7	a	0.4	0	0.01	5	-0.09	0.37	-0.10	0.14
7	a	0.4	0	0.01	5	-0.09	0.42	0.05	0.26
7	a	0.4	0	0.01	5	-0.08	0.49	0.01	0.12
7	a	0.4	0	0.01	5	-0.06	0.54	0.03	
7	a	0.4	0	0.01	5	-0.11	0.45		
7	a	0.4	0	0.01	5	-0.04	0.49		
7	a	0.4	0	0.01	5	-0.05	0.49		
7	b	0.4	0	0.01	5	-0.10	0.53	0.04	0.22
7	b	0.4	0	0.01	5	-0.09	0.49	0.61	0.31
7	b	0.4	0	0.01	5	0.01	0.45	-0.03	0.16
7	b	0.4	0	0.01	5	-0.08	0.52	0.15	0.29
7	b	0.4	0	0.01	5	-0.03	0.45	0.04	0.67
7	b	0.4	0	0.01	5	0.39	0.47	0.25	0.38
7	b	0.4	0	0.01	5	0.08	0.56	-0.07	0.55
7	b	0.4	0	0.01	5		0.55		0.62
7	b	0.4	0	0.01	5				0.18
8	a	0.4	0	0.01	0	0.05	0.35	-0.11	-0.03
8	a	0.4	0	0.01	0	0.09	0.36	-0.04	-0.08
8	a	0.4	0	0.01	0	-0.15	0.34	-0.14	0.01
8	a	0.4	0	0.01	0	-0.21	0.26	-0.05	0.02
8	a	0.4	0	0.01	0	-0.22	0.16	-0.06	-0.16
8	a	0.4	0	0.01	0	-0.07	0.23	-0.04	
8	b	0.4	0	0.01	0	0.15	0.59	0.09	0.21

8	b	0.4	0	0.01	0	-0.11	0.49	-0.05	-0.03
8	b	0.4	0	0.01	0	-0.19	0.43	-0.06	-0.08
8	b	0.4	0	0.01	0	-0.11	0.42	-0.04	0.01
8	b	0.4	0	0.01	0	-0.11	0.29	-0.16	-0.15
9	a	0.1	1	10	5	-0.12	0.51	-0.16	2.05
9	a	0.1	1	10	5	0.12	0.43	0.08	2.07
9	a	0.1	1	10	5	0.38	0.42	0.11	2.21
9	a	0.1	1	10	5	0.33	0.36	-0.19	2.45
9	a	0.1	1	10	5	0.07	0.33	-0.03	2.30
9	a	0.1	1	10	5			0.33	1.34
9	a	0.1	1	10	5			0.04	3.43
9	a	0.1	1	10	5				3.43
9	a	0.1	1	10	5				2.69
9	a	0.1	1	10	5				1.70
9	a	0.1	1	10	5				2.34
9	b	0.1	1	10	5	0.16	0.48	0.44	1.93
9	b	0.1	1	10	5	-0.08	0.54	0.07	2.78
9	b	0.1	1	10	5	0.40	0.57	-0.02	2.20
9	b	0.1	1	10	5	-0.06	0.48	0.20	1.22
9	b	0.1	1	10	5	-0.07	0.43	-0.46	2.45
9	b	0.1	1	10	5		0.30		2.86
9	b	0.1	1	10	5		0.26		
10	a	0.1	1	10	0	0.36	0.71	1.51	5.03
10	a	0.1	1	10	0	0.44	0.89	1.58	5.13
10	a	0.1	1	10	0	0.26	1.27	1.33	4.86
10	a	0.1	1	10	0	0.36	0.63	1.40	5.03
10	a	0.1	1	10	0	0.25	0.63	1.56	4.89
10	a	0.1	1	10	0		0.85	1.42	
10	b	0.1	1	10	0	0.30	0.86	2.41	5.13
10	b	0.1	1	10	0	0.37	0.89	1.51	5.43
10	b	0.1	1	10	0	0.54	0.78	1.58	5.03

10	b	0.1	1	10	0	0.18	0.73	1.33	5.13
10	b	0.1	1	10	0	0.42	1.27	1.40	4.86
10	b	0.1	1	10	0	0.29	0.66		
10	b	0.1	1	10	0		0.70		
11	a	0.1	0	10	5	-0.09	0.81	0.33	1.60
11	a	0.1	0	10	5	-0.03	0.71	0.40	1.57
11	a	0.1	0	10	5	-0.05	0.76	0.35	1.67
11	a	0.1	0	10	5	-0.01	0.80	0.37	1.73
11	a	0.1	0	10	5	0.00	0.45	0.41	1.91
11	a	0.1	0	10	5			0.48	
11	a	0.1	0	10	5			0.30	
11	b	0.1	0	10	5	0.19	0.69	0.29	1.61
11	b	0.1	0	10	5	0.11	0.79	0.25	1.55
11	b	0.1	0	10	5	0.22	0.80	0.38	1.65
11	b	0.1	0	10	5	0.17	0.86	0.27	1.40
11	b	0.1	0	10	5	-0.05	0.73	0.23	1.48
11	b	0.1	0	10	5	-0.03			1.16
11	b	0.1	0	10	5				1.35
11	b	0.1	0	10	5				1.39
11	b	0.1	0	10	5				1.44
12	a	0.1	0	10	0	0.91	1.36	2.40	4.83
12	a	0.1	0	10	0	0.93	1.34	2.20	4.53
12	a	0.1	0	10	0	0.71	1.12	2.18	4.83
12	a	0.1	0	10	0	0.72	1.34	2.22	4.83
12	a	0.1	0	10	0	0.98	1.02	2.28	4.83
12	a	0.1	0	10	0	1.11			
12	a	0.1	0	10	0	0.84			
12	a	0.1	0	10	0	1.01			
12	b	0.1	0	10	0	1.42	1.33	2.90	5.22
12	b	0.1	0	10	0	0.91	1.44	2.70	4.83
12	b	0.1	0	10	0	0.93	1.30	2.79	4.83

12	b	0.1	0	10	0	0.71	1.09	2.71	4.83
12	b	0.1	0	10	0	0.72	1.10	2.30	4.83
12	b	0.1	0	10	0		1.03	2.21	
12	b	0.1	0	10	0			2.65	
13	a	0.1	1	0.01	5	0.06	-0.11	0.07	1.37
13	a	0.1	1	0.01	5	0.08	-0.06	0.05	1.39
13	a	0.1	1	0.01	5	0.03	-0.07	0.17	1.36
13	a	0.1	1	0.01	5	0.11	-0.01	0.08	1.31
13	a	0.1	1	0.01	5	0.06	-0.03	0.27	1.49
13	a	0.1	1	0.01	5		0.15		
13	b	0.1	1	0.01	5	0.05	0.03	0.20	1.59
13	b	0.1	1	0.01	5	0.24	0.03	0.22	1.38
13	b	0.1	1	0.01	5	0.21	0.02	0.05	1.11
13	b	0.1	1	0.01	5	0.07	0.02	0.38	0.98
13	b	0.1	1	0.01	5	0.02	0.10	-0.02	1.14
13	b	0.1	1	0.01	5	0.07		0.03	
13	b	0.1	1	0.01	5			-0.28	
13	b	0.1	1	0.01	5			-0.16	
14	a	0.1	1	0.01	0	0.07	0.92	0.23	1.44
14	a	0.1	1	0.01	0	-0.04	1.04	0.19	1.31
14	a	0.1	1	0.01	0	0.38	0.98	0.08	1.07
14	a	0.1	1	0.01	0	0.12	0.86	0.39	1.01
14	a	0.1	1	0.01	0	0.09	1.09	0.25	1.51
14	a	0.1	1	0.01	0	0.01	1.05		0.99
14	a	0.1	1	0.01	0		1.03		1.27
14	b	0.1	1	0.01	0	0.14	1.07	0.28	1.26
14	b	0.1	1	0.01	0	0.57	0.86	0.26	0.86
14	b	0.1	1	0.01	0	0.35	1.02	0.09	1.44
14	b	0.1	1	0.01	0	0.01	0.97	0.23	1.31
14	b	0.1	1	0.01	0	-0.05	0.99	0.43	1.07
14	b	0.1	1	0.01	0		1.35		1.01

14	b	0.1	1	0.01	0		1.05		
14	b	0.1	1	0.01	0		1.05		
14	b	0.1	1	0.01	0		1.08		
15	a	0.1	0	0.01	5	0.46	0.30	0.21	0.40
15	a	0.1	0	0.01	5	0.16	0.34	0.09	0.31
15	a	0.1	0	0.01	5	0.26	0.24	0.11	0.41
15	a	0.1	0	0.01	5	0.23	0.10	0.19	0.44
15	a	0.1	0	0.01	5	0.07	0.68	0.19	0.44
15	b	0.1	0	0.01	5	0.24	0.59	0.33	1.19
15	b	0.1	0	0.01	5	0.27	0.43	0.24	0.78
15	b	0.1	0	0.01	5	0.34	0.37	-0.03	0.89
15	b	0.1	0	0.01	5	0.05	0.39	0.28	0.48
15	b	0.1	0	0.01	5	0.21	0.28	0.02	0.57
15	b	0.1	0	0.01	5	0.02	0.74		
16	a	0.1	0	0.01	0	-0.08	0.04	-0.06	0.35
16	a	0.1	0	0.01	0	0.15	0.12	-0.12	0.36
16	a	0.1	0	0.01	0	0.30	0.12	-0.20	0.31
16	a	0.1	0	0.01	0	0.02	0.08	0.03	0.31
16	a	0.1	0	0.01	0	0.04	0.07	0.00	0.17
16	a	0.1	0	0.01	0			-0.03	0.28
16	b	0.1	0	0.01	0	0.12	0.11	0.16	0.40
16	b	0.1	0	0.01	0	0.10	0.13	0.27	0.41
16	b	0.1	0	0.01	0	-0.14	-0.22	0.08	-0.24
16	b	0.1	0	0.01	0	-0.10	0.27	0.12	0.21
16	b	0.1	0	0.01	0	0.05	0.03	-0.17	0.21
16	b	0.1	0	0.01	0		0.21		0.21
16	b	0.1	0	0.01	0				0.05

*UC values represented by “1” for high UC media and “0” for low UC media

Appendix D – Experiment 3

Table C. 1. Bacterial Pathogen Removal (%) After Exposure to Each Water Matrix used in Experiment 3

Water Matrix	Column	Removal (%)		
		E. coli	*Pseudomonas*	*Salmonella*
GW	1	44	45	48
GW	1	31	64	46
GW	1	50	60	47
GW	1	37	44	49
GW	1	57	60	50
GW	1	31	50	52
GW	2	59	40	43
GW	2	46	50	42
GW	2	45	45	51
GW	2	44	27	52
GW	2	36	67	34
GW	2	45	70	47
AG	1	69	96	57
AG	1	73	94	58
AG	1	62	93	54
AG	1	57	96	47
AG	1	55	93	32
AG	1	61	91	41
AG	2	58	83	47
AG	2	64	90	55
AG	2	49	87	38
AG	2	61	84	49
AG	2	51	86	53
AG	2	55	91	59
URB	1	65	64	59
URB	1	53	55	57
URB	1	56	70	49
URB	1	59	54	56
URB	1	53	72	47
URB	1	61	62	56
URB	2	55	66	40
URB	2	51	58	37
URB	2	55	57	57
URB	2	56	56	55
URB	2	67	50	56

URB	2	59	56	64
WW	1	66	82	66
WW	1	83	80	49
WW	1	75	86	53
WW	1	74	84	47
WW	1	80	89	55
WW	1	74	90	60
WW	2	85	93	64
WW	2	85	88	70
WW	2	77	93	51
WW	2	81	86	66
WW	2	72	88	58
WW	2	81	86	47

Table C. 2. Standard Curve Measurements for Proteins using BSA.

Standard (BSA)	Reading				Mean
(mg/L)	1	2	3	4	
0	0.000	0.000	0.000	0.000	0.000
5	0.022	0.017			0.020
10	0.046	0.050	0.031	0.030	0.039
25	0.136	0.114			0.125
50	0.253	0.315	0.261	0.313	0.286
100	0.562	0.629	0.559	0.534	0.571
150	0.803		0.762	0.746	0.770
200	1.023		0.949	1.019	0.997

Table C. 3. Standard Curve Measurements for Carbohydrates using BSA.

Standard (Glucose)	Reading					Mean
(mg/L)	1	2	3	4	5	
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.081	0.105	0.130	0.112	0.092	0.104
25	0.232	0.235	0.261	0.226	0.235	0.238
50	0.477	0.499	0.557	0.466	0.446	0.489
75	0.682	0.886	0.804	0.715	0.721	0.762
100	0.765	0.992	0.918	0.984	1.062	0.944

Table C. 4. Protein Measurements from EPS Extracts

Water Matrix	Sample ID†	*E. coli*			*Pseudomonas*			*Salmonella*		
		A*	[Proteins] (mg/L)	Concentration (mg/10^{10} cells)	A*	[Proteins] (mg/L)	Concentration (mg/10^{10} cells)	A*	[Proteins] (mg/L)	Concent-ration (mg/10^{10} cells)
AG	bl1-a	0.015	3.00	0.72	0.031	6.20	3.08	0.03	5.60	2.51
AG	bl2-a	0.012	2.40	0.46	0.041	8.20	5.38	0.03	6.00	2.88
AG	bl3-b	0.009	1.80	0.26	0.019	3.80	1.16	0.01	2.00	0.32
AG	bl4-b	0.004	0.80	0.05	0.019	3.80	1.16	0.01	1.80	0.26
AG	1a	0.063	12.60	12.25	0.098	19.60	30.28	0.08	16.60	21.59
AG	2a	0.059	11.80	10.69	0.093	18.60	27.22	0.06	12.80	12.65
AG	3b	0.091	18.20	26.05	0.126	25.20	50.35	0.08	15.80	19.52
AG	4b	0.098	19.60	30.28	0.146	29.20	67.76	0.08	16.40	21.06
AG	5b	0.112	22.40	39.69	0.126	25.20	50.35	0.07	14.60	16.60
AG	6b	0.101	20.20	32.19	0.128	25.60	51.98	0.07	14.60	16.60
AG	free-a	0.106	21.20	35.50	0.122	24.40	47.18	0.12	23.40	43.35
AG	free-a	0.098	19.60	30.28	0.125	25.00	49.55	0.13	25.40	51.16
AG	free-b	0.215	43.00	147.47	0.160	32.00	81.47	0.13	26.40	55.30
AG	free-b	0.210	42.00	140.67	0.158	31.60	79.43	0.13	25.80	52.80
GW	bl1-a	0.026	5.20	2.16	0.025	5.00	2.00	0.01	2.00	0.32
GW	bl2-a	0.026	5.20	2.16	0.035	7.00	3.92	0.01	2.00	0.32
GW	bl3-b	0.007	1.40	0.16	0.011	2.20	0.39	0.01	1.40	0.16
GW	bl4-b	0.007	1.40	0.16	0.012	2.40	0.46	0.01	1.60	0.20
GW	1a	0.099	19.80	31.34	0.067	13.40	14.34	0.06	12.00	11.49
GW	2a	0.103	20.60	33.92	0.066	13.20	13.91	0.06	11.80	11.11
GW	3b	0.131	26.20	54.89	0.137	27.40	60.03	0.12	24.40	47.60
GW	4b	0.133	26.60	56.58	0.140	28.00	62.69	0.12	24.60	48.39
GW	5b	0.120	24.00	46.05	0.137	27.40	60.03	0.12	23.00	42.29

GW	6b	0.131	26.20	54.89	0.130	26.00	54.05	0.12	23.80	45.29
GW	free-a	0.064	12.80	13.08	0.107	21.40	36.61	0.06	11.40	10.37
GW	free-a	0.061	12.20	11.88	0.121	24.20	46.82	0.06	12.40	12.27
GW	free-b	0.129	25.80	53.22	0.099	19.80	31.34	0.14	27.80	61.80
GW	free-b	0.131	26.20	54.89	0.106	21.20	35.93	0.13	25.20	50.78
URB	bl1-a	0.018	3.60	1.04	0.002	0.40	0.01	0.02	4.20	1.41
URB	bl2-a	0.012	2.40	0.46	0.001	0.20	0.00	0.03	5.20	2.16
URB	bl3-b	0.004	0.80	0.05	0.009	1.80	0.26	0.01	1.80	0.26
URB	bl4-b	0.005	1.00	0.08	0.007	1.40	0.16	0.01	1.60	0.20
URB	1a	0.209	41.80	134.49	0.278	55.60	242.02	0.15	29.80	65.75
URB	2a	0.378	75.60	451.94	0.184	36.80	103.05	0.15	29.80	65.75
URB	3b	0.224	44.80	155.27	0.161	32.20	77.65	0.10	19.80	26.07
URB	4b	0.274	54.80	234.95	0.173	34.60	90.48	0.11	21.40	31.34
URB	free-a	0.097	19.40	24.82	0.119	23.80	40.02	0.10	19.80	26.07
URB	free-a	0.094	18.80	22.98	0.123	24.60	43.12	0.10	19.60	25.44
URB	free-b	0.117	23.40	38.51	0.119	23.80	40.02	0.12	23.60	39.26
URB	free-b	0.108	21.60	32.03	0.118	23.60	39.26	0.13	25.20	45.51
WW	bl1-a	0.029	5.80	2.69	0.023	4.60	1.69	0.02	4.60	1.69
WW	bl2-a	0.021	4.20	1.41	0.025	5.00	2.00	0.02	4.20	1.41
WW	bl3-b	0.010	2.00	0.32	0.020	4.00	1.28	0.02	4.80	1.84
WW	bl4-b	0.008	1.60	0.20	0.022	4.40	1.55	0.02	4.60	1.69
WW	1a	0.236	47.20	162.79	0.429	85.80	573.49	0.20	39.40	108.75
WW	2a	0.209	41.80	124.34	0.275	55.00	226.56	0.23	46.40	156.80
WW	3b	0.311	62.20	294.07	0.268	53.60	214.40	0.24	48.40	171.97
WW	4b	0.320	64.00	312.24	0.281	56.20	237.24	0.23	46.20	155.32
WW	free-a	0.115	23.00	26.88	0.112	22.40	24.70	0.12	23.00	26.88
WW	free-a	0.118	23.60	29.12	0.122	24.40	32.19	0.12	23.20	27.62
WW	free-b	0.13	25.20	35.37	0.13	26.80	42.02	0.13	25.40	36.18
WW	free-b	0.13	26.80	42.02	0.13	25.60	36.99	0.12	23.00	26.88

*Absorbance; † ‘a’ and ‘b’ represent separate replicates, “bl” indicates a blank sample and “free” indicates loosely-bound EPS measurement

Table C. 5. Carbohydrate Measurements from EPS Extracts

Water Matrix	Sample ID†	*E. coli*			*Pseudomonas*			*Salmonella*		
		A*	[Proteins] (mg/L)	Concentration (mg/10^{10} cells)	A*	[Proteins] (mg/L)	Concentration (mg/10^{10} cells)	A*	[Proteins] (mg/L)	Concent-ration (mg/10^{10} cells)
AG	bl1-a	0.017	1.70	0.23	0.031	3.10	0.77	0.018	1.80	0.26
AG	bl2-a	0.035	3.50	0.98	0.035	3.50	0.98	0.031	3.10	0.77
AG	bl3-b	0.025	2.50	0.50	0.027	2.70	0.58	0.039	3.90	1.22
AG	bl4-b	0.016	1.60	0.20	0.021	2.10	0.35	0.035	3.50	0.98
AG	1a	0.038	3.80	1.16	0.158	15.80	19.97	0.062	6.20	3.08
AG	2a	0.043	4.30	1.48	0.136	13.60	14.80	0.072	7.20	4.15
AG	3b	0.106	10.60	8.99	0.111	11.10	9.86	0.039	3.90	1.22
AG	4b	0.118	11.80	11.14	0.108	10.80	9.33	0.051	5.10	2.08
AG	free-a	0.095	9.50	7.22	0.008	0.80	0.05	0.071	7.10	4.03
AG	free-a	0.091	9.10	6.62	0.025	2.50	0.50	0.079	7.90	4.99
AG	free-b	0.065	6.50	3.38	0.032	3.20	0.82	0.076	7.60	4.62
AG	free-b	0.06	6.00	2.88	0.043	4.30	1.48	0.084	8.40	5.64
GW	bl1-a	0.008	0.80	0.05	0.021	2.10	0.35	0.048	4.80	1.84
GW	bl2-a	0.013	1.30	0.14	0.02	2.00	0.32	0.048	4.80	1.84
GW	bl3-b	0.041	4.10	1.34	0.025	2.50	0.50	0.035	3.50	0.98
GW	bl4-b	0.011	1.10	0.10	0.037	3.70	1.10	0.036	3.60	1.04
GW	1a	0.02	2.00	0.32	0.157	15.70	19.72	0.211	21.10	35.62
GW	2a	0.027	2.70	0.58	0.12	12.00	11.52	0.314	31.40	78.88
GW	3b	0.097	9.70	7.53	0.084	8.40	5.64	0.164	16.40	21.52
GW	4b	0.098	9.80	7.68	0.07	7.00	3.92	0.162	16.20	21.00
GW	free-a	0.033	3.30	0.87	0.03	3.00	0.72	0.022	2.20	0.39
GW	free-a	0.03	3.00	0.72	0.028	2.80	0.63	0.023	2.30	0.42
GW	free-b	0.028	2.80	0.63	0.035	3.50	0.98	0.018	1.80	0.26
GW	free-b	0.024	2.40	0.46	0.064	6.40	3.28	0.017	1.70	0.23

URB	bl1-a	0.019	1.90	0.29	0.024	2.40	0.46	0.013	1.30	0.14
URB	bl2-a	0.016	1.60	0.20	0.025	2.50	0.50	0.017	1.70	0.23
URB	bl3-b	0.024	2.40	0.46	0.019	1.90	0.29	0.02	2.00	0.32
URB	bl4-b	0.023	2.30	0.42	0.016	1.60	0.20	0.022	2.20	0.39
URB	1a	0.021	2.10	35.28	0.176	17.60	24.78	0.138	13.80	15.24
URB	2a	0.019	1.90	28.88	0.186	18.60	27.68	0.135	13.50	14.58
URB	3b	0.254	25.40	51.61	0.242	24.20	46.85	0.115	11.50	10.58
URB	4b	0.264	26.40	55.76	0.206	20.60	33.95	0.149	14.90	17.76
URB	free-a	0.12	12.00	11.52	0.164	16.40	21.52	0.131	13.10	13.73
URB	free-a	0.121	12.10	11.71	0.12	12.00	11.52	0.151	15.10	18.24
URB	free-b	0.195	19.50	30.42	0.234	23.40	43.80	0.17	17.00	23.12
URB	free-b	0.274	27.40	60.06	0.21	21.00	35.28	0.19	19.00	28.88
WW	bl1-a	0.007	0.70	0.04	0.014	1.4	0.1568	0.021	2.10	0.35
WW	bl2-a	0.01	1.00	0.08	0.012	1.2	0.1152	0.024	2.40	0.46
WW	bl3-b	0.021	2.10	0.35	0.017	1.7	0.2312	0.025	2.50	0.50
WW	bl4-b	0.02	2.00	0.32	0.019	1.9	0.2888	0.025	2.50	0.50
WW	1a	0.227	22.70	41.22	0.193	19.3	29.7992	0.076	7.60	4.62
WW	2a	0.256	25.60	52.43	0.197	19.7	31.0472	0.069	6.90	3.81
WW	3b	0.141	14.10	15.90	0.127	12.7	12.9032	0.119	11.90	11.33
WW	4b	0.131	13.10	13.73	0.121	12.1	11.7128	0.138	13.80	15.24
WW	free-a	0.179	17.90	25.63	0.178	17.8	25.3472	0.16	16.00	20.48
WW	free-a	0.167	16.70	22.31	0.179	17.9	25.6328	0.159	15.90	20.22
WW	free-b	0.223	22.30	39.78	0.278	27.8	61.8272	0.21	21.00	35.28
WW	free-b	0.211	21.10	35.62	0.256	25.6	52.4288	0.205	20.50	33.62

*Absorbance; † ‘a’ and ‘b’ represent separate replicates, “bl” indicates a blank sample and “free” indicates loosely-bound EPS measurement

Appendix E – Confirmation of *E. coli* and *P. aeruginosa* Bacterial Isolate Species

UNIVERSITY of GUELPH
LABORATORY SERVICES
Agriculture and Food Laboratory

Submitted By:
UNIVERSITY OF WATERLOO
NICOLE MCLELLAN
200 UNIVERSITY AVE W
CIVIL ENGINEERING
WATERLOO, ON N2L 3G1
Phone: 519 888-4567
Sampling Date: 2011-Aug-23

Owner:
NICOLE MCLELLAN

Received Date: 2011-Aug-26

Microbial ID by 16S/18S rRNA

Date Authorized: 2011-Sep-08 14:14

Sample ID	Client Sample ID	Specimen/ Sampling Date/Time	Best Similar species	Similarity %	Sequence l (bp)
0001	01	Culture 11-Aug-23	Escherichia coli	100%	>1200
0002	02	Culture 11-Aug-23	Pseudomonas aeruginosa	100%	>1200

Comments:

02 Best Similar species Sample 0002 also showed 1005 similarity to Ps

Test method(s): MOL-020

Supervisor: Shu Chen PhD 519 823 1268 ext. 57319 schen@uoguelph.ca

This report may not be reproduced except in full without written approval by L
These test results pertain only to the specimens tested.