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WR07R001

TRANSPORT AND SURVIVAL OF PATHOGENIC BACTERIA ASSOCIATED WITH DAIRY MANURE IN SOIL AND GROUNDWATER

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Project Completion Report

Transport and Survival of Pathogenic Bacteria Associated With Dairy Manure in Soil and Groundwater (WR07R001)

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PROJECT SUMMARY

Title: Transport and Survival of Pathogenic Bacteria Associated With Dairy Manure in Soil and Groundwater

Project I.D. WR07R001

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Period of Contract: July 1, 2007 – June 30, 2009

Background/Need: With over 15,000 dairy farms and 1.2 million cows, the dairy industry is of enormous historical, economical and cultural importance to the State of Wisconsin. Approximately 48 million tons of manure is produced in Wisconsin annually and nearly two-thirds of all farms spread manure immediately on the surrounding fields because of inadequate storage. In recent years, much concern has arisen over the release of human pathogens into the water and food supply due to the land application of manures for crop fertilization, wash water and stormwater runoff. On October 11, 2006, the EPA signed the Ground Water Rule into law, which requires drinking water systems using groundwater sources to monitor for indicator organisms of pathogenic viruses and bacteria from fecal contamination. Knowledge of the factors that affect and facilitate the transport of manure associated pathogens will be critical to making an accurate determination of groundwater vulnerability and providing adequate drinking water treatment to prevent waterborne disease.

Objective: The overall **goal** of this project was to evaluate the impact of various manure components, e.g., pH, organic matter, nutrients, particulate matter, and potential biofilm growth on the transport and survival characteristics of fecal indicators and pathogenic bacterium *E. coli* O157:H7. Research **objectives** focused on determining the relationship between the deposition of indicator organisms *E. coli* and pathogenic serotype *E. coli* O157:H7 through well controlled laboratory column studies and examining the interactions between manure suspensions and bacterial strains and their associated transport behavior under different solution chemistry. In addition, the potential of biofilm formation as a result of various manure components and its impact on bacterial attachment and survival mechanisms was investigated.

Methods: A series of column experiments was designed to systematically investigate the deposition of each bacterial strain in porous media over a range of pH, ionic strength, flow velocity, and initial cell concentrations. The surface electrokenetic property of indicator organism *E. coli* and serotype O157:H7 were determined under solution chemistry (ionic strength) that is relevant to groundwater, wash water and stormwater runoff by measuring the bacterial electrophoretic mobility and zeta potential. Data obtained from these measurements were applied to the extended DLVO model that accounts for acid-base (polar) interactions in addition to the van der Waals and electrostatic forces. The significance of non-DLVO forces, such as hydration effects, hydrophobic attraction, steric repulsion and polymer bridging, were identified by comparing the actual bacterial adhesion to the grain surface and the theoretical value

obtained from classic models. Bacterial breakthrough curves and retained cell profiles were obtained under pore fluid conditions representing both natural aquifers and those during heavy rainfall events.

Results and Discussion:

Ionic strength is an important factor in the initial adhesion, distribution, and detachment of bacteria in groundwater environment. Changing ionic strength of the buffer solutions used in our column studies is able to alter the surface charge of the bacteria and/or collector grain surface and thereby alter the adhesion and detachment of O157:H7 EDL933 Δ *stx1, stx2* and JM109 pGFP to glass bead surfaces. The attachment and growth of bacteria on biofilm-covered porous media is the norm rather than the exception and has important implications in environmental and man-made filtration processes. Information on the interaction between bacterial cells and biofilm surfaces may be utilized in designing biologically active filters, improving bioremediation, and predicting pathogen transport. Findings of this study suggest that the composition of *P. aeruginosa* biofilm EPS plays a pivotal role in controlling the initial attachment and deposition profiles of *E. coli* JM109 in porous media through non DLVO forces, including hydrophobicity, hydration forces and polymeric bridging. The presence of biofilm is also essential for the survival and growth of *E. coli* after their initial adhesion and biofilm detachment is the main mechanism through which *E. coli* was released back to the bulk fluid after the contamination event.

Conclusions/Implications/Recommendations:

The composition of manure can impact the deposition and survival of microorganisms, especially during events of heavy precipitation, through physical, chemical and microbiological mechanisms, e.g., changes of pH, salinity of pore fluid, blocking of the favorable deposition site by organic matters, aggregations between manure suspensions and bacterial cells, and formation of biofilms. An important phenomenon that we found in this study is that the transport and adhesion behavior of *E. coli* O157 differ significantly model prediction and from behavior of other *E. coli* strains. Therefore the use of indicator bacteria to predict the risk of pathogen contamination in groundwater needs to be evaluated in detail in future study.

Related Publications:

Liu, Y., Li, J. 2008, "Role of *Pseudomonas aeruginosa* biofilm in the initial adhesion, growth and detachment of *Escherichia coli* in porous media", *Environmental Science and Technology*, 42(2), 443–449.

Marissa Jablonski, 2009, "Comparison of the Role of Ionic Strength and Surface Charge Heterogeneity on the Initial Adhesion, Distribution and Detachment of *E. coli* O157 and JM109 in Glass Beads-Packed Column Experiments", Master Thesis

Key Words: Bacterial Transport, Bacterial Adhesion, Groundwater, Manure, *E. coli, E. coli* O157

Funding: UWS

1.Introduction

Land application of animal manure is a cost effective and environmentally sustainable means to treat and reuse dairy farms by-products. Being an excellent resource for plant nutrients and organic matter, manure has the potential to improve soil fertility and productivity. However, improper management of animal wastes can severely threaten groundwater quality due to the presence of a variety of enteric pathogenic microorganisms including bacteria, protozoa and viruses in manure (Bradford et al., 2002, 2006). In the epidemiology of *E. coli* O157:H7 outbreaks in the United States (1982-2002), 10 out of the 39 waterborne outbreaks were associated with drinking water; many of them were directly or indirectly contaminated with animal manure (Islam et al., 2004; Muniesa et al., 2006). Recent outbreaks linked to the consumption of leafy greens further manifested that manure compost and irrigation water contaminated with manure are important vehicles by which pathogens can be introduced to groundwater and crops grown in the field. Studies have shown that cattle are the principal source of *E. coli* O157 infection with 15% of healthy cattle carrying O157. Cells spiked in compost and irrigation waters were persistent in soils and pre-harvest contamination can also occur through plant roots (Wachtel et al., 2002). Unfortunately, little monitoring of the occurrence of pathogens in complex environment involving groundwater and manure has been documented due to the low number of pathogens needed to cause illness and the limited sensitivity of detection methods. As a result, mechanisms governing the transport and survival of manure associated O157:H7 in soil and groundwater are poorly understood.

The transport and deposition of biocolloids, i.e., bacteria, viruses and protozoa, in porous media are commonly predicted using the classical filtration theory developed by Yao et al. (1971). In this "clean bed" filtration model, two separate rate limiting processes are responsible for the removal of suspended microbial particles: (1) the physical processes of diffusion, interception and gravitation settling that result in collisions between the colloids and collector grains and (2) the chemical factors controlling the interaction forces, e.g., van der Waals and electrostatic forces, that result in attachment of the biocolloids to the grain surface. According to this conceptual model, microbial particle concentration in the fluid phase is represented by first order kinetics with a spatially and temporally constant colloid deposition rate coefficient. The suspended and retained particle concentrations in the porous media are therefore predicted to decrease exponentially with transport distance. Despite widespread use in modeling and process design, a growing number of studies have demonstrated that discrepancies exist between experimental data and the log-linear decrease pattern anticipated from the "clean bed" theory under certain experimental conditions by investigating the concentration profiles of retained colloids (Li et al., 2004; Li et al., 2006; Liu et al., 2007; Tong et al., 2005). The divergence of experimental data from theory prediction has been attributed to a variety of factors, including straining, heterogeneity in collector grain and microbial population, cell motility, surface roughness, time dependent attachment, and colloid detachment. Previous studies have found that bacterial cell surface characteristics including outer membrane proteins, lipopolysaccharide (LPS), fimbrae, flagella and extracellular polymeric substances (EPS), may significantly contribute to the microbial surface heterogeneity and interfere with Derjaguin-Landau-Verwey-Overbeek (DLVO) forces, i.e. van der Waals and electrostatic forces, which govern bacterial attachment to surfaces. Therefore, the transport and deposition behavior of indicator organisms, e.g., *E. coli* and fecal coliforms, may not necessary represent those of the pathogenic organisms. To date, much attention had been focused on *Cryptospordium* and *Giardia* because of the obvious size difference that exists between bacteria and protozoa (McCarthy et al., 2004). Little information is available in the literature regarding the relationship between the transport and survival characteristics of fecal indicator organisms and bacterial pathogen *E. coli* O157:H7 even though the later has been found to be considerably more environmentally resistant and has extended survival in water, feces and soil.

In addition to nutrients, manure suspensions consist of a complex mixture of partially digested organic matter and microbial biomass, and therefore encompass a large variety of particle sizes (Bradford et al., 2006). Moreover, animal waste is likely to be exposed to a wide range of solution chemistry as a result of mixing of animal urine and rainfall or groundwater. Site specific hydrologic conditions and manure management practices, e.g., storage and composting, will likely influence the composition and salinity of the manure as well. Such complex and dynamic composition of manure may have significant impacts on the deposition and survival characteristics of pathogens in porous media. Previous studies have found that the fate and transport of microbial particles in the subsurface were affected by solution chemistry, including pH and ionic strength. Higher ionic strength promotes bacterial adhesion by reducing the electrostatic double layer repulsion between microbial particles and grains, as predicted by the classic DLVO theory. The presence of dissolved organic matters such as humic and fulvic acids in the pore fluid has been reported in the literature to shift the maximum retained profiles of bacteria and viruses down gradient from the filter inlet. The blocking of favorable deposition sites by the organic matter near the filter entrance has been argued as the cause for this enhanced microbial transport. Moreover, the presence of particulate matter in animal waste may facilitate pathogen transport through particle aggregation. Lastly, microbial growth in the form of biofilm as a result of nutrients and organic matters in manure and its impact on colloid transport in porous media has largely been neglected in previous studies. Recent research from our laboratory and other researchers has demonstrated that biofilm growth may significantly alter the deposition pattern and survival mechanisms of pathogens through pore size reduction, modification of grain surface properties and microbial competition (Chabaud et al., 2006; Li unpublished; Searcy et al., 2006).

2. Procedures and Methods

Escherichia coli JM109 strain, *Escherichia coli* O157:H7 *EDL933* strain wildtype (toxic), and *Escherichia coli* O157:H7 *EDL933* strain Δ *stx1, stx2* mutant(non-toxic) were used for bacterial transport studies in glass beads packed columns. *Pseudomonas aeruginosa* wild type PAO1 and its isogenic mucoid alginate-overproducing strain PDO300 were used for growing biofilm. The bacteria were streaked on Luria-Bertani (LB) agar plate supplemented with antibiotics and incubated at 37°C for 12 to 16 hours. A single isolated colony was then placed in 10 mL of LB broth with antibiotics and shaken at 210 rpm until reaching stationary growth phase after 14 hours growth in the incubator (New Brunswick Scientific Co., NJ) at 37°C. The mature bacteria was then centrifuged (Avanti J-201, Beckman Coulter, CA) at 4°C, 3750 rpm for 10 minutes and decanted. The bacteria pellets were then resuspended and rinsed with NaCl-MOPS buffer solution, and re-centrifuged three times. NaCl-MOPS (3-(*N*-morpholino)-propanesulfonic acid) buffer solution was created with100mM NaCl and 2.2mM MOPS mixed with deionized water and diluted to 1 M, 0.1 M and 0.01 M strengths to run the separate experiments. The pH of the deionized water was measured to be 5.27. The pH of the respective buffer solutions were not altered from their natural pH upon dilution. The 1 M NaCl-MOPS was mixed and pH levels raised to 6.99 to 7.02, the 1 M solution was then diluted 10 times to 0.1 M NaCl-MOPS whose pH measured 6.46 to 6.69. The 0.1 M solution was then diluted 10 times to 0.01 M NaCl-MOPS whose pH measured 5.19 to 5.42. The optical density (OD) of the bacteria solution was measured using a Visible spectrophotometer (Varian, Inc., CA) at 600nm to create a solution of 10^7 to 10^8 colony forming units (CFU) per mL. The solution was then stored in the refrigerator prior to its experimental use to prevent bacterial cell growth.

Cylindrical polycarbonate plastic columns (26 cm long, 2.54 cm internal diameter) were wet packed to a porosity of 0.4 with glass beads of an average diameter of 550 μm (MO-SCI Specialty

Products, MO). Three different ionic strengths of NaCl-MOPS buffer(1M, 0.1M, and 0.01M) solution was injected at a velocity of 0.011 cm/sec. A peristaltic pump (Cole Parmer, IL), in the upflow direction, pumped approximately 12 pore volumes at an influent velocity of 0.011 cm/sec of buffer solution overnight to fully saturate the ionic strength of the column and beads, followed by 6 pore volumes of bacterial solution. Finally two pore volumes of buffer solution were injected through the glass bead-packed polycarbonate plastic columns. The effluence was then vortexed for at least 45 seconds to ensure sample mixing. The samples were then diluted in 10 fold increments and each dilution was drop plated in triplicate 10 μL samples. The plated bacterial growth was quantitatively counted using the drop plate method and a visible light microscope. Figure 1 shows a schematic of the flow system.

The inoculation was conducted by injecting 450 mL *P. aeruginosa* suspension in a LB broth with a cell concentration of 108 colony forming unit (CFU)/mL, into the column. Bacteria were allowed to attach to the glass beads by recycling 100% of the cell suspension for 12 h using a peristaltic pump. After 12 h, the bacterial suspension was replaced with a synthetic nutrient solution, whose composition was reported in a previous paper, to facilitate biofilm growth. The system was operated continuously for five days under a constant flow rate of 3.0 mL/min. The flow injection direction was switched between upflow and downflow directions every 12 h to ensure uniform biofilm distribution inside the column. The biofilm was grown at room temperature (20–25 °C) in the dark, and its establishment was monitored daily by measuring the concentration of suspended bacteria in the column effluent. After initial inoculation, bacterial counts in the column effluent were as high as 10^{10} CFU/mL during the first two days for both types of P. aeruginosa biofilms. After five days, bacterial concentration in the column effluent reached a pseudosteady state and remained at 10^5–10^6 CFU/mL.

To analyze the biofilm bacterial and EPS distribution in the column, column media were evenly dissected into five segments and each segment of the glass beads was placed into a beaker containing 50 mL NaCl-MOPS (3-(N-morpholino)-propanesulfonic acid) buffer, composed of 100 mM NaCl and 2.2 mM MOPS in deionized water. This buffer was chosen because it is free of phosphate, which is known to interfere with cell deposition. The pH was adjusted to 6.9–7.1 using NaOH. The biofilm bacterial count was measured by the drop plate method as previously described. The biofilm EPS was further extracted using the high-speed centrifugation method described by Brown and Lester. PAO1 biofilm EPS carbohydrate content was quantified using the phenol-sulfuric acid method with glucose as the standard and PDO300 biofilm EPS uronic acid concentration was determined using the m-hydroxydiphenyl sulfuric acid method with d-glucuronic acid as the standard. The zeta-potentials of JM109, PAO1, PDO300 bacteria and crushed glass beads were measured 10 times using 20 cycles per analysis (ZetaPALS analyzer, Brookhaven Instruments Corp., Holtzville, NY).

After biofilm establishment, JM109 transport and survival experiments were conducted in a total of six packed beds (two clean columns, two columns coated with PAO1 biofilm, and two columns coated with PDO300 biofilm). For each set of experiments, *E. coli* cells were simultaneously injected into two identical columns; one used for bacterial deposition analysis and the other used for bacterial survival and detachment study. Approximately two pore volumes (PV) of *E. coli* suspension were injected after equilibrating each column for at least 20 PV of bacteria-free background 100 mM NaCl-MOPS buffer in the upflow direction at a constant approach velocity of 0.011 cm/s. Following bacterial injection, the column was eluted with additional 8 PV of the background buffer solution. Every half PV of the column effluent was collected in a 50 mL polystyrene tube and immediately placed on ice prior to microbiological analysis.

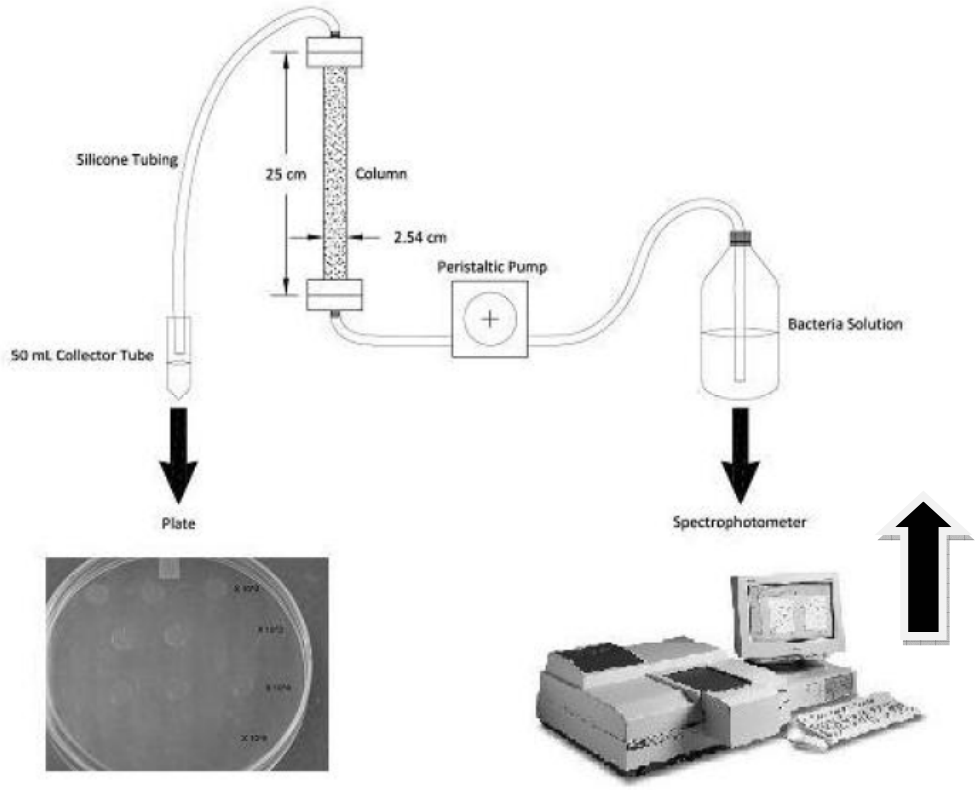

Figure 2.1 Schematic of the Flow System

After completing the transport experiment, porous media from one of the two columns were evenly dissected into five segments and the retained JM109 cells were recovered by sonication in the same manner as biofilm extraction from glass beads surfaces. Quantitative assessment of *E. coli* concentrations in the column effluents and cell distribution within the column media was carried out by viewing samples on a Leitz Diaplan microscope fitted with a Leitz Plemopak fluorescence attachment with a 20× objective (Leitz Microsystems, Wetzlar). The GFP-conferred fluorescence was visualized under illumination with a blue laser (excitation 488 nm; suppression 515 nm). The number of cells in a minimum of 20 randomly chosen fields of view was determined for each sample.

Colloid filtration theory (CFT) was used to obtain the theoretical particle retention pattern in the packed columns based on *E. coli* breakthrough curves. The particle distribution S(X), i.e., the number of deposited bacteria per mass of the granular collector, was calculated using the following equation:

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

Here, X is the column depth, t_0 is the duration of continuous particle injection, ε is the bed porosity, C_0 is the initial cell concentration, ρ_b is the porous medium bulk density, U is the approach (superficial) velocity, and k_d is the deposition rate coefficient, which was estimated based on the steady state breakthrough concentration.

3 Results and Discussion

Figure 3.1 and 3.2 show breakthrough curves plotted using the normalized effluent concentration for *E. coli* O157:H7 and *E.coli* JM109, respectively. The data points were quantified by dividing effluent concentration (C) by influent concentration (C_o) and then plotted versus pore volume, acting as time. Steady-state conditions can be seen in both figures through the graphic plateau quickly reached once bacteria flow entered the column. Influent concentration, C_o, was controlled in the range from 10^7 to 10^8 colony forming units (CFUs) per ml. Effluent concentration as seen in Figures 3.2 and 3.3 varies greatly with changing ionic strength despite the influent concentration similarity. For *E. coli* O157:H7, 72 % normalized effluent concentration with 1M NaCl-MOPS, 83 % normalized effluent concentration with 0.1M NaCl-MOPS and 90% normalized effluent concentration with 0.01M NaCl-MOPS. For *E. coli* JM109, 40 % normalized effluent concentration with 1M NaCl-MOPS, 57 % normalized effluent concentration with 0.1M NaCl-MOPS and 60 % normalized effluent concentration with 0.01M NaCl-MOPS. Comparing O157:H7 to JM109, the trend in common is the normalized effluent concentration decreases with increased ionic strength, that is, the adhesion of bacteria to the glass beads gets stronger in higher ionic strength conditions. Besides that, degrees of adhesion can be seen change with bacteria strain, JM109 is more adhesive to glass beads than O157 under all the ionic strength conditions.

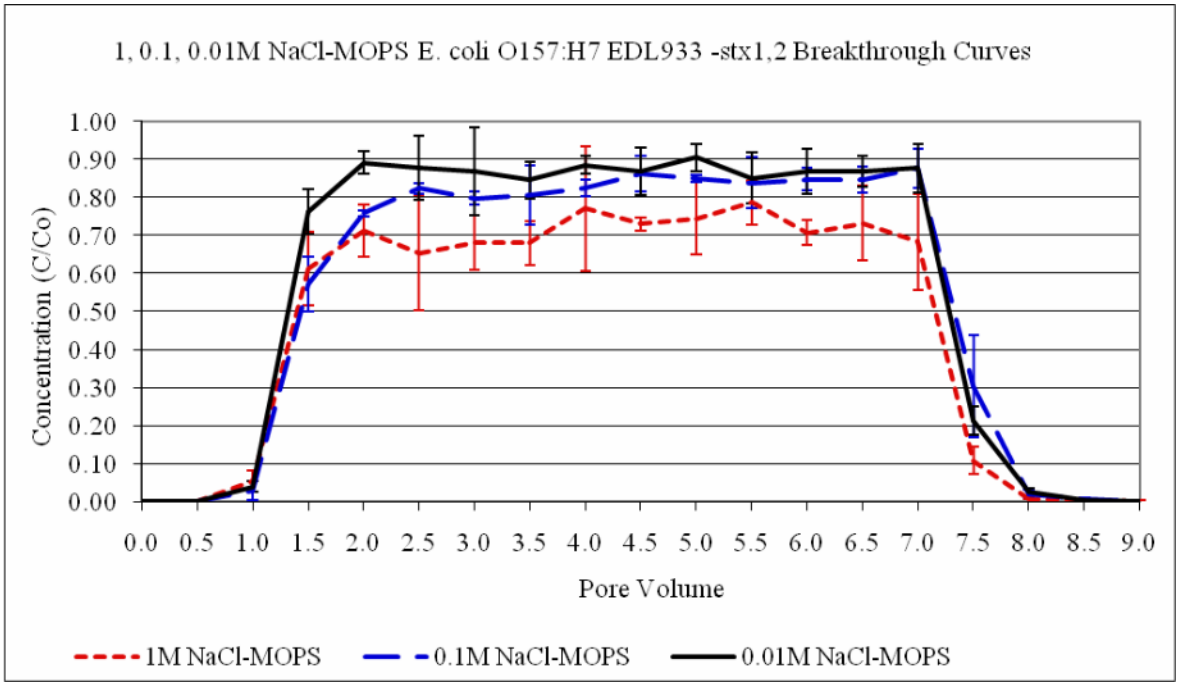

Figure 3.1 *E. coli* O157:H7 breakthrough curves suspended in 1, 0.1, 0.01M NaCl-MOPS buffer with pH 5.42 to 7.02. Bacteria found in the effluent is approximately 72 %, 83 %, and 90% of total injected bacteria.

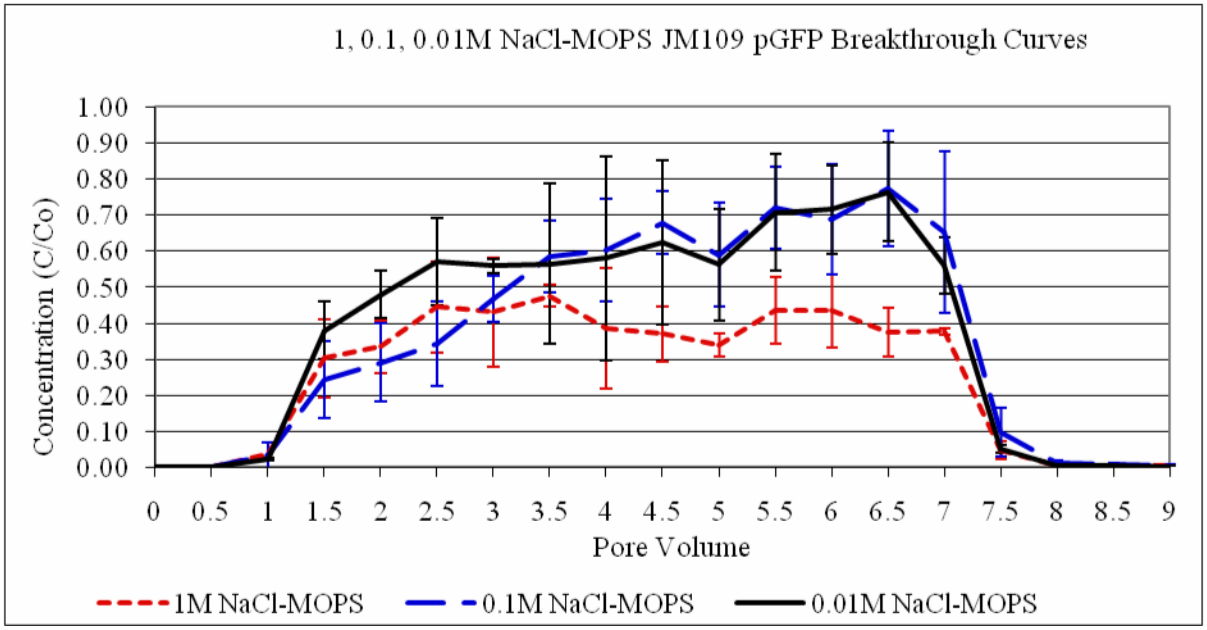

Figure 3.2 *E.coli* JM109 breakthrough curves suspended in 1M, 0.1M, and 0.01M NaCl-MOPS buffer with pH 5.19-7.02. Bacteria found in the effluent are approximately 40%, 57%, and 60% of total injected bacteria.

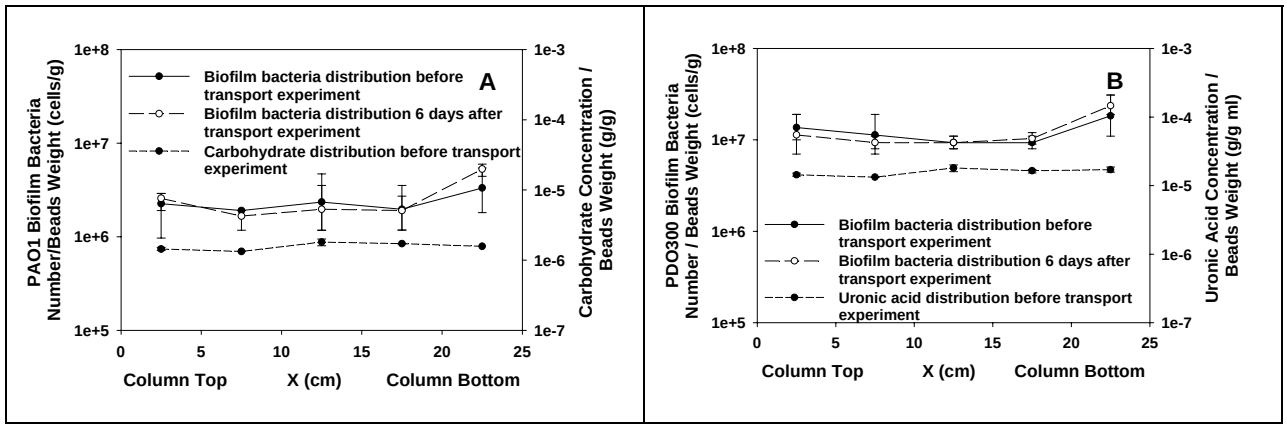

Figure 3.3 Distribution of retained biofilm bacteria and biofilm EPS (carbohydrate is the main EPS component for PAO1 biofilm, uronic acid is the main EPS component for PDO300 bioflim) in PAO1 bioflim coated column (A) and PDO300 biofilm coated column (B). Error bars represent standard deviations of trilicate measurements. Arrows indicate the column section close to the six day low-nutrient solution injection inlet.

Figure 3.3 shows the bacteria count and EPS concentration of *P. aeruginosa* biofilms along the columns. The results show that the main EPS contents, i.e., carbohydrate for PAO1 biofilm (Figure 3.3A) and uronic acid for PDO300 biofilm (Figure 3.3B), had relatively even distribution along the columns before the *E. coli* JM109 injection based on statistical estimates of the 95% confidence intervals. It is also noted that the PDO300 biofilm contained more cells than the PAO1 biofilm, which maybe attributed to

the higher PDO300 initial attachment compared with the PAO1 bacteria, considering their similar growth rates. Six days after the *E. coli* transport experiment, the distribution of biofilm bacteria in the columns remained the same except for a slight increase of bacterial count near the bottom of the column where the nutrient solution was injected.

Figure 3.4 presents the breakthrough curves showing the normalized effluent concentration, C/C_0, during the injection and elution of *E. coli* cells. The breakthrough plateau was constant at 2.5–3 PV, indicating a steady-state condition in all three types of columns. The magnitude of the steady-state breakthrough plateau for the PAO1 biofilm was lower compared with those for the PDO300 biofilm and clean column, indicating that a higher number of *E. coli* cells were retained in the PAO1 biofilm-coated column. The PDO300 biofilm, by contrast, had a very small impact on the *E. coli* bacterial removal, despite that the PDO300 biofilm had a higher bacterial count and EPS content than the PAO1 biofilm. During the MOPS-NaCl buffer elution, tailing of *E. coli* was observed in all columns with similar magnitudes despite different column media types and breakthrough concentrations. The detachment of previously attached bacteria likely contributed to the tailing. A control experiment using a conservative dye tracer did not exhibit any skewed breakthrough or tailing, indicating that physical nonequilibrium was not significant for the tracer in the columns.

Figure 3.5 shows the retained *E. coli* profiles compared with those predicted by the CFT. A log-linear relationship was observed between the number of retained bacteria and the transport distance in the clean column, which indicates a constant deposition rate coefficient as implicated in the "clean-bed" theory. In the biofilm-coated columns, the retained *E. coli* concentrations decreased hyper-exponentially as a function of transport distance (Figures B, C), indicating that the deposition rate coefficients decreased with increasing transport distance. Recently, various studies examining the retained colloid profiles in porous media reported decreasing deposition rate coefficients. The apparent change in deposition rate coefficients with distance has been attributed to the heterogeneity in bacterial surface properties, distribution in the interaction energies between bacteria and porous media, and particle deposition dynamics. In our study, the occurrence of an energy barrier associated with the negatively charged biofilm surface may have caused the monotonic derivation from CFT. Another possibility is that the biofilm surface roughness amplified the distribution heterogeneity among the *E. coli* population.

The column effluent biofilm and *E. coli* bacterial densities were monitored every 12 h for six days after the *E. coli* injection. As shown in Figure 3.6 A, the number of *E. coli* bacteria in the effluent decreased rapidly and dropped below the sensitivity limit of the analytical method (102 cells/mL) four days after the transport study in the clean column. Extended tailing with low *E. coli* concentrations following the breakthrough-elution was observed in both columns with biofilm (Figures 3.6 B and C). In both columns, the effluent *E. coli* concentration experienced fluctuation, which occurred concurrently with biofilm detachment. Detachment is known to be the primary process that balances biofilm growth in the filter media through the release of cells and aggregates into the bulk liquid. Our observation implies that the *E. coli* bacteria became an integral part of the biofilm and that biofilm detachment was the phenomenon that most likely influenced the fate of *E. coli* bacteria long after the contamination event.

Despite the fluctuation of bacterial counts, the average *E. coli* bacterial number in the column effluent decreased by approximately 55 and 66% in the PDO300-coated column and the PAO1-coated column, respectively, and became relatively constant four days after the cell injection. Overall, the effluent *E. coli* count was about 2 orders of magnitude less than, but with a similar pattern to that of the P. aeruginosa. The extended tailing of *E. coli* also demonstrated that cells could remain in the biofilm matrix for a prolonged period of time following the passage of a pulse injection.

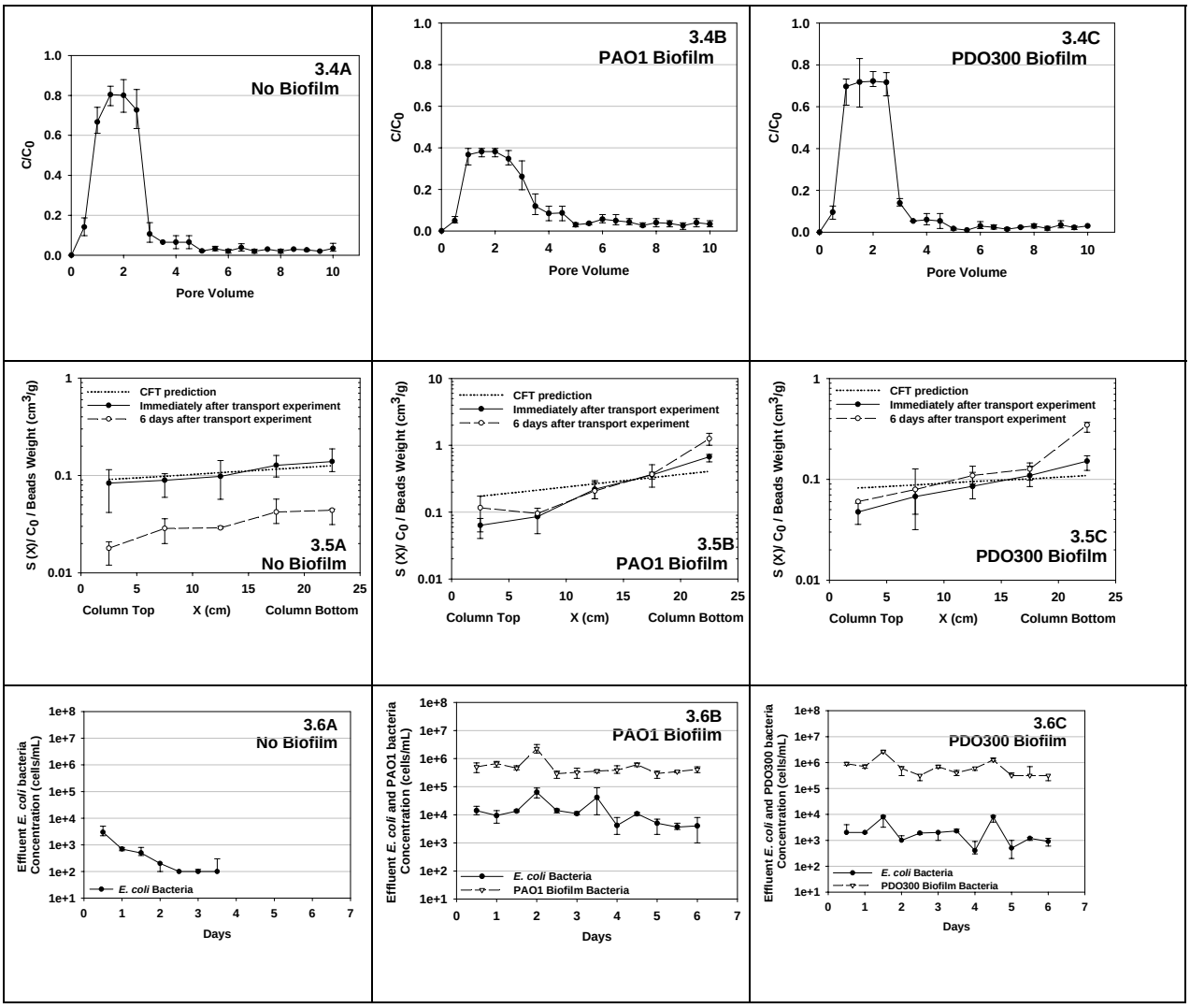

Figure 3.4. *E. coli* bacterial breakthrough-elution curves in the clean column (A); PAO1 biofilm-coated column (B); and PDO300 biofilm-coated column (C). Error bars represent standard deviations of triplicate measurements.

Figure 3.5. Retained *E. coli* JM109 profiles in the clean column (3A); PAO1 biofilm-coated column (3B); and PDO300 biofilm-coated column (3C) immediately and six days after the *E. coli* transport experiment. Error bars represent standard deviations of triplicate measurements. Arrows indicate the column section close to *E. coli* cells and low-nutrient solution injection inlet after the *E. coli* transport experiment.

Figure 3.6. Effluent biofilm bacterial and *E. coli* JM109 concentrations over six days of low-nutrient injection from the clean column (A); the PAO1 biofilm-coated column (B); and the PDO300 biofilm-coated column (C). Error bars represent standard deviations of triplicate measurements.

The retained *E. coli* profiles six days after the injection are shown in Figure 3.5. Compared with the *E. coli* distribution immediately after the transport experiment, the *E. coli* cell number decreased in all layers in the clean column (Figure A), but increased in most layers in both biofilm-coated columns (Figure B and C).

Table 3.1. Normalized percent recovery of *E. coli* bacteria (recovered cell concentration / initial cell concentration) in (a) column effluent during *E. coli* bacteria injection and elution, (b) column media immediately after *E. coli* bacteria injection, (c) column effluent over six days after injection, and (d) column media six days after injection, in PAO1 biofilm-coated columns, PDO300 biofilm-coated columns, and clean columns. Bacterial growth rate in column media were calculated by the formula $\mu = \frac{1}{(t_2 - t_1)} \ln\left(\frac{n_2}{n_1}\right)$, where n_2 is the cell number six days after *E. coli* injection (time t_2), and n_1 is the cell number immediate after *E. coli* cell injection (time t_1). Data represents the average recovery of three replicate experiments.

		No Biofilm	PAO1 Biofilm	PDO300 Biofilm
Transport experiment	Column effluent[(a)]	80.40±0.71%	53.56±0.93%	81.40±1.25%
	Column media[(b)]	20.10±1.07%	42.67±1.65%	15.20±0.72%
	Recovery rate[(a+b)]	100.5±1.78%	96.23±2.58%	96.6±1.97%
Survival experiment	Column effluent[(c)]	1.00±0.78%	76.68±2.34%	12.70±2.56%
	Column media[(d)]	3.00±0.45%	81.85±1.76%	31.25±2.46%
	Recovery rate[(a+c+d)]	84.40±1.94%	212.09±4.03%	125.35±6.27%
E. coli growth rate (day^{-1})		-0.32	0.11	0.12

Table 3.1 compares the total *E. coli* bacterial cells extracted from the column media immediately and six days after *E. coli* transport experiment. Despite the low nutrient supply, only 3% of the total injected *E. coli* cells were recovered from the clean column media after six days compared with the 20.1% recovery rate immediately after the E. coli transport experiment. As shown in the table, the total *E. coli* recovery rate (column media plus effluent) was 100.5% immediately after the cell transport experiment and 84.4% after Day 6 in the clean column, indicating that some of the *E. coli* lost their fluorescent signal after six days. Both biofilm-coated columns had more of the *E. coli* bacteria six days after the pulse injection, which led to the conclusion that the injected *E. coli* bacteria were able to grow in biofilms. The specific growth rates (Table 1) for *E. coli* were virtually identical in both types of biofilm ($P = 0.01$ by paired-sample t test), indicating that the amount and composition of *P. aeruginosa* biofilm EPS had little effect on the survival and growth of *E. coli* bacteria.

4. Conclusions and Recommendations

The composition of manure can impact the deposition and survival of microorganisms, especially during events of heavy precipitation, through physical, chemical and microbiological mechanisms, e.g., changes of pH, salinity of pore fluid, blocking of the favorable deposition site by organic matters, aggregations between manure suspensions and bacterial cells, and formation of biofilms. Ionic strength is an important factor in the initial adhesion, distribution, and detachment of bacteria in groundwater environment. Changing ionic strength of the buffer solutions used in our column studies is able to alter the surface charge of the bacteria and/or collector grain surface and thereby alter the adhesion and detachment of O157:H7 EDL933 Δ *stx1, stx2* and JM109 pGFP to glass bead surfaces. The attachment and growth of bacteria on biofilm-covered porous media is the norm rather than the exception and has important implications in environmental and man-made filtration processes. Information on the interaction between bacterial cells and biofilm surfaces may be utilized in designing biologically active filters, improving bioremediation, and predicting pathogen transport. Findings of this study suggest that the composition of *P. aeruginosa* biofilm EPS plays a pivotal role in controlling the initial attachment and deposition profiles of *E. coli* JM109 in porous media through non DLVO forces, including hydrophobicity, hydration forces and polymeric bridging. The presence of biofilm is also essential for the survival and growth of *E. coli* after their initial adhesion and biofilm detachment is the main mechanism through which *E. coli* was released back to the bulk fluid after the contamination event. An important phenomenon that we found in this study is that the transport and adhesion behavior of *E. coli* O157 differ significantly model prediction and from behavior of other *E. coli* strains. Therefore the use of indicator bacteria to predict the risk of pathogen contamination in groundwater needs to be evaluated in detail in future study.

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