ABSTRACT

Title of Dissertation: TRANSPORT AND CAPTURE OF BACTERIA FROM URBAN STORMWATER RUNOFF USING BIORETENTION

Lan Zhang, Doctor of Philosophy, 2008

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Bioretention, a nature-based treatment practice, has significant potential for reducing the threat of microbial pollutants from urban stormwater runoff to receiving water bodies. The overall goal of this research was to evaluate the removal efficiency for bacteria from urban stormwater runoff in bioretention systems and the potential of an engineered media (iron oxide-coated sand (IOCS)) for enhancing bacterial removal. This investigation was accomplished through laboratory column studies coupled with field tests.

Column studies on the transport and destruction of *Escherichia coli* O157:H7 strain B6914 (a surrogate of pathogenic *E. coli*) in conventional bioretention media (CBM) and IOCS demonstrated that the bacteria were well removed in CBM (a mean 70% efficiency), but IOCS significantly enhanced the capture of strain B6914 (a mean 99.4% efficiency) due to the greater positive charge and surface roughness.

However, the decay of trapped strain B6914 cells was much faster in CBM compared to the IOCS. More than 99.98% of B6914 cells attached to CBM died off within one week, while approximately 48% of trapped cells still survived in the IOCS after one week. Predation by indigenous protozoa in the CBM appears to play a dominant role in the faster decline of the number of trapped B6914 cells in CBM. Additionally, long-term (18 months) column experiments indicated that during the periodic application of simulated rainfall, the removal efficiency for strain B6914 improved over time, achieving 97% or higher efficiency after six months.

Consistent with the laboratory studies, two years of field studies showed that bioretention systems reduced the concentration of indicator bacteria in the outflow during most storm events and increased the probability of meeting specific water quality criteria. The concentration of indicator bacteria in the input flow generally increased with higher daily temperature. No clear trend for the bacterial removal efficiency with respect to temperature was found in laboratory and field studies. However, the bacterial decay coefficients in CBM increased exponentially with elevated temperature.

Based on these results, it is concluded that CBM not only achieves good removal for bacteria, but also has the potential to render the process sustainable.

TRANSPORT AND CAPTURE OF BACTERIA FROM URBAN STORMWATER RUNOFF USING BIORETENTION

By

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Chapter 1: Introduction

As the result of growing populations and increased urbanization, the impact of contamination from urban stormwater runoff on the water quality of receiving waters has received increasing attention in recent years. Specifically, as urban stormwater runoff flows across the earth's surface, it can accumulate high levels of many pollutants, such as suspended solids, fertilizers/nutrients, toxic hydrocarbons, heavy metals, and pathogens (USEPA, 2002; Davis, 2005). In fact, urban stormwater runoff has become one of the primary sources of pollutant loadings to rivers, estuaries, and oceans, as further improvements are made in controlling point sources such as municipal sewage and industrial waste (Ahn *et al.*, 2005).

An important component of the contaminant load in urban stormwater runoff is microbial pollutants, which mainly come from the fecal matter of dogs, cats, pigeons, squirrels, and other urban animals, as well as humans (Mallin *et al.*, 2000). Although not all microorganisms are harmful, a number of pathogenic bacteria (such as *Shigella* spp., *Salmonella* spp., *Pseudomonas aeruginosa* and certain *Escherichia coli* species) and viruses (such as enteroviruses, and adenovirus) are found in stormwater runoff and can directly cause diseases in humans (Field *et al.*, 1993; CDT, 2006). In addition, some protozoan pathogens such as *Giardia* and *Cryptosporidium* in urban stormwater are also a concern, although *Cryptosporidium* is found in less than 50 percent of stormwater samples (USEPA, 2002). Disease outcomes associated with

water borne infections include mild to life-threatening gastroenteritis (includes diarrhea and abdominal pain), salmonellosis, urinary tract infection, dysentery and hepatitis (Stoner and Dorfman, 2006.).

Because of the challenges associated with monitoring for potential pathogens of concern, many different microorganisms or groups of microorganisms (e.g., total coliform bacteria, fecal coliform bacteria, fecal streptococci, enterococci and *E. coli*) have been suggested as indicator organisms to signal conditions in recreational and shellfish-harvesting water that might lead to adverse health consequences due to fecal contamination. Two of the most common indicator organisms used worldwide are fecal coliform (i.e., thermotolerant coliform) and *E. coli* (Foppen and Schijven, 2006). In the case of urban stormwater runoff, Young and Thackson (1999) found that the levels of fecal coliform and *E. coli* bacteria in urban streams could be correlated with the housing density, population, percent impervious area, and apparent domestic animal density. Furthermore, concentrations of fecal coliform bacteria in urban stormwater are highly variable even within a given monitoring site (Schueler, 1999). For example, the mean concentration of fecal coliform bacteria in urban stormwater for 34 studies across the United States was reported at 15,038 MPN/100 mL (Schueler, 1999). Similarly, another national database of 1,600 samples (mostly Nationwide Urban Runoff Program data collected in the 1980s) estimates the mean concentration of fecal coliform at 20,000 MPN/100 mL (Pitt, 1998). However, the Maryland Department of the Environment (MDE) reported

average concentrations of fecal coliform bacteria and *E. coli* in urban runoff at 3600 MPN/100 mL and 1450 MPN/100 mL, respectively at typical sites (MDE, 2000).

Microbial pollution in urban stormwater runoff is of concern because it can lead to water quality impairment in estuaries, coastal waters, and other water bodies, which, in turn, cause beach closings, restrictions on shellfish harvest and the need for increased treatment of drinking water to decrease the risk of human health problems (USEPA, 2001a; USEPA, 2001b). To guard against human health risks associated with such water quality impairment, the U.S. Environmental Protection Agency (USEPA) sets the limit on the allowable concentrations of total coliform bacteria in drinking water at 0 CFU in 100 mL. For primary contact water, the USEPA maximum geometric mean criterion is 200 fecal coliforms in 100 mL, and for designated freshwater beaches, the USEPA steady-state geometric mean criterion is 126 *Escherichia coli*/100 mL (USEPA, 1986).

The magnitude of the water quality problem posed by stormwater runoff and the application of these water quality standards for microbial pollutants are demonstrated by the example of beach closings and advisories. The growing number of closings/advisories is an indication of serious water resource pollution at national beaches (Figure 1.1). In 2005, there were a total of 20,397 beach closings and advisory days in the U.S., of which 75 percent were based on monitoring that detected bacterial levels exceeding beachwater quality standards. Polluted runoff and stormwater caused or contributed to 5,333 closing/advisory days (Stoner and

Dorfman, 2006). Therefore, proper control and management of stormwater runoff to reduce the microbial pollutant load from impervious urban sources has significant potential for improving the quality of receiving waters.

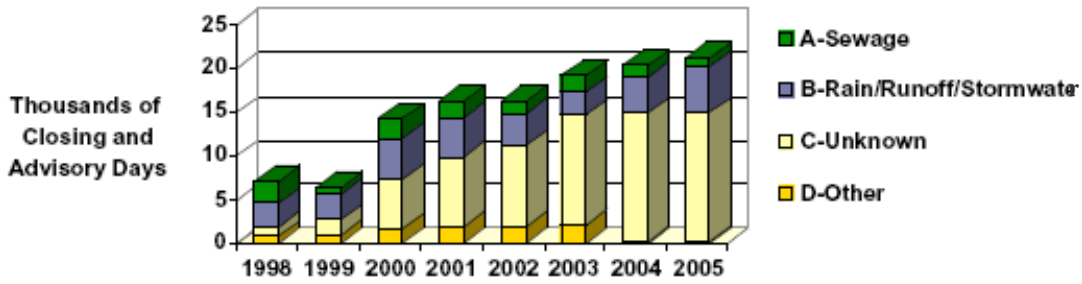


Figure 1.1 Source of pollution that caused beach closings and advisories in the U.S. (Stoner and Dorfman, 2006). (A) Sewage spills and overflows; (B) Polluted runoff, stormwater, or preemptive due to rain; (C) Unknown; (D) Other sources (including those with no source information provided).

Bioretention for treatment of urban stormwater runoff

One potential approach for addressing urban stormwater runoff pollution is the use of bioretention systems. Bioretention, a nature-based stormwater best management practice (BMP), is designed for water filtration and evapotranspiration, and pollutant removal by soil filtering, sorption, biological uptake, precipitation and other mechanisms (USEPA, 2004). As shown in Figure 1.2, a bioretention cell generally consists of a porous media layer (soil and sand mixture), supporting a vegetative layer, with a surface layer of hardwood mulch. During storm events, a ponding area serves as reserve space for runoff storage and provides extra time for

infiltration of water into the media (Hsieh and Davis, 2005a). Mulch provides a nutrient-rich environment for microbial growth, decomposition of organic material, and adsorption and bonding of heavy metals. Soils support vegetative growth along with promoting nutrient uptake and providing additional water storage. Coarse sand provides for drainage and aeration of the planting soil (DER, 2001).

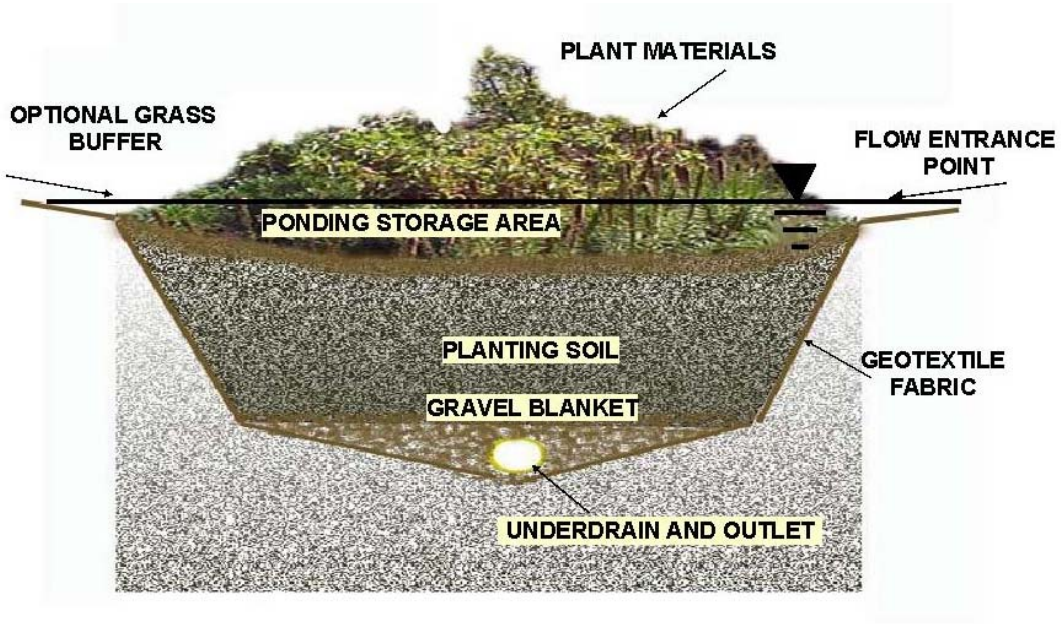


Figure 1.2 Typical bioretention system (DER, 2001)

Previous studies of bioretention systems have investigated their infiltration capacity and their ability to remove pollutants from stormwater runoff, both in the laboratory and in the field. These studies have demonstrated good removal of a variety of relevant contaminants, including heavy metals, nutrients (e.g., nitrogen and phosphorus species), oil and grease, and suspended solids (Davis *et al.*, 2001; Davis *et al.*, 2003; Hsieh and Davis, 2005b; Davis *et al*., 2006; Hong *et al*., 2006).

Nevertheless, while bioretention monitoring studies typically monitor for parameters such as total phosphorus, heavy metals, and suspended solids, there are few monitoring data available for parameters such as bacteria, compared to other BMPs such as wet ponds and constructed wetland systems (USEPA, 1999). Limited data published to date have evaluated the capacity of bioretention systems to remove microbial pollutants from stormwater runoff (Rusciano and Obropta, 2007).

Research objectives and approach

Given the magnitude of the threat to water quality posed by microbial pollutants in urban stormwater runoff and the untested potential for their removal in bioretention systems, the overall goal of this research was to evaluate the removal efficiency for pathogenic bacteria in bioretention systems and the potential for enhancing that treatment. The work to accomplish this goal was driven by four hypotheses. First, we hypothesized that bioretention should exhibit a high removal efficiency for bacterial pollutants. This was based on the data collected from 13 stormwater infiltration systems; the removal efficiency for bacteria varied over a range of 36% to 83% (average 37%) (USEPA, 1999). Bioretention could potentially achieve even higher removal of input bacteria than these values determined for infiltration systems due to the enhanced biological activity in conventional bioretention media, which is composed of sandy loam soil, sand and mulch (leaf compost) (MDE, 2000).

However, as the result of growing populations and increased urbanization and associated increased pollutant loads, it is possible that a conventional bioretention facility will not be able to meet future discharge criteria. Thus, developing a novel engineered bioretention media may be important to maximizing bacterial attenuation. For example, given that bacteria generally are negatively charged, porous media coated with ferric and/or aluminum oxides (or hydroxides) that possess positively charged surfaces can potentially strongly adsorb microorganisms (Scholl *et al.*, 1990; Knapp *et al.*, 1998). Therefore, the second hypothesis of this research was that a novel engineered media (such as iron oxide coated sand) layer will improve bacterial removal in bioretention and reduce the effluent discharge level.

Direct removal of bacteria from stormwater runoff is not the only important criterion in evaluating BMP performance. Specifically, optimizing subsequent bacterial destruction between storm events is also necessary to make the process sustainable. The application of compost leaf to soil can modify the composition of the bacterial taxa in soil, as well as increase the general level of microbial activity, which result in increased competition and/or antagonism among microbes (Tiquia *et al.*, 2002). Predation by indigenous microbes has been reported to be a contributory factor to the bacterial survival (Jiang *et al.*, 2002). Therefore, the third hypothesis driving this research was that the enhanced biological activity and other characteristics of bioretention should contribute to bacterial destruction between storm events, resulting in a sustainable process.

As hypothesized above, enhanced biological activities in conventional bioretention media may result in a good removal of introduced bacteria and make bioretention systems sustainable. Microbial activities (such as metabolism, predation, and proliferation) are significantly influenced by temperature. Generally, the activity of microorganisms increases with temperature until a maximal tolerable temperature is reached (Boyd and Hoerl, 1986). Therefore, the fourth hypothesis of this research was that bioretention media at high temperature could achieve a greater reduction of trapped bacteria due to the faster metabolism and die-off of bacteria, and enhanced nutrient competition and predation from indigenous microbes.

To achieve the overall goal of this research, three specific objectives were designed to address the four hypotheses outlined above: (1) to evaluate and compare the capture and destruction of pathogenic bacteria by a conventional bioretention media and iron-oxide coated sands during simulated storm events using column studies (hypotheses 1 and 2), (2) to evaluate the sustainability of bacterial removal from simulated urban stormwater runoff in conventional bioretention media using long-term column studies (hypothesis 3), (3) to investigate the effect of temperature on capture and destruction of pathogenic bacteria from urban stormwater runoff in conventional bioretention media using laboratory column studies coupled with field tests (hypothesis 4). The research approach used to accomplish these objectives is summarized in the flow chart presented in Figure 1.3 and described briefly in the following paragraphs.

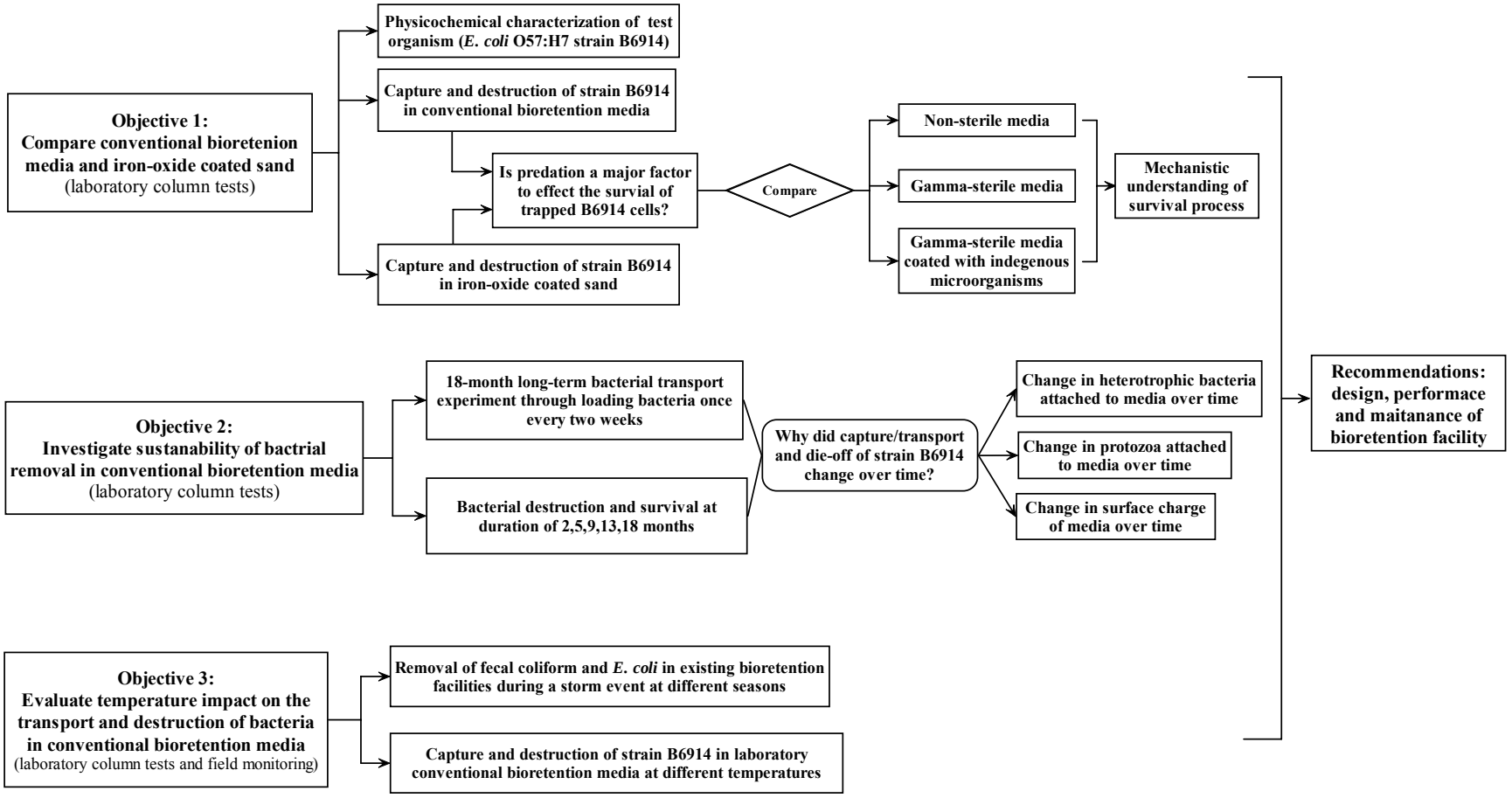


Figure 1.3 Flow chart of research objectives and approaches for this research

Escherichia coli has been demonstrated to be a more specific indicator for the presence of fecal contamination than the fecal coliform group of bacteria (Cheremisinoff and Cheremisinoff, 1993; Medema *et al.*, 2003). In particular, *E. coli* O157:H7 is an enterohemorrhagic *E. coli*, one of the major pathogenic waterborne bacteria of concern (USEPA, 2001b). *E. coli* O157:H7 breakouts have been reported in many countries and it has become a worldwide threat to public health. For example, from mid-December 1989 to mid-January 1990, 243 cases of gastrointestinal illness from antibiotic-resistant *E coli* O157:H7 occurred in a rural Missouri township as a result of an unchlorinated water supply (Swerdlow *et al.*, 1992). In May 2000, an outbreak of *E. coli* O157:H7 and *Campylobacter* spp. occurred in Walkerton, Ontario (Canada) associated with a contaminated municipal water supply (Bruce-Grey-Owen Sound Health Unit, 2000). Additionally, an outbreak of *E coli* O157:H7 associated with swimming in natural water bodies also has been reported (Bruneau *et al.*, 2004). Thus, a derivative of *E. coli* O157:H7 strain B6914 was used as a surrogate for pathogenic *E. coli* in the laboratory component of this research.

A comparison of conventional bioretention media and iron oxide-coated sand for the capture and destruction of strain B6914 from simulated urban stormwater runoff was performed using laboratory column studies (Chapter 3). A mathematical filtration model was applied to simulate and explain the experimental transport data. Predation from native microbes in the media may be a major reason for the different rates of destruction of trapped B6914 cells in these two media. Therefore, to investigate the effect of protozoa and bacterial predators and competition on the

capture and survival of strain B6914, additional transport/destruction experiments were conducted in parallel columns packed with non-sterile media, γ-irradiated sterile media, or γ-irradiated sterile media coated with cultured native microorganisms.

An 18-month long-term column study was used to evaluate the sustainability of bacterial removal in bioretention systems (Chapter 4). In this long-term study, five columns packed with conventional bioretention media were loaded once every two weeks with the B6914 suspension in synthetic stormwater runoff. During each loading event, strain B6914 cells were enumerated in the influent and the effluent to provide information on the capture behavior of the media. In addition, at durations of 2, 5, 9, 13, 18 months, one column was sacrificed, and the media were evaluated for the number of amount of trapped B6914 cells, heterotrophic bacteria, and protozoa and the surface charge. Temporal trends in these data were used to evaluate change in the capture and die-off of trapped B6914 cells over time.

Another important part of this research was to investigate transport and capture of microbial pollutants in existing bioretention facilities using field-scale studies (Chapter 5). In this component of the research, during storm events at different seasons of the year, samples were collected from input and output flows using appropriate methods, and the concentrations of indicator organisms (fecal coliform and *E. coli*) were analyzed. Temperature significantly influenced the level of indicator bacteria observed in the input flow and the concentration-based and total organism-based removals. Therefore, to systematically evaluate the impact of temperature on bacterial capture and destruction in conventional bioretention media, the results of the field tests were coupled with data from laboratory column studies on

the capture and destruction of stain B6914 during simulated storm events under different environmental temperatures (5, 15, 25, and 37 ºC).

Finally, based on the overall project results for the performance of bacterial treatment in bioretention systems, recommendations on bacterial capture layer design and maintenance are presented in Chapter 6, along with suggestion for future work.

Chapter 2: Literature review

Bioretention systems are designed with the assumption that the media can act as a biological filter, reducing runoff pollutants by processes such as physical filtration, adsorption, and chemical and biological reactions. Knowledge of the mechanisms and factors that influence the bacterial transport and survival in porous media is of great importance for successful implementation of bacterial removal in bioretention systems.

In this chapter, background information on bacteria in stormwater runoff, the movement and retention of bacteria in water percolating through porous media, the survival of bacteria in porous media under different environmental conditions (such as moisture, temperature, pH), and the improvement of bacterial removal using modified porous media are reviewed, along with the filtration models used for simulating the transport and fate of bacteria in porous media. This review is specifically focused on the transport and survival of *E. coli* because *E. coli* was chosen as the test organism in this research.

2.1 Pathogenic bacteria in urban stormwater runoff

As discussed in Chapter 1, the limited data available on the levels of pathogenic bacteria in urban stormwater runoff across the United States suggest that the concentrations of bacteria are highly variable (Pitt, 1998; Schueler, 1999; MDE, 2000). For example, the mean concentration of indicator bacteria (fecal coliform

bacteria) observed has varied from a few thousand to tens of thousands MPN in 100 mL of urban storm runoff.

What is the source of these organisms? As noted in Chapter 1, pathogenic bacteria in urban stormwater mainly appear to come from the fecal matter of dogs, cats, pigeons, squirrels, and other urban animals, as well as humans. Young and Thackston (1999) found that the fecal bacterial levels in urban stormwater runoff could be correlated with the housing density, population, percent impervious area, and density of domestic animals and wildlife. Bannerman *et al.* (1993) evaluated the concentrations of indicator bacteria in stormwater runoff from different sources within residential, commercial, and industrial land uses. The data showed that fecal coliform levels from residential areas were much higher than the other two areas, with feeder and collector streets found to be more important sources for the total bacterial loads than roofs, driveways, or lawns within residential areas. These data support the hypothesis that pets and wildlife animals in residential areas are the main sources of pathogenic bacteria in urban stormwater runoff.

Roof tops, roads, parking lots, driveways and other impervious surfaces no longer allow rainfall to soak into the ground. Rainfall that flows across these surfaces washes away the accumulated animal wastes and converts it directly to a stormwater runoff load to receiving waters. However, if before urban stormwater runoff flows into receiving waters, it passes through a filtration-type BMP, such as bioretention, the transport and fate of pathogenic bacteria in porous media becomes critical to understand.

2.2 Transport and retention of bacteria in porous media

Transport of microorganisms through porous media is governed by a variety of abiotic and biotic factors. The main mechanisms responsible for immobilization of bacteria moving through porous media are physical straining or filtration of cells by small pores, microbial adsorption to medium surfaces, and sedimentation (Newby *et al.*, 2000). Sedimentation, the process of gravitational deposition of colloids on matrix surfaces, may play a role in bacterial transport, but only in very slow-moving water over extended periods of time (Wan *et al.,* 1995; Newby *et al.*, 2000). Therefore, sedimentation is not discussed further here, and the other two mechanisms are presented in detail below.

2.2.1 Physical straining/filtration of cells by porous media

The physical straining mechanism of immobilization involves the blocking of movement through pores smaller than the bacteria. Straining of bacteria has been shown to be correlated with bacterial size and shape, media particle size and clogging of filter media (Stevik *et al.*, 2004).

Filtration of bacterial cells has been shown to be statistically proportional to bacterial size, with larger cells removed more effectively by filtration. Generally, straining becomes an important mechanism when the limiting dimension of the bacteria is greater than 5% of the mean diameter of the media particles (Herzig *et al.*, 1970). Bacterial shape, as quantified by the ratio of cell width to cell length, also influences the transport of bacterial cells through porous media (Weiss *et al.*, 1995).

Some reports suggest that cell attachment to media for long rod-shape cells is better than for spherical cells (Feldner *et al.*, 1983; van Loosdrecht *et al.*, 1989).

The physiological state of bacterial cells plays a significant role in determining their effective diameter. Under starvation conditions, bacteria typically decrease in size to 0.3 μm or even smaller, which may increase their transport potential (Newby *et al.*, 2000). When nutrients are not limiting, most bacterial cells will produce exopolymers outside of the cell. Exopolymers increase the effective diameter of a cell, which may promote attachment.

E. coli is a gram-negative, facultatively anaerobic, straight, rod-shaped bacterium of 2.0-6.0 μm × 1.1-1.5 μm (Bergey *et al.*, 1984). A scanning electron micrograph of *E. coli* is shown in Figure 2.1. For sand with particle diameters of 0.05 to 2.0 mm (USDA soil texture classification system), straining should have a relatively small impact on the retention of *E. coli*, based on the guidelines presented above. However, in a soil that contains a significant portion of silt or clay, straining may be a major mechanism of bacterial immobilization. Additionally, the presence of micropores in porous media may reduce the filtration effectiveness and allow a more rapid and greater extent of bacterial movement, because bacteria cells are physically excluded from micropores when the micropores or pore throats located in the micropore domain are smaller than the cells (Newby *et al.*, 2000).

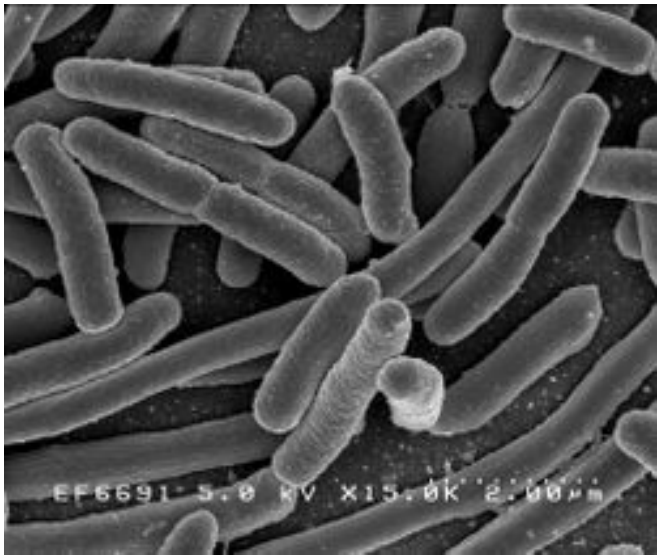


Figure 2.1 Scanning electron micrograph of *Escherichia coli* bacteria (source: Rocky Mountain Laboratories, NIAID, NIH, 2006)

2.2.2 Adhesion of bacteria to porous media

Bacterial adhesion to media surface consists of the initial attraction of the cells to the surface followed by adsorption and attachment (Rijnaarts *et al.*, 1995). The initial transfer of the bacterial cells to a particle surface is by one of three ways: diffusion, advective transport, or active movement of the cell (Newby *et al.*, 2000). Diffusion is a result of Brownian motion, and advective transport is primarily due to water movement. Additionally, cells may actively migrate close to a surface in response to a chemotactic chemical gradient. Whatever the mechanism, once a cell contacts a particle surface, bacterial adsorption and attachment to a surface can take place.

Bacterial adsorption is a physicochemical process, which can be divided into reversible adsorption and irreversible adsorption depending on the mechanisms involved. Derjaguin-Landau-Verwey-Overbeek (DLVO) theory is commonly used in

explaining particle adsorption (Stevik *et al*., 2004). A schematic diagram of this theory is shown in Figure 2.2. The theory assumes that particle attraction occurs over a primary minimum distance (≤ 1 nm) and a secondary minimum distance (5-10 nm). There is a zone of maximum electrostatic repulsion between these two minima, and the width of this zone decreases with increasing ionic strength of the aqueous solution (Marshall, 1971).

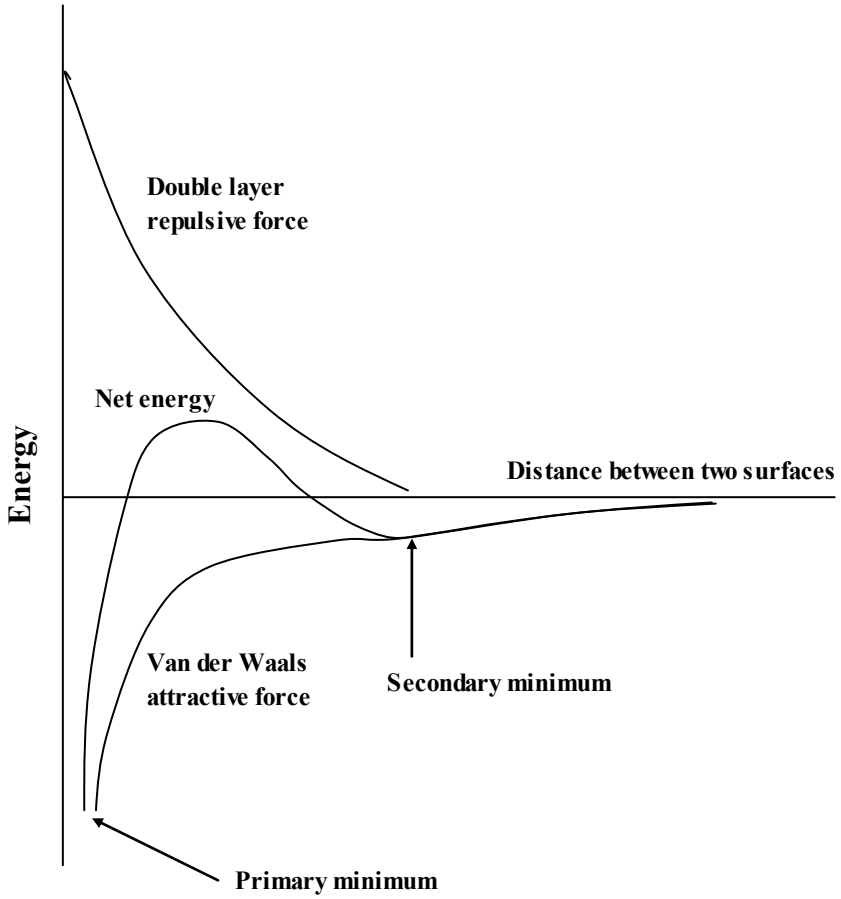


Figure 2.2 Schematic diagram of the variation of free energy with particle separation distance according to DLVO theory. The net energy is given by the sum of the double layer repulsive force and the van der Waals attractive force (Adapted from van Loosdrecht *et al.*, 1990b).

Bacterial adsorption and attachment to the surface of porous media is a two-step process. The first step is reversible adsorption, which takes place when bacteria overcome the secondary repulsive force and adsorb to the surface at the secondary minimum (van Loosdrecht *et al.*, 1990a). It is a weak interaction, and controlled by electrostatic forces, hydrophobic interactions and van der Waals forces. Reversibly adsorbed bacteria can be detached from the particle surface and returned to the water phase, for example, by increasing the water flow velocity or by changing the chemical composition of the aqueous solution. The second step, irreversible attachment, often thought of as adhesion, is a permanent process that occurs at the primary minimum. Subsequently, a bacterium becomes firmly attached to the surface as a result of the production of specialized structures (e.g., fimbriae, flagella) and exopolymers that cement the bacterial cell to the adsorbent (Newby *et al.*, 2000).

Factors that influence the adhesion of bacterial cells to porous media mainly include surface characteristics of the media (e.g., physicochemical properties, topography-roughness, formation of biomass), cell surface characteristics (e.g., hydrophobicity, electrostatic charges, etc.), environmental factors (e.g., temperature, pH, organic matter characteristics, ionic strength and cationic species present), as well as hydraulic factors (e.g., water flow velocity) (Katsikogianni and Missirlis, 2004b; Stevik *et al.*, 2004). These key factors are reviewed in the following subsections.

2.2.2.1 Surface characteristics of porous media

The size, surface texture, and surface charge of porous media and biomass accumulation in the media may greatly affect bacterial adsorption. Smaller particles

have a larger specific surface area, thus, providing more adsorption sites. For example, bacteria can be significantly adsorbed to clay particles in porous media due to the very small size of the clay particles and their large cation exchange capacity (Stevik *et al.*, 2004).

The surface roughness of porous media may increase bacterial adsorption as a result of reduced shear forces; therefore, roughening particle surfaces greatly increases bacterial colonization. Bacteria also adhere more to grooved and braided materials than flat ones, probably partially due to increased surface area (Scheuerman *et al.*, 1998; Katsikogianni and Missirlis, 2004b).

Generally, hydrophilic materials are more resistant to bacterial adhesion than hydrophobic materials. It has been shown that large numbers of bacteria attached to hydrophobic plastics with little or no surface charge, moderate numbers attached to hydrophilic metals with a positive or neutral surface charge, and very few attached to hydrophilic, negatively charged substrata (Katsikogianni and Missirlis, 2004a). The hydrophobicity of a particle surface is controlled to a large extent by the amount of organic matter present. Specifically, increasing amounts of organic matter increase the degree of hydrophobicity of the porous media (Newby *et al.*, 2000).

In addition, microbial biomass in the media is an important factor controlling transport of bacteria in soils (Murphy and Tate, 1996). The build-up of biofilm may restrict pore sizes. The higher the biomass, the greater the probability of soil pore plugging and reductions in bed porosity and hydraulic conductivity, and thus, enhance straining (Taylor and Jaffé, 1990; Vandevivere and Baveye, 1992). The biofilm coating on the porous media also may increase the roughness of the media

surface (Liu and Li, 2008). Biofilm may act as an additional sorbent, thereby enhancing bacterial adhesion (Bellin and Rao, 1993). In addition, the presence of extracellular polymer substances (e.g., polysaccharides, proteins, nucleic acids) in bioflims can greatly enhance bacterial adhesion by hydrogen bonding and polymer bridging between bacterial cells and the film-coated porous media (Stevik *et al.*, 2004; Liu and Li, 2008). Extracellular polymer substances may also alter porous media surface electrostatic charge, therefore changing the electrostatic and van der Waals interactions between bacterial surfaces and porous media (Liu and Li, 2008).

2.2.2.2 Cell surface characteristics

Previous studies have indicated that the cell surface characteristics that affect bacterial attachment to media surfaces primarily include the hydrophobicity, bacterial surface charge and presence of particular surface structures such as flagella, fimbriae, and extracellular lipopolysaccharides (Dong *et al.*, 2002; Foppen and Schijven, 2006). These characteristics are reviewed in detail below.

Some previous research has indicated that hydrophobic cells tend to partition from the aqueous phase and accumulate more readily at the solid-water interface compared to hydrophilic bacteria, which results in the increased adhesion of hydrophobic cells to soil particles (van Loosdrecht *et al.*, 1990b; Huysman and Verstraete, 1993). However, for relatively hydrophilic organisms, such as *E. coli*, there was no correlation between hydrophobicity and bacterial adhesion and transport in soil porous media (Gilbert *et al.*, 1991; Foppen and Schijven, 2006). Scholl *et al.* (1990) and Scholl and Harvey (1992) further indicated that for relatively hydrophilic

organisms, the major factor controlling the initial adhesion of bacteria is the surface charge of the minerals in the aquifer material.

Electrostatic charges on bacterial surfaces develop from dissociation of various functional groups, like carboxyl and amino groups located on the outer surface and in deeper layers of the cell wall (van Loosdrecht *et al.*, 1987b). Accordingly, the surface charge of a bacterium is influenced by the growth medium, the pH and the ionic strength of the suspending solutions, bacterial age, and bacterial surface structure (Katsikogianni and Missirlis, 2004b). In natural environments, most bacteria are negatively charged. Soil particle surfaces also often carry negative electrostatic charges. Thus, the dominant electrostatic interaction between bacterial cells and soil particles is repulsion (Newby *et al.*, 2000).

Bacteria can be classified into gram-positive and gram-negative bacteria based on differences in their cell wall. Most pathogenic bacteria in humans are gram-negative, such as *E. coli* and *Salmonella*. Pathogenic gram-positive bacteria include *Listeria* and *Streptococcus* (Gladwin and Trattler, 2007). The gram-positive cell wall is characterized by the presence of a very thick peptidoglycan layer. Imbedded in the gram-positive cell wall are polyalcohols called teichoic acids, which give the gram-positive cell wall an overall negative charge due to the presence of phosphodiester bonds between teichoic acid monomers. Also, teichoic acids can serve to act as chelating agents, and contribute to the attachment of bacteria to media (Madigan and Martinko, 2005).

In addition to the peptidoglycan layer, the gram-negative cell wall also contains an additional outer membrane composed of phospholipids and lipopolysaccharides

(LPS). When LPS are highly-charged, a gram-negative cell wall has an overall negative charge. It is reported that LPS is a key factor in the attachment of *E. coli* to media surfaces (Foppen and Schijven, 2006). LPS consists of three structurally distinct domains: (1) a hydrophobic lipid A, the membrane anchor and endotoxic portion of LPS; (2) the core saccharide consisting of a branched chain of nonrepeating hexose and heptose sugars; and (3) the polysaccharide (O-antigen) side chain, a repeating unit of sugars that extends into the extracellular milieu (Amor *et al.*, 2000). The core can be further divided into the outer core and the inner core. The inner core is mainly composed of $_{L}$-glycero-$_{D}$-manno-heptose and 3-deoxy-$_{D}$-manno-oct-2-ulosonic (or 2-keto-3-deoxyoctulasonic) acid (KDO) residues, which are phosphorylated in *E. coli*. Most of the charges on the LPS come from the phosphate groups of the inner core, and to a lesser extent on carboxylic acid groups of KDO (Kastowsky *et al.,* 1991). The molecular interactions of these surface functional groups control attachment to media surfaces. The presence of extracellular polymer substances (polysaccharides) can induce bacterial cell-to-cell contact that generates biofilm formation, and enhance bacterial adhesion by hydrogen bonding and polymer bridging between bacterial cells and the film-coated porous media (Davey and Duncan, 2006; Liu and Li, 2008).

Other cell surface structures, such as flagella and fimbriae (or pili) also may influence the transport of bacterial cells in porous media. Flagella are extracellular appendages, and are made of a single type of protein. Flagella can be found on both gram-positive and gram-negative bacteria. If present, it contains two rings for support in gram-positive bacteria, while flagella have four rings in gram-negative

bacteria (Madigan and Martinko, 2005). Flagella typically aid bacteria with motility. The motive force generated at the membrane is used to turn the flagellar filament, in the manner of a turbine driven by the flow of hydrogen ions through the basal body into the cell (Encyclopædia Britannica, 2008). Chemotaxis allows a bacterium to adjust its swimming behavior so that it can sense and migrate toward increasing levels of an attractant chemical or away from a repellent one (Encyclopædia Britannica, 2008). The flagella movement allows the cell to migrate close to a surface, and it is important in controlling the initial interaction between cells and particle surfaces (Stevik *et al*., 2004). Fimbriae, a proteinaceous appendage, is present in gram-negative bacteria. They are thinner and shorter than flagella. The appendages allow bacteria to adhere to media surfaces and keep from being washed away by flowing fluid. Fimibriae also are in part responsible for microbial colonization on particle surfaces as well as the formation of biofilms (Encyclopædia Britannica, 2008).

2.2.2.3 Environmental factors

2.2.2.3.1 Temperature

Bacterial adhesion is sensitive to temperature. For example, Hendricks *et al.* (1979) reported that adsorption of bacteria to soil particles was substantially greater at higher temperature. Some of the explanation for this observation may be provided by Gallardo-Moreno *et al.* (2002) who studied the effect of temperature on the surface physicochemical characteristics and adhesion behaviour of *Enterococcus faecalis* ATCC29212 to glass and silicone. Their results showed that when the temperature

was increased from 22°C to 37°C, water contact angles on bacterial lawns increased, indicating an increase in hydrophobicity. As a result, the increase of temperature resulted in higher adhesion to the hydrophobic silicone, and lower adhesion to the hydrophilic glass.

2.2.2.3.2 Solution chemistry

The pH and ionic strength in the aqueous phase have a large impact on the electrophoretic mobilities of bacteria. Different bacterial species have different isoelectrical points (or points of zero charge). For example, the isoelectric point of *E. coli* O157:H7 was observed to occur at a pH of approximately 4.3 (Lytle *et al*., 1999). When the pH in the aqueous phase is higher than the point of zero charge, the surface charge of a bacterium is negative. The pH of stormwater runoff typically is within the range of 6.5 to 8.5 (Morrison, 1989), thus, *E. coli* in stormwater runoff are normally expected to have a net negative surface potential.

On the other hand, the surface charge of colloid surfaces depends on the ionization of surface groups and/or adsorption of ions from the aqueous phase. Therefore, the surface charge on a bacterium or porous media particle can be neutralized by accumulation of oppositely charged ions in solution. As described above, bacterial adsorption can be explained by DLVO theory (Figure 2.2). With increasing the ionic strength of a soil solution primarily, the double layer repulsive force will reduce. While, the van der Waals attractive force is almost independent of the ionic strength (Newby *et al.*, 2000). Therefore, increasing the ionic strength can lead to a decrease the width of the zone between primary minimum and secondary

minimum (or the thickness of the diffusion layer). As a result of this double layer compression, the bacterial cells may be brought closer to the media, thus increasing the chance of attachment. Previous studies have verified this mechanism (Gannon *et al*., 1991; Powelson and Mills, 2001). At the same time, increasing the ionic strength can make *E. coli* less negative, thus enhancing the adsorption of *E. coli* to porous media (Foppen and Schijven, 2006). However, different species of cations in the solution may produce different effects on the transport of bacteria to media surface at the same ionic strength. Generally, divalent or trivalent cations (e.g., Ca^{2+}, Mg^{2+}, Fe^{3+}) with smaller radii of hydration increase the attachment of bacteria more compared to monovalent cations (Powelson and Mills, 2001). Common anions (e.g., Cl^-, SO_4^{2-}, HCO_3^-) have little influence on adsorption of bacteria (Boyd *et al.*, 1969).

Additionally, organic compounds in soil solutions have been shown to affect the attachment of bacteria to media. This is because dissolved organic matter (DOM) is negatively charged in natural waters (Jardine *et al.*, 1989). For example, Scholl and Harvey (1992) observed an increase in bacterial adsorption after the removal of dissolved organic carbon from the bacterial suspension used in adsorption experiments. This may have been due to competition between dissolved organic carbon and bacteria for positively charged surface sites on the sand. Furthermore, Johnson and Logan (1996) and Johnson *et al.* (1996) also found that dissolved the organic matter inhibited adsorption of strain A1264 on quartz and Fe-quartz particles. They concluded that organic matter increased the negative surface charge of quartz and the bacteria and made the bacteria–quartz interaction electrostatically unfavorable.

2.2.2.3.3 Hydraulic factors

Flow conditions are considered one of dominant factors that influence the number of attached bacteria, as well as biofilm performance in porous media (Duddridge *et al.*, 1982, Stoodley *et al.*, 1999). It is generally considered that higher flow rates, and correspondingly higher shear rates, create higher detachment forces that result in a decrease in the number of attached bacteria and a correspondingly thinner biofilm (Rittmann, 1982). Higher flow velocities also reduce the contact time, thus decreasing the likelihood of bacterial adsorption.

2.3 The survival of bacterial in porous media

Pathogens migrating through porous media can be captured by filtration, adsorption and sedimentation, as described above. However, unlike abiotic pollutants (e.g., heavy metals, suspended solids), once removed from solution, the number of trapped enteric bacteria dramatically varies under different environmental conditions due to growth or decay of these bacteria and predation by other microorganisms. The die-off rates of various *E. coli* strains under different conditions are present in Table 2.1. As reviewed in the following subsections, the survival of enteric bacteria in soil is primarily influenced by the availability of nutrients, temperature, moisture content, pH, heavy metal concentrations, oxygen levels, sunlight and biotic factors (e.g., competition from other bacteria and predation by bacterial predators and protozoa) (Bitton and Harvey, 1992).

Table 2.1 Effect of media, temperature, moisture, pH and oxygen on the die-off rate of *E. coli* (adapted from Foppen and Schijven, 2006).

Strain	Media	T (ºC)	Moisture (% field capacity)	pH	Die-off rate (d^{-1})	Reference
Effect of media						
E. coli	Ground water	20			0.12	Kudryavseva, 1972
E. coli	Seawater	20		7.1	0.42	Althaus *et al.*, 1982
E. coli ATCC 25922	Estuarine waters	20			1.57	Hood and Ness, 1982
E. coli ATCC 25922	Sediment	20			0.20	
E. coli	Intact soil core (depth=25cm)	15	50		0.069	Oliver *et al.,* 2006
E. coli	Repacked soil core	15	50		0.096	
E. coli	Repacked soil core with slurry incorporated	15	50		0.094	
E. coli	Repacked soil core with feces incorporated	15	50		0.054	
Effect of temperature and moisture						
E. coli O157:H7	Sandy loam soil with manure amended (mass ratio 10:1) during the first 40 days	5			0.06	Jiang *et al.*, 2002
E. coli O157:H7		15			0.15	
E. coli O157:H7		21			0.21	
E. coli	Loamy sand soil mixed with fresh pig manure (mass ratio 9.3:1)	5	80	5.2	0.08	Cools *et al*., 2001
E. coli		25	80	5.2	0.25	
E. coli	Sandy soil mixed with fresh pig manure (mass ratio 9.3:1)	5	80	4.6	0.04	
E. coli		25	80	4.6	0.21	
E. coli	Loamy soil mixed with fresh pig manure (mass ratio 9.3:1)	5	80	6.7	0.08	
E. coli		25	80	6.7	0.26	
E. coli		5	60	6.7	0.30	
E. coli		5	100	6.7	0.05	
Effect of pH						
E. coli MH3427	Deionized water	10		4	0.58	McFeters and Stuart, 1972
E. coli MH3427	Deionized water	10		5	0.4	
E. coli MH3427	Deionized water	10		6	0.3	
E. coli MH3427	Deionized water	10		7	0.32	
E. coli MH3427	Deionized water	10		10	0.71	
Effect of oxygen						
E. coli	0.003M phosphate buffer (1-18% oxygen saturation)	20		6	0.58	Althaus *et al.,* 1982
E. coli	0.003M phosphate buffer (100% oxygen saturation)	20		6	0.09	

2.3.1 Available nutrients

E. coli cells have been observed to be not well-adapted to survive in soil (Cools *et al.*, 2001; Oliver *et al.*, 2006). For example, typical laboratory column studies indicate a rapid decline in the numbers of inoculated cells and a decrease in the average activity per cell for the surviving microbes (Newby *et al.*, 2000). Klein and Casida (1967) suggested that the major death factor of *E. coli* in natural soil was their inability to step down their metabolic rate to meet the low availability of nutrients in soil. This is supported by the observation that the decrease of *E. coli* populations in non-sterile soils could be reversed, in part, by the addition of organic nutrients (Krasovsky and Stotzky, 1987). Similarly, Oliver *et al. (*2006) found that the die-off rate of *E. coli* in soil decreases with the addition of nutrients (slurry (liquid mixture of excrement and urine produced by housed livestock) or feces), that is, *E. coli* can survive longer time when plentiful nutrients are provided (Table 2.1). Tate (1978) also indicated that when *E. coli* ATCC15597 was introduced into an organic soil (Histosols, containing greater than 80% organic matter), it survived longer than in a fine sand. However, as discussed further below, when these nutrients (such as manure, slurry, feces) are introduced, the effect of the native microorganisms in these nutrient sources on the growth of *E. coli* has to be considered, because these microorganisms may contribute to decreases in the number of bacteria by predation of antagonistic organisms and competition for nutrients with *E. coli*.

2.3.2 Temperature and moisture content

In general, a lower incubation temperature and a higher soil moisture content favor the survival of bacteria (Table 2.1). For example, laboratory studies by Cools *et al.* (2001) investigated survival of *E. coli* and *Enterococcus* spp. in three soils of different texture (a sandy soil, a loamy soil and a loamy sand soil) at three different incubation temperatures (5, 15, and 25ºC). Specifically, they found that the number of the two species declined more rapidly at 25ºC than 5ºC in each soil with the same moisture content. Similarly, *E. coli* O157:H7 in bovine feces survived for 42 to 49 days at 37ºC, for 49 to 56 days at 22ºC, and for 63 to 70 days at 5ºC (Wang *et al.*, 1996). Other researchers observed similar results (e.g., Sjogren, 1994; Sørensen *et al.*, 1999). Jiang *et al.* (2002) also found that the die-off rate of *E. coli* O157:H7 in manure-amended soil was lower at 5 ºC than 15 or 21ºC during the first 40 days. The maximum day at which the concentration of *E. coli* O157:H7 decreased below the detection limit at 5, 15 and 21ºC was 56, 152, and 193 days, respectively. The low growth or decay rate of *E. coli* itself and the lack of antagonistic activity of predators at cooler conditions may contribute to the longer survival of *E. coli* at 5ºC (Cools *et al.*, 2001).

Various bacterial species differ in their abilities to withstand low or high water contents. However, in general, bacteria can survive longer in moist soil then in dry soil (Campbell and Beiderbeck, 1976). Cools *et al.* (2001) found no significant difference in survival of *E. coli* in soil at 60, 80 and 100% of the field capacity at 15ºC, but at 5 and 25ºC, survival at 100% field capacity was significantly higher than at the other two moisture contents (data at 5 ºC are presented in Table 2.1).

Nevertheless, moisture content increases above a certain optimum level have been shown to result in a decrease in microbial cells in natural soil (Postma and Veen, 1990), possibly due to oxygen depletion as pores become water saturated and to the higher motility of predators at higher soil moisture contents.

2.3.3 Other abiotic factors

The survival of pathogens in porous media is affected by a variety of other abiotic factors. Generally, soil pH that is too low or high decreases the survival of most bacteria (Table 2.1). In the case of *E. coli*, survival is better in more neutral-to-alkaline soil than in more acidic soil (Sjogren, 1994). Heavy metals are also known to have a toxic effect on bacteria. For example, the die-off rate of *E. coli* O157:H7 in groundwater with a copper concentration of 3.91 mg/L was double that in groundwater with 0.61 mg Cu/L (Artz and Killham, 2002). The die-off rate of *E. coli* in water with a low oxygen saturation was significantly higher than in water saturated with oxygen (Table 2.1). Additionally, in the absence of sunlight, fecal coliforms in seawater survived for days, whereas 90% of the fecal coliforms were inactivated within 30 to 90 min in the presence of sunlight. Visible light rather than the ultraviolet light spectrum of sunlight was primarily responsible for the bactericidal effect (Fujioka *et al.*, 1981).

2.3.4 Biotic factors

In addition to the abiotic factors described above, several biotic factors also negatively influence bacterial survival, such as substrate competition between

microorganisms and predation by protozoa. In water, enteric bacteria appear to be unable to adequately compete with natural microflora for low concentrations of nutrients (Sinclair and Alexander, 1984). As mentioned above, this is probably because the enteric bacteria are not able to step down their metabolic rate to meet the low availability of nutrients, thus resulting in the decline of their numbers (Klein and Casida, 1967).

Additionally, predation by protozoa and bacterial predators has been suggested as a regulating factor for population size of pathogenic bacteria introduced to soil environments (Sørensen *et al.*, 1999). Similarly for wastewater treatment, it was also found that protozoan grazing plays an important role as an enteric bacteria removal mechanism in sand infiltration systems (Stevik *et al.*, 1998; Bomo *et al.*, 2004). Enzinger and Cooper (1976) studied the relative role of protozoa and bacterial predators on the survival of *E. coli* in estuarine water samples by adding different antibiotics. Their investigation indicated that predation mainly results from protozoa, whereas bacterial predators were not important for coliform destruction. However, the studies of McCambridge and McMeekin (1980) suggested that interacting microbial predators each played a distinct role in the destruction of fecal bacteria after *E. coli* was introduced into estuarine water, with predacious protozoa exerting their major influence on *E. coli* destruction during the first 2 days. It is reported that bacteria may maintain at a minimum population size in the presence of predators. If the number of bacteria falls lowers than this level, predation has little or no effect on the decline of the bacterial population. If the number of bacteria is higher than this

level, the bacterial population may be reduced to the minimum level by predation (Alexander, 1981; Stevik *et al.*, 2004).

Protozoa have been identified as major consumers of primary producers (both algae and bacteria) in food chains. They, in turn, constitute an important food resource for metazoan plankters (Warren *et al.*, 1997). Predation by protozoa on bacteria can have positive or negative effects on bacterial populations and their activity. Protozoan grazing may select for bacterial populations based on size, cell surface characteristics, or other bacterial properties (Tso and Taghon, 2006). Selective grazing can cause changes in the bacterial community structure, effectively eliminating some types of bacteria while allowing others to flourish. As described above, predation enhances elimination of *E. coli*, which is desirable for pathogen treatment in bioretention systems. Alternatively, predation may stimulate the activity and growth of some bacteria through increased recycling of limiting nutrients and nitrogen mineralization, or prevention of bacterial stasis (Bonkowski, 2003; Tso and Taghon, 2006).

Protozoa are the most effective of bacterial feeders because of their feeding and growth habits. Protozoa eat first, and then multiply through division, with generation times potentially as short as bacteria. As a result, they quickly develop a high grazing pressure (Clarholm, 2002). Sørensen *et al.* (1999) reported that protozoa density in an uninoculated soil remained constant, while the protozoa multiplied to a 10-100 fold higher level after addition of *E. coli* to the soil.

Correspondingly, it is natural that bacteria would employ various strategies to evade predation, such as adopting a very small or larger size, forming superstructures

(e.g., filaments, or flocs), or in habiting the small pores that exclude the protozoa (Wang *et al.*, 2005). It was reported that bacteria tend to have a somewhat greater abundance in the fraction of soils with pores smaller than 6 μm and that such pores may protect bacteria from predation (Ranjard and Richaume, 2001). However, bacteria in such environments face the added disadvantage of a more limited supply of nutrients diffusing into dead volumes (Wright *et al.*, 1995). As described in Section 2.3.2, temperature significantly influences the growth and decay of *E. coli* in porous media. Consistently, protozoa are also temperature sensitive. Most of the protozoa grow best at room temperature (18 to 22ºC), although some protozoa species can grow well at temperatures up to and above 30ºC. For example, genera *Naegleria* and *Acanthamoeba* can be cultured at temperatures in the range of 37 to 45ºC (Warren *et al.*, 1997). However, most protozoa began to encyst at 37ºC (Darby *et al.*, 2006).

Many researchers have evaluated the impact of indigenous microbes on the survival of introduced bacteria by comparing the survival of pathogens in non-sterile media to that in sterilized media (McCambridge and McMeekin, 1980; Hood and Ness, 1982; Jiang *et al.*, 2002). Autoclaving and gamma-irradiation are commonly used to sterilize natural media, although compared to autoclaving, gamma irradiation may have less of an affect on the chemical and physical properties of the media (McNamara *et al.*, 2003). However, both of the two methods can increase the amount of available nutrients in the media, such as soluble organic carbon, ammonium nitrogen, exchangeable sulfur and exchangeable Mn (Salonius *et al.*, 1967; Lensi *et al.*, 1991; McNamara *et al.*, 2003). Based on these previous studies, in autoclaved

soils, sediments, or estuarine water, *E. coil* can survive longer, or the number of *E. coli* declines more slowly, compared to non-sterile media (McCambridge and McMeekin, 1980; Hood and Ness, 1982; Jiang *et al.*, 2002). These results can be attributed to eliminating antagonistic microbial activity, and increasing the nutrient levels in the sterilized media.

An alternative research method for evaluating the impact of native microbes on introduced bacteria, without introducing nutrients or other microorganisms, is to add a protozoan antibiotic. Cycloheximide (Actidione), which can inhibit protein biosynthesis in eukaryotic organisms, is one of the most widely used protozoa inhibitors (McCambridge and McMeekin, 1980; Sørensen *et al.*, 1999; Bomo et al., 2004). However, the treatment does not inhibit all protozoa. For example, Sørensen *et al.* (1999) indicated that compared to the protozoan density (10^4 to 10^5 protozoa per gram of soil) in untreated soil, the protozoa in the soil treated with cycloheximide decreased at least 10 fold, but approximately 10^3 protozoa per gram of soil survived. Additionally, a persistent problem with using chemicals to inhibit eukaryotic cell function is the possibility that other components of the indigenous microbial communities are also altered. For example, inhibition of protozoa may result in a marked increase in the number of indigenous bacterial predators compared to an untreated system, and the growth of indigenous anaerobic bacteria (McCambridge and McMeekin, 1980; Tso and Taghon, 2006). Thus, whatever technique is used, it is a challenge to evaluate the impact of protozoa on the survival of pathogenic bacteria introduced to porous media, while maintaining the physical, chemical, and biological conditions in the porous media.

2.4 Surface-modified media for bacterial removal

Removal of pathogenic bacteria via media filtration is difficult, because these colloids carry a negative surface charge in the pH range of natural waters and most filter media (e.g., sand, diatomaceous earth) are also negatively charged in this pH range. Therefore, in recent years, concentrated efforts have been made to improve the performance of common negatively charged filter media for removing microbial contaminants from water by utilizing some modified media, such as sand coated with (hydro)oxide iron or aluminum, composite minerals, and activated carbon (Abudalo *et al.*, 2005; Morrow *et al.*, 2005; Pal *et al.*, 2006). The performance of these modified media for bacterial removal is presented in detail below.

As described above in Section 2.2.2.2, bacteria generally are negatively charged under natural environmental conditions. Therefore, bacteria can be strongly bound to positively charged media. Iron and aluminum oxides possess a positively charged surface at the pH values of interest ($pH_{zpc} \approx 8.5$, Stumm and Morgan, 1981). As a result, coatings of iron or aluminum oxides on the media surface can significantly increase the bacterial adsorption by altering the surface charge of the media, thus, making the interaction between bacteria and media more favorable. Many previous studies have demonstrated that iron or aluminum hydroxide coated sand increases the sticking efficiency (α), and can achieve a high bacterial removal efficiency (Knapp *et al.*, 1998; Bolster *et al.*, 2001; Abudalo *et al.*, 2005, Foppen and Schijven, 2005). However, the performance of metallic hydroxides coated media has been shown to decrease significantly over prolonged use when the media was continuously exposed to wastewater (Chen *et al.*, 1998). For example, the effective lifetime of aluminum

hydroxide coated sand was found to be approximately 4 months when the sand was exposed to chlorinated wastewater in continuous flow-through columns. This change could have been caused by the decrease of the zeta potential of coated sands after being exposed to wastewater.

Morrow *et al.* (2005) examined several candidate materials - α-alumina, silica, dolomite, pyrophyllite and Pyrax - which varied widely in their surface characteristics (hydrophobicity and surface charge), for their potential to attenuate the migration of pathogens in a biobarrier. Interestingly, the negatively charged, hydrophobic pyrophyllite ($Al_2Si_4O_{10}(OH)_2$) achieved a higher bacterial retention (93%) and a higher deposition coefficient for *E. coli* O157:H7 than α-alumina (hydrophilic and positively charged), silica (hydrophilic and negatively charged) or dolomite (hydrophobic and positively charged). They also tested another composite mineral, the negatively charged Pyrax, consisting of mica (5%), kaolinite (5-10%), quartz (35-45%), and pyrophyllite (40%). This relatively inexpensive mineral also demonstrated high bacterial retention, which was attributed to the hydrophobic pyrophyllite component of this composite mineral. However, pyrophyllite is a phyllosilicate mineral species belonging to the clay family (Chisholm, 1911). High contents of clay may restrict water infiltration, thus resulting in clogging in the filtration system.

In addition, activated carbon has also been widely used in point-of-use filtration systems due to its high adsorption rates and capabilities for reducing concentrations of toxic organic compounds. Foppen and Schijven (2005) found that, compared to goethite-coated sand and a mixture of quartz sand with calcite, a mixture of quartz with grains of activated carbon had a higher sticking efficiency and removal

efficiency for *E. coli* at lower concentrations of *E. coli* (10^3-10^4 cells/mL). In addition, Pal *et al.* (2006) also indicate that modification of activated carbon by aluminium hydrooxychloride can change the zeta potential of activated carbon from negative to positive, thus enhancing bacteria removal. A greater than 6-log removal of *E. coli* was achieved using activated carbon modified with 1-10% aluminium hydrooxychloride.

However, the inherent drawback of activated carbon is its excellent biocompatibility with bacteria. Bacteria may proliferate on activated carbon during the transport process, thus, eventually causing the carbon materials themselves to become pollutants. Some researchers have tried to fix this problem by modifying the filter matrix with an antibacterial coating (e.g., silver, diallyl dimethyl ammonium chloride, or zinc hydroxide). The studies by Pal *et al.* (2006) showed that no bacterial growth was observed on the media modified with each antibacterial material. Nevertheless, the process of modifying activated carbon with a silver coating is expensive, and introducing these antibacterial materials may result in subsequent environmental risks due to leaching.

2.5 Bacterial transport models

2.5.1 Transport equation

In general, the advection-dispersion-sorption (ADS) equation can be used to describe the transport of bacteria in porous media (Harvey and Garabedian, 1991; Tan *et al*., 1994; Tufenkji, 2007). Because residence times in laboratory-scale column experiments are usually short, inactivation of microorganism in aqueous phase is

negligible (Tufenkji, 2003). Thus, the one-dimensional ADS equation for transport of bacteria can be written as:

$$\frac{\partial c}{\partial t} + \frac{\rho}{\theta} \cdot \frac{\partial S}{\partial t} = D \frac{\partial^2 c}{\partial^2 x} - \nu \frac{\partial c}{\partial x} \tag{2.1}$$

where c is the concentration of bacteria suspended in aqueous solution (M_{cells}/mL), ρ is the bulk density of the porous medium (M_{solid}/L^3), θ is the porosity, S is the concentration of bacteria attached to the solid phase (M_{cells}/M_{solid}), D is the hydrodynamic dispersion coefficient (L^2/T), x is the distance (measured from the entrance of the column), ν is the average pore water velocity (L/T).

Bacterial adsorption can be described by a linear equilibrium or kinetic expression (Reddy and Ford, 1996). If only single-component equilibrium is considered, the general adsorption isotherm is described by a linear adsorption equation (Harvey and Garabedian, 1991; Reddy and Ford, 1996; Tufenkji, 2007):

$$S = k_D \cdot c \tag{2.2}$$

$$\frac{\rho}{\theta} \cdot \frac{\partial S}{\partial t} = \frac{\rho k_D}{\theta} \cdot \frac{\partial c}{\partial t} \tag{2.3}$$

where k_D is the diffusion coefficient. Thus, Equation 2.1 can be rewritten as

$$R \frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial^2 x} - \nu \frac{\partial c}{\partial x} \tag{2.4}$$

$$R = 1 + \frac{\rho k_D}{\theta} = \frac{\nu_{water}}{\nu_{microbe}} \tag{2.5}$$

where R is the retardation factor, which can be defined as the ratio of the mean water flow velocity (ν_{water}) to the mean transport velocity of microorganisms ($\nu_{microbe}$) (Harvey, 1997). Foppen and Schijven (2006) reviewed a number of studies

regarding *E. coli* transport in laboratory columns and in field tests, concluding that the retardation factor was in a range between 0.1 and 2.81 and that the average of all values evaluated was 1.15.

However, an equilibrium adsorption state is not reached instantaneously in a suspension of microbes and colloid particles (Tufenkji, 2007). Therefore, kinetic adsorption can express the bacterial transport better, which will be used in the data analysis of this research. The kinetic attachment mechanism is composed of attachment and detachment, and controls the removal of microorganisms from the aqueous phase. Assuming a first-order kinetic reaction for the rate of attachment and detachment, equation 2.1 can be described as follows (Deshpande and Shonnard, 1999; Tufenkji, 2007):

$$\frac{\rho}{\theta}\frac{\partial S}{\partial t} = k_a c - \frac{\rho}{\theta} k_{\text{det}} S \tag{2.6}$$

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial^2 x} - \nu\frac{\partial c}{\partial x} - k_a c + \frac{\rho}{\theta} k_{\text{det}} S \tag{2.7}$$

where k_a is the adsorption/deposition rate coefficient (T^{-1}), and k_{det} is the detachment rate coefficient (T^{-1}). Equation 2.1 can also be rewritten as:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial^2 x} - \nu\frac{\partial c}{\partial x} - k_a c + R_d \tag{2.8}$$

where $R_d = \frac{\rho}{\theta} k_{\text{det}} S$. R_d represents the rate at which bacteria desorb from the collector ($M_{cells} \cdot L^{-3} \cdot T^{-1}$).

2.5.2 Adsorption/deposition rate coefficient (k_a)

The adsorption/deposition rate coefficient k_a can be calculated based on colloid filtration theory (Tien *et al.*, 1979; Bolster *et al.,* 1998; Tufenkji and Elimelech, 2004):

$$k_a = \frac{3(1-\theta)}{2}\frac{\nu}{d_c}\eta_0\alpha \tag{2.9}$$

where η_0 is the single-collector collision efficiency, α is the sticking coefficient, and d_c is the diameter of the collector. Martin *et al.* (1996) suggested that characteristic collector diameters d_c based on the smaller particles (arithmetic mean d_a, geometric mean d_g, or 10^{th} percentile of the cumulative grain size d_{10}) can most accurately described bacterial transport in different-sized porous media. The other two key parameters in Equation 2.2, η_0 and α, are discussed in the following two subsections.

2.5.3 Single collector collision efficiency (η_0)

The single collector collision efficiency (η_0) is defined as the fraction of approaching particles which collide with the collector (Deshpande and Shonnard, 1999). The Rajagopalan and Tien (RT) model, which is based on a model that restricts fluid flow to a concentric spherical space surrounding the collectors, is widely used to calculate the single collector collision efficiency η_0 (Rajagopalan and Tien, 1976; Logan *et al.*, 1995). Tufenkji and Elimelech (2004) further optimized the RT model. Their correlation equation was developed assuming that the overall single-collector efficiency can be calculated as the sum of the contributions of the

individual transport mechanisms - Brownian diffusion (η_D), interception (η_I), and gravitational sedimentation (η_G):

$$\eta_0 = \eta_D + \eta_I + \eta_G \tag{2.10}$$

These transport mechanisms are defined by Tufenkji and Elimelech (2004) as:

$$\eta_D = 2.4 A_s^{1/3} N_{pe}^{-0.715} N_R^{-0.081} N_{vdw}^{0.052} \tag{2.11}$$

$$\eta_I = 0.55 A_s N_R^{1.675} N_A^{0.125} \tag{2.12}$$

$$\eta_G = 0.22 N_R^{-0.24} N_G^{1.11} N_{vdw}^{0.053} \tag{2.13}$$

where:

$$A_s = 2(1-\gamma^5)/(2-3\gamma+3\gamma^5-2\gamma^6) \tag{2.14}$$

$$\gamma = (1-\theta)^{1/3} \tag{2.15}$$

$$N_{pe} = \frac{U d_c}{D_f} \tag{2.16}$$

$$U = \nu \cdot \theta \tag{2.17}$$

$$D_f = \frac{k_B (T+273)}{3\pi d_p \mu} \tag{2.18}$$

$$N_R = d_p / d_c \tag{2.19}$$

$$N_{vdw} = \frac{A}{k_B (T+273)} \tag{2.20}$$

$$N_A = \frac{A}{12\pi\mu d_p^{\,2} U} \tag{2.21}$$

$$N_G = \frac{2}{9} \frac{d_p^{\,2} (\rho_p - \rho_f) g}{\mu U} \tag{2.22}$$

where: k_B is Boltzmann's constant (1.38×10^{-23} J/K), T is temperature (°C), d_p is the bacterial diameter, and μ is dynamic viscosity; A is the Hamaker constant (J), which is equal to 6.2×10^{-21} J for the bacterium-glass-water interface (Rijnaarts *et al.*, 1995); and ρ_p and ρ_f are the bacterial density and fluid density, respectively.

2.5.4 Sticking coefficient (α)

The sticking coefficient (α) is defined as the ratio of the rate that bacteria stick to a collector to the rate they strike the collector (Logan *et al.*, 1995). Because current theories are inadequate to predict α, it is common to use column experiments to determine α under given physicochemical conditions by using the following relationship (Tufenkji and Elimelech, 2004):

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-\theta)L\eta_0}\ln\frac{c}{c_0} \tag{2.23}$$

where L is the filter medium packed length, and c/c_o is the column outlet normalized bacterial concentration at the initial stage of the particle breakthrough curve (Tufenkji and Elimelech, 2004). Ideally, α is equal to 1 in a completely destabilized system (Yao *et al.*, 1971).

As discussed above, the surface charge difference between the collector and *E. coli* is one of the most important factors determining the α for these two materials (Foppen and Schijven, 2006). The charge differences depend on the solution chemistry, ionic strength, geochemical heterogeneity and surface roughness of media, and the composition of lipopolysaccharides on the outer membrane of *E. coli*. Increasing the ionic strength or increasing the fraction of positively charged materials on sand will greatly increase the sticking coefficient, while increasing the dissolved organic matter in the bacterial suspension will decrease α (Foppen and Schijven, 2005; Foppen and Schijven, 2006), as discussed in Section 2.2.2.3.2.

2.5.5 Desorption or detachment rate (R_d)

As described above, R_d is related to the detachment rate coefficient k_{det}. The detachment rate coefficient, k_{det} can be determined empirically from detachment functions, which describe the change in bacterial fraction retained, N/N_0, with time (Blue *et al.*, 1995):

$$N / N_0 = e^{-k_{det} t} \qquad t < t_i$$
$$N = \text{Constant} \qquad t \geq t_i \tag{2.24}$$

Here, N_0 is the number of bacteria trapped on the collector at the time of initial adsorption; N is the number of that trapped bacteria remaining at times thereafter; and t_i is a specific time when N starts to become a constant.

The detachment of *E. coli* has not been studied systematically. Due to the deficiency in the current understanding of microbial detachment processes, detachment was often ignored in the classical colloid filtration theory to predict microbe migration and fate in saturated porous media. Slow release of microorganisms has been observed in laboratory and field-scale transport experiments of bacteria, viruses, and protozoa. Therefore, detachment occurs, but as a process it is considered relatively unimportant (Tufenkji, 2007).

2.5.6 Solution of the transport equation

To solve the ADS equation (2.8), it is necessary to select the appropriate boundary conditions. For a continuously constant feeding process in a column study, the following initial and boundary conditions apply (van Genuchten and Alves, 1982):

$$
\begin{aligned}
&c(x,0) = c_i \\
&-D\frac{\partial c}{\partial x} + vc\big|_{x=0} = \begin{cases} vc_0 & 0 \le t \le t_0 \\ 0 & t \ge t_0 \end{cases} \\
&\frac{\partial c(\infty,t)}{\partial x} = 0
\end{aligned}
\tag{2.25}
$$

where t_o is the end of the time of continuously feeding. The analytical solution of equation (2.8) subject to these conditions can be written as (van Genuchten and Alves, 1982):

$$
c(x,t) = \begin{cases} \frac{R_d}{k_a} + (c_i - \frac{R_d}{K_a})A(x,t) + (c_0 - \frac{R_d}{K_a})B(x,t) & 0 < t < t_0 \\ \frac{R_d}{K_a} + (c_i - \frac{R_d}{K_a})A(x,t) + (c_0 - \frac{R_d}{K_a})B(x,t) - c_0 B(x,t-t_0) & t \ge t_0 \end{cases}
\tag{2.26}
$$

where

$$
\begin{aligned}
A(x,t) = \exp(-k_a t)\{1 - \frac{1}{2}erfc\left[\frac{x - vt}{2(Dt)^{1/2}}\right] - (\frac{v^2 t}{\pi D})^{1/2}\exp\left[-\frac{(x-vt)^2}{4Dt}\right] \\
+ \frac{1}{2}(1 + \frac{vx}{D} + \frac{v^2 t}{D})\exp(\frac{vx}{D})erfc\left[\frac{x - vt}{2(Dt)^{1/2}}\right]\}
\end{aligned}
\tag{2.27}
$$

$$
\begin{aligned}
B(x,t) = \frac{v}{v+u}\exp[-\frac{(v-u)x}{2D}]\ erfc\left[\frac{x-ut}{2(Dt)^{1/2}}\right] + \frac{v}{v-u}\exp[-\frac{(v+u)x}{2D}]\ erfc\left[\frac{x+ut}{2(Dt)^{1/2}}\right] \\
+ \frac{v^2}{2k_a D})^{1/2}\exp(\frac{vx}{D} - k_a t)\,erfc\left[\frac{x+vt}{2(Dt)^{1/2}}\right]
\end{aligned}
\tag{2.28}
$$

$$
u = v(1 + \frac{4k_a D}{v^2})^{1/2}
\tag{2.29}
$$

Alternatively, Equation (2.8) can also be converted to the following dimensionless form:

$$\frac{\partial C}{\partial T} = \frac{1}{Pe}\frac{\partial^2 C}{\partial X^2} - \frac{\partial C}{\partial X} - \kappa_1 C + \kappa_2 \tag{2.30}$$

where Pe =νL/D, X =x/L, T=ν·t/L, $\kappa_1=k_a\cdot L/\nu$, $\kappa_2=R_d\cdot L/\nu$, and C= c/c_o.

After further neglecting detachment (κ_2), Bolster *et al.* (1998) solved equation (2.30) for the normalized bacterial flux concentration, C(X,T), at the end of a column (X =1 or x=L) for a step input by modifying the solution of Parlange *et al.* (1992):

$$\begin{aligned} C(1,T) = {} & 0.5\exp\left[\frac{Pe}{2}\left(1-\sqrt{1+4\kappa_1 Pe^{-1}}\right)\right] erfc\left[\frac{1-T\sqrt{1+4\kappa_1 Pe^{-1}}}{\sqrt{4\kappa_1 Pe^{-1}}}\right] \\ & + 0.5\exp\left[\frac{Pe}{2}\left(1+\sqrt{1+4\kappa_1 Pe^{-1}}\right)\right] erfc\left[\frac{1+T\sqrt{1+4\kappa_1 Pe^{-1}}}{\sqrt{4\kappa_1 Pe^{-1}}}\right] \end{aligned} \tag{2.31}$$

Chapter 3: The capture and destruction of pathogenic bacteria from simulated urban stormwater runoff using conventional bioretention media and iron oxide-coated sand

3.1 Introduction

Elevated concentrations of microbial pollutants in urban stormwater runoff is becoming one of the primary sources of water quality impairment in estuaries, coastal waters, and other receiving water bodies. Beach closings, restrictions on shellfish harvesting, and the need for increased treatment of drinking water to decrease the risk to human health are commonly related to pathogen problems (USEPA, 2001b; Stoner and Dorfman, 2006). As described in Chapter 2, physical filtration and biological adsorption are the main mechanisms of bacterial capture in porous media (Newby *et al.*, 2000). Bioretention (sandy loam soil, sand, mulch media mixture) may potentially achieve higher removal of input bacteria than those determined for traditional infiltration systems due to the enhanced biological activity in conventional bioretention media (CBM). Nevertheless, limited data evaluating the capacity of bioretention systems to remove pathogenic bacteria from stormwater runoff have been published to date (Rusciano and Obropta, 2007; Hunt *et al.*, 2008).

Generally, soil surfaces carry negative electrostatic charges, while most microorganisms are also negatively charged, creating an unfavorable environment for bacterial attachment and immobilization. Therefore, in recent years, efforts have been made to improve filter media performance for removing microbial contaminants from water by employing coatings of metal (hydr)oxides on porous media (e.g., Mills

et al.,1994; Abudalo *et al.*, 2005). Some metal (hydr)oxides possess positively charged surfaces at neutral pH, with the potential for high bacterial adsorption. This makes them good candidates for exploitation in engineered bioretention layers for bacterial removal.

The overall goal of research was to evaluate the capture and destruction of pathogenic bacteria in CBM using *E. coli* O157:H7 strain B6914 as a surrogate of pathogenic *E. coli*. The specific objectives of this research were to evaluate via column transport experiments the effectiveness of the capture of strain B6914 by CBM, investigate the potential of an engineered media (iron oxide-coated sand (IOCS)) for enhancing bacterial removal, and evaluate the sustainability of the process by monitoring the subsequent survival/destruction of the trapped bacterial cells in the two media. In addition, in order to elucidate the effect of indigenous microorganisms on the capture and destruction of strain B6914, parallel transport and survival experiments were conducted using γ-irradiated sterile CBM. Though γ-irradiation killed all microorganisms in the media, it may cause increases in available nutrients, which may be favorable for the growth of captured bacteria (Salonius *et al.*, 1967; Lensi *et al.*, 1991; McNamara *et al.*, 2003). Thus, complementary transport and survival experiments were conducted using the γ-irradiated media coated with cultured native microorganisms from CBM.

3.2 Materials and methods

3.2.1 Growth and physiochemical characteristics of test microorganism

3.2.1.1 Microorganism

As described in Chapter 1, *E. coli* has been demonstrated to be a most common indicator for the presence of fecal contamination, and *E. coli* O157:H7 is of interest because of its severe pathogenic potential. Castro and Tufenkji (2007) indicated that the physicochemical characterizations (cell surface hydrophobicity and eletrophoretic mobility) of nontoxigenic strains and toxigenic strains of *E. coli* O157:H7 had no observable difference at high solution ionic strength (>1 mM), and nontoxigenic strains and toxigenic strains had similar attachment to granular media. The ionic strength of synthetic urban stormwater runoff was approximately 5.8 mM. The composition of synthetic runoff is listed below. Therefore, a derivative of *E. coli* O157:H7 strain B6914 that has lost the shiga-toxin genes and been engineered to contain a green fluorescent protein gene (Fratamico *et al*., 1997) was used as the test organism in this study.

The original inoculum of strain B6914 was provided by Dr. J. S. Karns (Environmental Microbial Safety Laboratory, USDA, Beltsville, MD). The culture was maintained by streaking strain B6914 onto Luria-Bertani (LB) agar plates (10 g of tryptone, 5 g of yeast extract, 10 g of sodium chloride, 15 g of agar per liter (Miller, 1972)) containing 100 μg ampicillin (Ap) per mL, incubating overnight at 37 °C, and then storing the plates in a refrigerator (4°C). The culture was transferred to fresh plates every one month.

3.2.1.2 Bacterial growth conditions

To prepare the stock solution of strain B6914 for use in the transport and survival experiments, a single colony of strain B6914 from a LB agar plate was aseptically transferred using a sterile inoculating loop into 5 mL of LB broth (same ingredients as LB agar without the agar) with 100 μg/mL Ap in a sterile centrifuge tube. The culture was then grown overnight in a reciprocating water bath shaker (Model R76, New Brunswick Scientific Co. Inc., Edison, NJ, U.S.A.) at 37°C and 120 rpm. Then, a 2 mL sample from this liquid culture was aseptically transferred into 100 mL of LB broth (with Ap at 100 μg/mL) in a sterile 500 mL side-arm flask with a cotton plug, and incubated at 37°C with shaking (120 rpm). Subsequently, the optical density value of the culture was monitored using a Spectronic 21 spectrophotometer (Bausch & Lomb, U.S.A) at 600 nm (OD_{600}). A typical growth curve of strain B6914 under these conditions is shown in Figure 3.1(a). The bacteria grew exponentially in the OD range of 0 to 0.9, after which the number of bacteria was stable and the culture entered the stationary phase. To maintain a stable bacterial feed for the column studies, strain B6914 was cultured and harvested at the late exponential phase ($OD_{600nm} \approx 0.9$). Then, 10 mL of culture solution was aseptically transferred into a 15 mL sterile centrifuge tube, centrifuged (4,500 rpm/2,038 $\times$g) for 10 min, after which the cell pellet was washed with phosphate buffered saline (PBS, pH=7.5) and then centrifuged again. The PBS was prepared by dissolving 1.236 g sodium phosphate dibasic (Na_2HPO_4), 0.180 g sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) and 8.500 g sodium chloride (NaCl) in 1 L sterile deionized water (Gerhardt *et al.*, 1994). After pouring off the supernatant, the cells were suspended in

4 mL PBS, and the cell numbers determined by spread-plate-counts method described below. Once prepared, the stock solution was always used within a half hour.

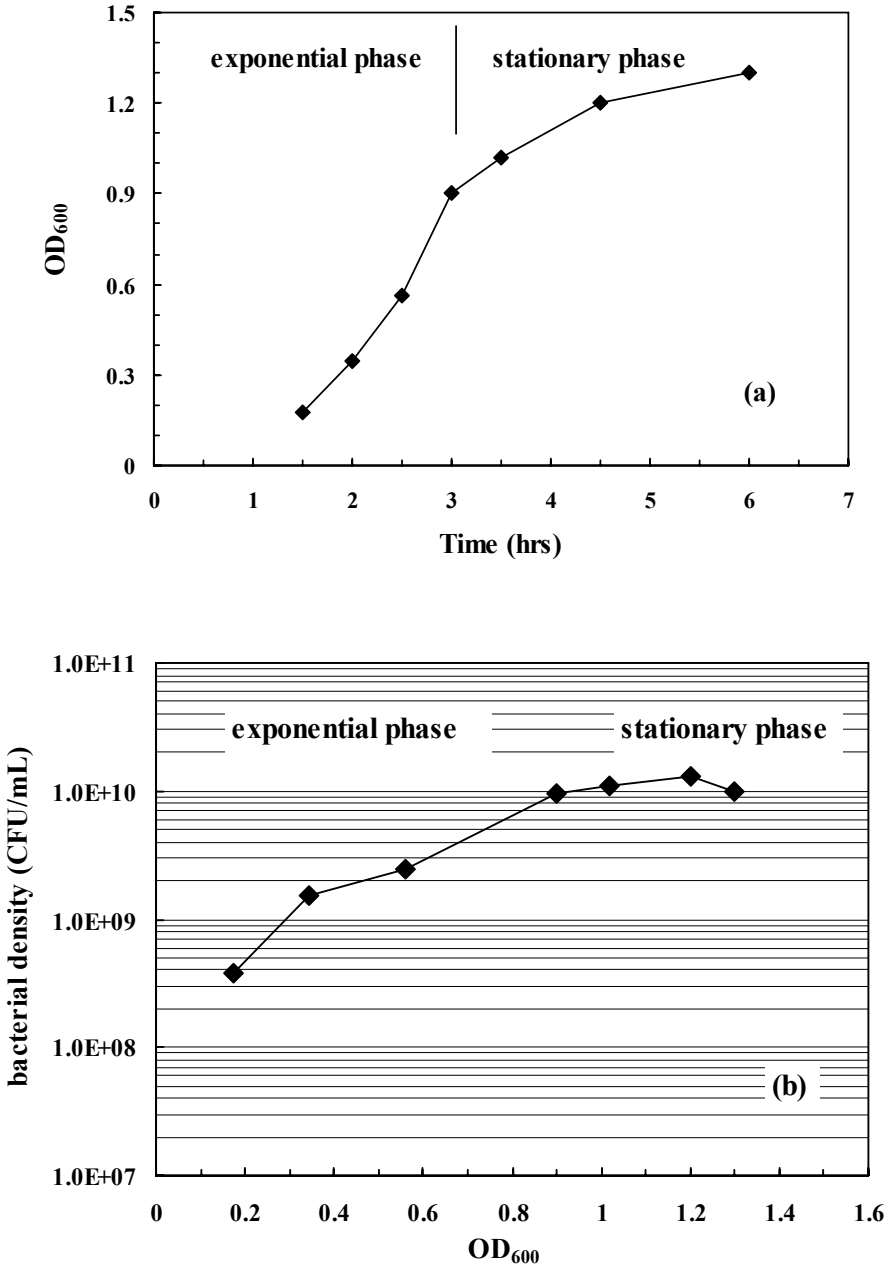


Figure 3.1 (a) A typical growth curve of *E. coli* O157:H7 strain B6914 in LB broth as OD_{600} vs. time, (b) the relationship between bacterial density (CFU/mL) and OD_{600} under these conditions.

To obtain a quantitative relationship between OD_{600} and cell numbers for strain B6914 at the beginning of the project, a batch growth curve was performed as described above. After 1.5 h incubation, at every succeeding half-hour, the absorbance was checked. Each-time the OD_{600} was measured, and a 10 mL culture was centrifuged twice and resuspended in 4 mL PBS solution and the cell plate count determined. The resulting relationship is presented in Figure 3.1 (b). This relationship was tested several times and similar results were always obtained.

3.2.1.3 Bacterial surface physicochemical characterizations

Bacterial physicochemical characterizations of strain B6914 were completed to assist in understanding the transport of strain B6914 through porous media. Specifically, the cell surface hydrophobicity and eletrophoretic mobility were measured.

Cell surface hydrophobicity was determined by measuring the surface contact angle and performing the bacterial adherence to hydrocarbons (BATH) test at room temperature. Measurement of the surface contact angle was performed as described by van Loosdrecht *et al.* (1987a). First, a 10 mL cell suspension, grown as described above, was filtered through a 0.45 μm-pore-size membrane filter (Pall Corporation, MI). Subsequently, the filter, which now was covered with a continuous bacterial layer, was mounted on a glass slide and dried in a desiccator for a half hour to obtain a stable water contact angle. Then, a water droplet (1 μL) was applied on the bacterial layer, and the contact angle was immediately measured with a Goniometer (Model 100, Rame-hart Inc., NJ). Six replicate measurements were conducted.

The BATH test was conducted using *n*-hexadecane, *n*-octane and *o*-xylene as the assay hydrocarbons (Rosenberg *et al.,* 1980; Gannon *et al.,* 1991). In the first step of the BATH procedure, a B6914 cell suspension, grown as described above, was diluted to a concentration of approximately 1.1×10^8 cells/mL using PBS. A mixture of 5.0 mL of this cell suspension and 0.00, 0.25, 0.50, 0.75, 1.0, 1.5, or 2.0 mL of the test hydrocarbon was then added to sterilized 12-mL test tubes and mixed with a vortex mixer for 2 min at room temperature. After allowing 30 min for the separation of the hydrocarbon and aqueous phases, the hydrocarbon phase was carefully removed with a pipette, and then the absorbance of the aqueous phase was determined at 400 nm using the Spectronic 21 spectrophotometer. The reduced absorbance of the aqueous phase was used as the measure of cell surface hydrophobicity. In addition, the lower aqueous phase and the upper "cream" were examined microscopically to ensure that the cells were indeed attached to the hydrocarbon.

The electrophoretic mobility of strain B6914 in synthetic urban stormwater runoff (described below) was measured with a Zetasizer 3000 (Malvern Instruments Ltd., Malvern, UK), which was provided by Prof. Otto Wilson (The Catholic University of America, Washington, D.C.). Electrophoretic mobility was converted to zeta potential by the Helmhotz-Smoluchowski method (Hiemenz, 1986). The cell suspensions, grown as described above, were diluted in synthetic runoff to a concentration of approximately 1.1×10^8 cells per mL before determination of the electrophoretic mobility. The determination was then performed at 25°C, with a potential difference between the electrodes of 200 V. Four replicate determinations were completed.

3.2.2 Preparation of laboratory CBM

CBM is composed of sandy loam soil, sand and mulch (DER, 2001). The sandy loam soil was provided by the Construction Service Division, Department of Public Works and Transportation of Prince George's County, Maryland. The soil was collected from the soil layer at a depth of 15-35 cm at Tantallon, Maryland. Soils from this location were mixed in a large container, air-dried at room temperature, and then crushed to pass a 1.18 mm sieve. The soil was determined by the University of Delaware Soil Testing Program to have a pH of 5.4, organic matter content of 1.2% and the following composition: 63% sand, 18% silt, and 19% clay. The sand (U.S. Silica® Mystic White II pool filter sand) was obtained from a local home supply store. The mulch was obtained from the College Park, Maryland, Department of Public Works. It was produced through composting of municipal yard wastes (leaves and grass clippings). The mulch was stored in a plastic bag, and then put into a bucket with a lid, and stored at room temperature until use. Before preparing CBM, a small quantity of mulch was taken out from the bag and air-dried at room temperature. Subsequently, the sand and mulch were also sieved through a 1.18 mm sieve, afterwhich the sand, soil, and mulch were mixed at a volume ratio of 5:3:2 (MDE, 2000; DER, 2001) to make the laboratory CBM. The particle-size distributions of these media were analyzed using dry-sieving technique (Das, 1992). The results of these analyses are presented in Table 3.1.

Table 3.1 Size distribution of the media used in the column tests

	d_{10} (mm)	D_{50} (mm)	d_{60} (mm)	d_{60}/d_{10}
Sandy loam soil	0.16	0.58	0.68	4.25
U.S. Silica® Mystic White II pool filter sand	0.61	0.85	0.90	1.48
U.S. Silica® Unground fine sand	0.33	0.46	0.49	1.48
Laboratory CBM	0.15	0.74	0.81	5.40

3.2.3 Preparation of IOCS

In this study, two sands (U.S. Silica® Mystic White II pool filter sand and unground fine sand) were used to synthesize IOCS. The particle size characteristics of these two sands are presented in Table 3.1. The procedures of Mills *et al.* (1994) were followed to prepare the IOCS. Sand was first rinsed several times with deionized water until the rinse water became clear, and then dried overnight in an oven at 90ºC. The sand was combined with 10% nitric acid (v/v) in a flask, and shaken for 2 h. After that, the supernatant was decanted, and the sand was rinsed with deionized water to a near neutral pH. Then, the sand was combined with 0.5 N NaCl, shaken for 2 h, and rinsed with deionized water again to near a neutral pH. Finally, the clean sand was dried overnight in an oven at 90ºC.

To perform the IOCS synthesis, 15 g (dry mass) of the clean sand and 30 mL of 0.11 M ferric chloride solution were combined in a 50 mL polypropylene centrifuge tubes. The slurry was slowly titrated with 5 N NaOH to a final pH between 4.5 and 5.0, and then shaken vigorously for 36 h to ensure complete coating of the sand by iron-(hydr)oxide precipitate. The newly coated sand was rinsed repeatedly in deionized water, dried in an oven at 90°C, rinsed, and dried again before use in the experiments (Mills *et al*., 1994; Knapp *et al.*, 1998).

The amount of iron deposited on the sand was determined by extraction using the citrate-dithionite and acid ammonium oxalate methods (Loeppert and Inskeep, 1996). The citrate-dithionite method is used to extract total free iron oxide, which includes crystalline and noncrystalline (amorphous) compounds of iron (e.g., hematite, goethite). The acid ammonium oxalate method is used to extract amorphous iron oxide. The detailed procedures of the citrate-dithionite and acid ammonium oxalate methods are presented below.

The surface charges of sand and IOCS were determined using the back-titration method of Duquette and Hendershot (1993). The detailed method is presented in Section 3.2.7.4. Additionally, the surface morphology of the clean sand and the IOCS was observed with a FEI Quanta 200F Environmental Scanning Electron Micrograph (E-SEM, FEI Company, Eindhoven, The Netherlands) in the CALCE Test Services and Failure Analysis (TSFA) laboratory, University of Maryland.

3.2.4 Column transport experiments

3.2.4.1 Column setup

All column experiments were performed in glass chromatography columns (Cat. No. 420401-2520, Kontes, Vineland, NJ). Each column had a 2.5 cm inner diameter and was 23 cm in height, with a 20 μm pore size polyethylene bed support. No cells were lost during flow through this bed support in a preliminary test, in which a bacterial suspension was pumped through a column without media. Before each transport experiment, the media were dry-packed into a column by pouring the mixture into the column in small batches and then tapping the column to achieve a

consistent final media depth (about 21.7 cm) and bulk density (approximately 1.34 g/cm^3).

The column experimental set up for the transport experiments is shown schematically in Figure 3.2 and as a photograph in Figure 3.3. All glassware (reservoir, vials, etc.) and tubing were sterilized by autoclaving prior to use to avoid the introduction of exogenous microbes. Prior to initiating a transport experiment, the packed column was flushed with sterile synthetic runoff to reach saturation of the pore volume. The content of the synthetic runoff (Table 3.2) was based on references on urban stormwater runoff chemistry (Davis *et al.,* 2001). To simulate rainfall application, the influent was pumped into the column from the top using a Masterplex® L/S peristaltic pump (Cole-parmer Instrument Company, Catalog No. 7553-30) at a flow rate of 40 mL/hr (equal to an approach velocity of 8 cm/hr) for 6 hrs. This hydraulic loading represents a common storm (0.4 cm/hr; return period < 1 year) concentrated by a factor of 20 from the drainage area to the treatment facility (DER, 1993; Davis *et al.*, 2001). The effluent was collected from the bottom of the column. All experiments were performed at room temperature (25 ± 3ºC).

Table 3.2 Matrix for synthetic runoff used in this study (Davis *et al.*, 2001).

Component	Value	Source	Component	Value	Source
pH	7.0	HCl/NaOH	Zinc	0.1 mg/L	$ZnCl_2$
NO_3^-	2 mg/L as N	$NaNO_3$	Copper	0.05 mg/L	$CuCl_2$
Organic N	4 mg/L as N	Glycine	Lead	0.05 mg/L	$PbCl_2$
Phosphorus	0.6 mg/L as P	Na_2HPO_4	Oil	20 mg/L	Used motor oil
Dissolved solids	120 mg/L	$CaCl_2$			

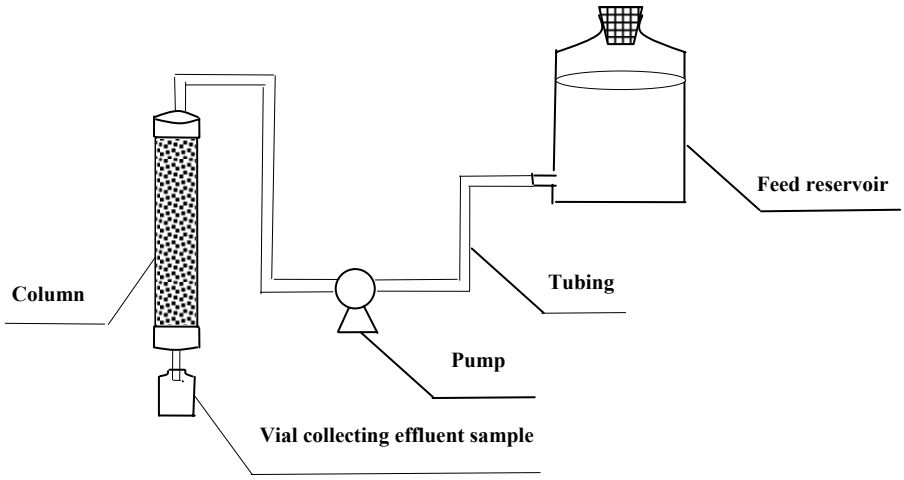


Figure 3.2 Schematic diagram of column experiment setup.

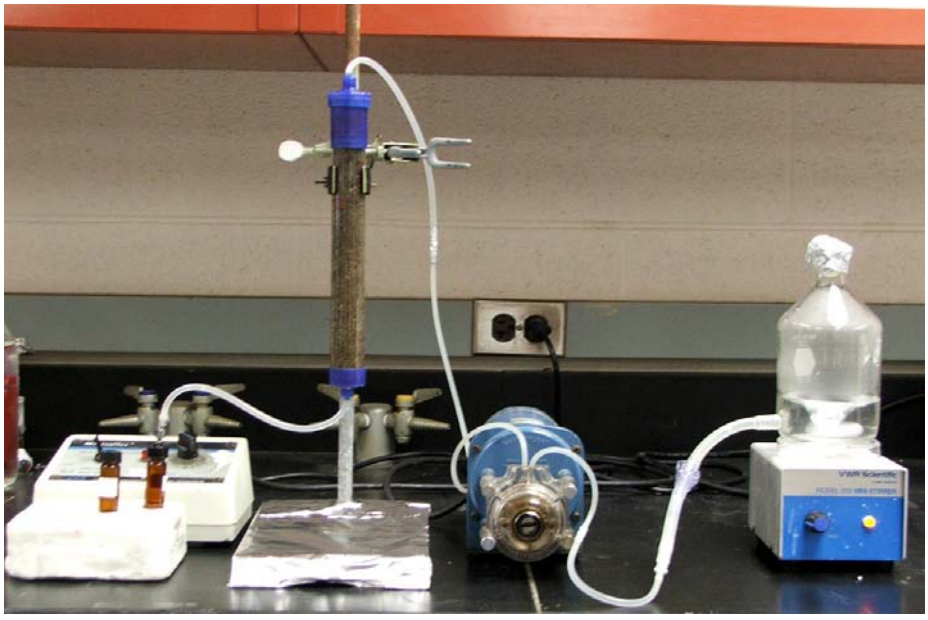


Figure 3.3 Photograph of the column experimental setup.

3.2.4.2 Bromide tracer experiments

During most bacterial transport experiments, bromide tracer tests were used to support the microbial transport and capture studies by providing data for estimating the average pore water velocity and hydrodynamic dispersion in the columns. For these tests, sodium bromide (NaBr) was dissolved in the synthetic runoff to obtain a bromide concentration of 200 mg/L, and the solution was stirred and continuously pumped into the column from the top. Effluent samples were collected every quarter-hour or half-hour from the bottom of the column, and the bromide concentration determined.

A FORTRAN program "trafit1d" was used to fit to the experimental bromide tracer data (Seagren, 1994). The best-fit parameters (hydrodynamic dispersion coefficient D, and pore velocity ν) were obtained by minimizing the sum of the squares of absolute residuals between the normalized experimental bromide tracer data and the normalized flux-averaged concentration calculated using the one-dimensional non-reactive solute transport model of Parker and van Genuchten (1984). Then porosity θ, was calculated based on the best-fit ν, experimentally controlled flow rate Q, and the across sectional area A, by using the equation:

$$\theta = \frac{Q}{A \cdot \nu} \tag{3.1}$$

3.2.4.3 Bacterial transport experiments

For the bacterial transport and capture experiments, *E. coli* O157:H7 strain B6914 in PBS stock solution was diluted in the synthetic runoff to a concentration of $1.1 \pm 0.3 \times 10^6$ CFU/mL (mean ± standard deviation). The synthetic runoff was

stirred and continuously pumped into the column from the top at the flow rate of 40 mL/hr (8 cm/hr) for the 6 h simulated rainfall events. Influent samples were collected aseptically in autoclaved vials every hour, and the effluent samples were collected every half-hour or hour from the bottom of the column. Strain B6914 cells in the influent and effluent samples were enumerated by the method described in Section 3.2.7.1. Based on these data, the mass removal efficiency of strain B6914 was calculated as follows:

$$\text{Mass removal efficiency} = \left(1 - \frac{\sum_i C_{effluent,i} \cdot \Delta t_i}{\sum_j C_{influent,j} \cdot \Delta t_j}\right) \times 100\% \qquad (3.2)$$

where $C_{influent}$ and $C_{effluent}$ are the concentration of strain B6914 in the influent and effluent at the time of t_i or t_j, respectively; Δt is the time internal between two sampling.

Based on the best-fit parameters from the bromide tracer tests and the bacterial transport experimental data, bacterial transport parameters (sticking efficiency α, single collector collision efficiency η_0, and adsorption rate coefficient k_a) were calculated using the equations 2.9-2.23 presented in Section 2.4.

In addition, the effect of ionic strength on bacteria transport and capture in CBM was investigated because ionic strength of stormwater runoff in winter will greatly increase due to salt application to melt snow. In this experiment, the ionic strength of synthetic runoff (Table 3.2) containing 200 mg/L sodium bromide was adjusted in the range of 5.8 mM to 50 mM by adding different $CaCl_2$ concentrations. First, B6914 suspended in synthetic runoff with an ionic strength of 5.8 mM was loaded into a CBM column to observe the transport and capture of B6914 by following the same

procedures as described above. Following the 6 hr-loading, the media in the column were flushed using synthetic runoff without bromide and B6914 for 14 hr to ensure that all of bromide in the media was washed out of the column, after which the column was allowed to sit stagnant for two weeks. Then, B6914 suspended in synthetic runoff with an ionic strength of 50 mM was loaded into the column to observe the transport and capture of B6914 by following the same procedures.

3.2.5 Bacterial survival experiments

After the 6 hr stimulated rainfall application described in Section 3.2.4, the survival of trapped bacteria over time was investigated. The columns were gravity drained for variable times (0.5 to 28 days), depending on the media. At the end of the selected drainage time, the column was sacrificed and soil samples from seven different depths (0-2, 2-4, 4-6, 6-9, 9-13, 13-17, and 17-21.7 cm, measured from the top) in the column were aseptically removed and transferred to seven sterile aluminum dishes. A 10 g wet soil sample from each dish was then diluted at a ratio of 1:10 (by mass) in sterile deionized water and aseptically extracted for 2 min by using a Waring blender with a 1-liter glass container. After extraction, the mixtures were allowed to settle for 1-2 minutes before dilution and plating from the middle fraction in the blender container (Gagliardi and Karns, 2000). Strain B6914 cells in the mixtures were enumerated by the method as described in Section 3.2.7.1. The remaining soil samples in the dishes were dried at 105 ºC for one day to determine the soil moisture content (Black, 1965). Additionally, the heterotrophic bacteria and protozoa in the column media were enumerated using the methods described below.

3.2.6 Media sterilization and coating with microorganisms

For comparison to the bacterial transport and survival experiments in the non-sterile columns described above, parallel experiments employing γ-irradiated sterile columns were conducted to investigate the effects of native microorganisms in CBM on bacterial transport and survival. The sterile columns were packed with dry CBM as described in Section 3.2.4.1, and then were γ-irradiated with a dose of 30 kGy from a Co^{60} source in the Department of Materials Science and Engineering, University of Maryland, College Park.

As discussed in Section 3.1, γ-irradiation may cause increases in available nutrients, which may be favorable for the growth of captured bacteria. To investigate this effect, two experiments were performed. First, when the sterile and non-sterile columns were flushed using synthetic stormwater runoff to reach saturation before loading the bacterial suspension, the wash water was collected in sterile culture tubes. To evaluate growth of B6914 on the nutrients available in the media, a stock solution of strain B6914 was added into each tube to reach the same concentration of bacteria, and then the OD value of the tubes were observed for one week.

In addition, a complementary transport and survival experiment was conducted using the γ-irradiated media, but coated with cultured native microorganisms. For culturing the native microorganisms in CBM, 10 rice grains were added to 100 mL sterile water in 1 L flasks with a cotton plug, and then it was boiled for five minutes. After the culture media cooled, 1 g CBM media was added into each flask, and cultured for two weeks at room temperature. After the two-week incubation, the culture was filtered through a stainless steel mesh with a pore size of 0.5 mm to

remove large bacterial aggregates and rice debris (Wang *et al.*, 2005). The γ-irradiated CBM and culture solution were mixed at a ratio of 1:1 (by mass) in a large flask with a cotton plug for two days. The mixtures were aseptically transferred to a sterile tray covered with foil, and air-dried in a fume hood. Protozoa and heterotrophic bacteria in the new media were enumerated using the Singh MPN method and the method of heterotrophic spread-plate counts respectively, which are described in Section 3.2.7.1. The dry γ-irradiated media coated with native microorganisms was packed into columns, and the transport and survival experiments of strain B6914 were conducted as described above.

To further to evaluate the effect of protozoa, the CBM culture solution described above was filtered twice through 5 μm membrane filters to lower the concentration of protozoa in the filtrate (Kreuzer *et al.*, 2006). The filtrate was then coated to the γ-irradiated media following the same procedure as described above. Subsequently, the transport and survival experiments of strain B6914 were conducted.

3.2.7 Analytical methods

3.2.7.1 Microbiological measurements

Strain B6914 cells in the influent and effluent solution or media extraction mixtures were enumerated by dilution in PBS and plating on LB agar containing 100 μg/mL ampicillin using the method of heterotrophic spread-plate counts adapted from Standard Method 9215 (APHA *et al.*, 1995). The plates were incubated at 37ºC for one day, and then the colonies were counted. B6914 colonies were confirmed by

checking fluorescence under long wave UV (365 nm) and disregarding any colonies that did not glow green.

Total heterotrophic bacteria cells in the influent and effluent solution or media extraction mixtures enumerated by dilution in PBS and plating on R2A agar using the method of heterotrophic spread-plate counts adapted from Standard Method 9215 (APHA *et al.*, 1995). The R2A agar plates were incubated at 25 °C for five days, and then the colonies were counted.

The number of protozoa in the media extraction mixtures was determined using a modified Singh MPN method (Singh, 1946; Stout *et al.*, 1982; Sinclair and Ghiorse, 1987). Approximately 15 mL of 1% agar containing 5 g/L NaCl was poured into a 9-cm-diameter Petri dish, and then five sterile, 2-cm-diameter, 1-cm-high glass rings were placed in the Petri dish before the agar solidified. Exactly 0.7 mL of diluted sample was added to each of the five replicate rings. Additionally, 0.1 mL of approximate 1.0×10^{10} CFU/mL *Enterobacter aerogenes* (ATCC 49469) at the stationary phase was added into each ring as bacterial food source. *E. aerogenes* cells were cultured and harvested following the batch growth curve procedure for strain B6914, as descried in Section 3.2.2. One Petri dish was used for each dilution level, and five dilution levels (10^0 to 10^{-4}) were used for each sample. All samples were cultured at room temperature for 2 weeks. To confirm the presence (positive) or absence (negative) of protozoa, samples were removed aseptically from the ring and examined under a microscope. Typically, the presence of protozoa was also visible by disappearance of the food bacteria. Then, based on the numbers of positive and

negative results at each dilution, the number of protozoa in the media extraction mixtures was obtained from a most probable number (MPN) table.

3.2.7.2 Iron determinations for IOCS

The amount of iron deposited on the sand during IOCS synthesis was determined by extraction using the citrate-dithionite and acid-ammonium-oxalate methods of Loeppert and Inskeep (1996).

In the citrate-dithionite extraction, 0.5 g of soil, 0.5 g sodium dithionite ($Na_2S_2O_4$), 6 g sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 30 mL of deionized water were added into a 50 mL polypropylene centrifuge tube. Then, the mixture was shaken overnight (16 h) on a reciprocating shaker. Subsequently, the supernatant was transferred to a 50 mL volumetric flask, and one or two drops of Superfloc polyacrylamide flocculating agent (Cytec Industries, Inc., West Paterson, NJ, 2 g/L in deionized water) were added. After vigorously shaking the mixture for 15 s, the supernatant was diluted to 50 mL and shaken again. The suspension was allowed to settle for 1 h, and centrifuged. After centrifuging, the supernatant was filtered through a 0.2 μm membrane filter, and then diluted with deionized water to 50 mL. Subsequently, the concentration of iron in the dilution solution was determined by a Perkin-Elmer 5100ZL atomic absorption spectrophotometer (AAS) on the frame module, against four standards in the range between 0.5 and 5 mg/L, according to Standard Methods 3110 and 3111A (APHA *et al.,* 1995). A standards check was performed after every 10 analyzed samples with an acceptable difference ≤ 5 %. The detection limit for iron concentrations with this technique is 0.02 mg/L.

The extraction solution for the method of acid-ammonium-oxalate extraction was made up of 0.175 M ammonium oxalate and 0.1 M oxalic acid, adjusted to pH 3.0 by addition of HCl. 0.5 g of sand and 30 mL of this solution were added into a 50 mL polypropylene centrifuge tube covered by foil, and then shaken for 2 h. After centrifuging, the supernatant was filtered through a 0.2 μm membrane filter, and then diluted with deionized water to 50 mL. The concentration of iron in the dilution solution was determined by AAS as described above.

3.2.7.3 Bromide analysis

Bromide concentrations in the column influent and effluent samples were measured using a Cole-Parmer 27502-04, 05 bromide electrode (Cole-Parmer Instrument Company, Vernon Hills, IL). The calibration was conducted each time by measuring four bromide standard solutions (1, 10, 100, and 1000 mg/L) to obtain the calibration curve. The detection limit for the bromide probe is 0.01 mg/L in the presence of other ions (based on manufacturer information).

3.2.7.4 Surface charge of media

The surface charge of sand and IOCS was determined using the back-titration method of Duquette and Hendershot (1993). In this method, 0.5 g media and 25 mL of 0.01 M $Ca(NO_3)_2$ solution were added into a 50 mL beaker, and the suspension stirred until the pH stabilized. The suspension was then titrated with 0.1 M HNO_3 until a pH of 3 was reached. After allowing the suspension to equilibrate for 2 min, the back-titration was performed with 0.005 M $Ca(OH)_2$ at a rate of 0.5 mL/min to

pH 10. During the titration and back-titration processes, the volumes of acid and base, respectively, were recorded along with the pH. In addition, a reference titration was needed to correct for the H^+/OH^- consumption due to metal hydrolysis and other known reactions in the solution. The reference titration was performed by synthesizing the media suspension and titrating to pH 3, as described above. Then, the suspension was transferred into a 50 mL centrifuge tube and centrifuged (15 min, 1000 × g), after which the supernatant was filtered through a 0.2 μm Millipore filter. The volume of suspernatant was determined, and then it was transferred into a 50 mL beaker and allowed to equilibrate for 10 min with continuous stirring. Then, the solution was back-titrated as described above. The surface charge q in $cmol_c/kg$ can be calculated as:

$$q = -a\left(\frac{C_{base}V_s}{W_{soil}} - \frac{C_{base}V_r}{W_{soil}} \cdot \frac{V_o}{V_{sn}}\right) \tag{3.3}$$

where: a is a unit conversion factor, 100 (1000 g/kg × 0.001 L/mL × 100 $cmol_c/mol_c$); C_{base} is the concentration of the base used to back-titrate, mol/L; V_s is the volume of base added during the reference titration, mL; W_{soil} is the mass of the soil, g; V_r is the volume of the base added during the reference titration, mL; V_o is the volume of the supporting electrolyte and added acid, mL; and V_{sn} is the volume of the supernatant, mL.

3.2.8 Statistical analyses

Data were analyzed using SAS version 8.01 (SAS Institute, Cary, NC, USA). The differences between means (n=4) for bacterial removal efficiency and hydraulic

parameters (such as porosity and dispersion coefficient) in CBM and IOCS were tested using the student's t-test. A significance level of $P < 0.05$ was selected for the determination of statistical difference.

3.3 Results and discussion

3.3.1 Bacterial physicochemical characterizations

The surface contact angle of strain B6914 was found to be 12 ± 1° (mean ± standard deviation), which indicates that strain B6914 is poorly hydrophobic. Similarly, Morrow *et al.* (2005) measured a contact angle in water of 18.2 ± 0.4° for *E. coli* O157:H7 (ATCC200728). This finding is also consistent with previous studies by Gilbert *et al.* (1991) and van Loosdrecht *et al.* (1987b) who reported that *E. coli* NCTC 9002 and *E. coli* ATCC 8793, respectively, also have low surface contact angles. Thus, it can be concluded that *E. coli* O157:H7 strain B6914 is a more hydrophilic than a hydrophobic organism.

Consistent with the contact angle observation, the results of the BATH experiment illustrated no significant affinity of strain B6914 towards *n*-hexadecane or *n*-octane, with only approximately 25% of strain B6914 removed from the aqueous phase by *o*-xylene (Figure 3.4). The use of different volumes of test hydrocarbons did not seem to significantly affect the results. Similar results were obtained with *E coli* B *ilvA thy* and *E coli* K-12 CSH 57 (Rosenberg *et al.*, 1980). Additionally, after the hydrocarbon was mixed with the cell suspensions for 2 min, a creamy upper layer was formed. Microscopic examination of the upper layer revealed that the emulsion

of *o*-xylene droplets contained rod-shaped bacteria, while there were no bacteria in the emulsion of *n*-hexadecane or *n*-octane droplets. Thus, the results of the BATH experiment further verified that the cell surface of strain B6914, at least as grown for these studies, has a low degree of hydrophobicity.

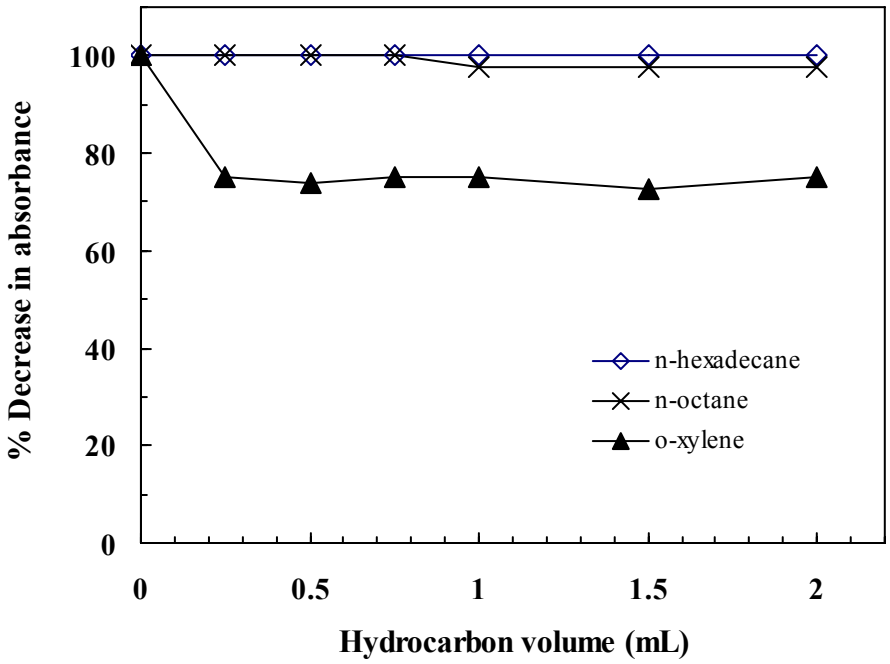


Figure 3.4 The percentage of decrease in absorbance of the aqueous phase after aqueous bacterial suspension was mixed with varying volumes of hydrocarbon (n-hexadecane, n-octane, or o-xylene).

Strain B6914 in synthetic runoff (pH = 7, ionic strength = 5.8 mM) displayed a negative zeta potential of -2.6 ± 0.1 mV, which indicates that this bacterium possesses a very weak negative charge. Foppen and Schijven (2006) determined the zeta potential of *E. coli* ATTCC 25922 as function of the ionic strength of various solutions, and similarly found that the zeta potential of *E. coli* ATTCC 25922 is close to -20 mV in $CaCl_2$ solution with ionic strength values ranging from 2 to 200 mM at a

pH of 7. Similarly, Morrow *et al.* (2005) measured a negative zeta potential of -4.44 ± 2.37 mV for *E. coli* O157:H7 (ATCC200728). Lytle *et al.* (1999) also found the electrophoretic mobilities (EPM) of *E. coli* O157:H7 strains ranged from -0.22 to -0.46 μm·cm/V·s and averaged -0.36 μm·cm/V·s. The negative EPM values represent a negative surface charge.

3.3.2 Stability of strain B6914 in the synthetic runoff

Strain B6914 inoculated into the synthetic runoff at room temperature decayed rather quickly, with apparently first-order endogenous decay kinetics and a half-life of approximately 8 hr (Figure 3.5). The die-off rate constant of B6914 in the synthetic runoff is 0.084 hr^{-1} (or 2.01 d^{-1}), which is higher than the values reported for other *E. coli* strains in ground water, seawater or estuarine water (Table 2.1). This high rate of decay may be attributed to the scarcity of nutrients and/or toxicity from the used motor oil or heavy metals in the synthetic runoff. Previous studies have shown that when *E. coli* bacteria are introduced into water environments, morphological, physiological properties and biochemical activity of cells significantly change, such as shrinking of the cell and the reduction of cytoplasmatic content, damage to the integrity of the cell, and decrease of respiration and dehydrogenase activity (Foppen and Schijven, 2006). While not surprising, this observation translated into difficulties in maintaining constant cell concentrations in the influent solution for the duration of the column experiments. Therefore, to help maintain relatively stable concentrations of strain B6914 in the influent during the 6-hr stimulated rainfall event, new synthetic runoff with a suspension of fresh strain

B6914 cells was made and applied to the column every three hours. With this regimen, the influent concentration of B6914 cells declined by less than 25% in any given experiment.

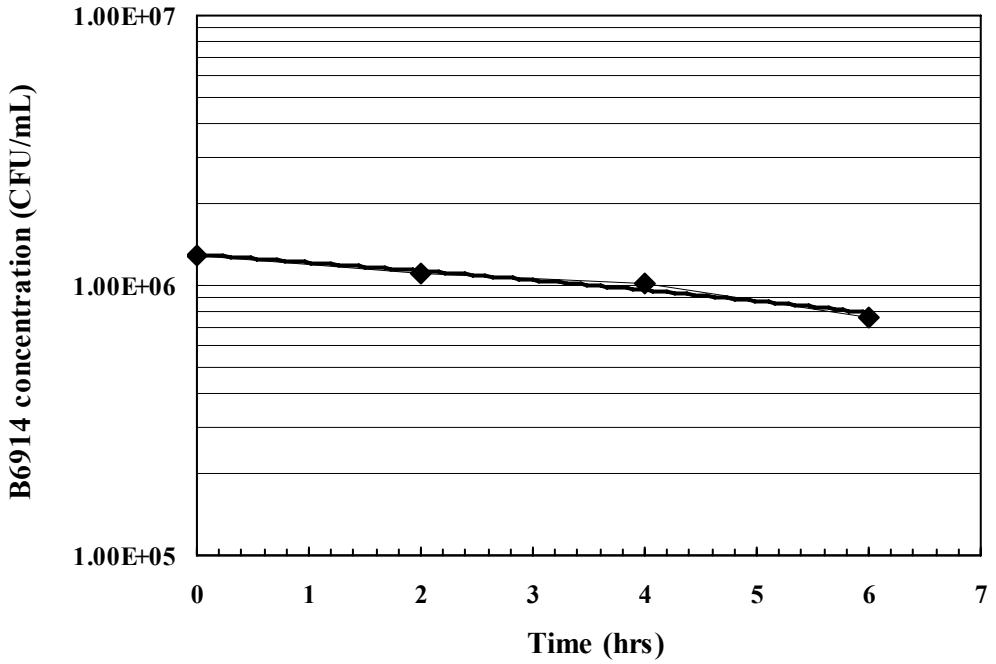


Figure 3.5 Strain B6914 concentrations in the synthetic runoff with respect to time.

3.3.3 Bacterial transport experiments

3.3.3.1 Bacterial transport and capture by CBM

Bacterial transport and capture in the media was quantified through enumerating B6914 in the influent and effluent of bacterial transport experiments in columns packed with CBM. The composite breakthrough curve for strain B6914 in CBM during the initial period of this project is shown in the Figure 3.6. Based on these experimental data, the removal efficiency of strain B6914 in CBM was calculated

using the Equation 3.1 to be approximately 80%. Thus, with an influent concentration of 1.1 ± 0.3 $\times 10^6$ CFU/mL, strain B6914 can be effectively reduced by CBM.

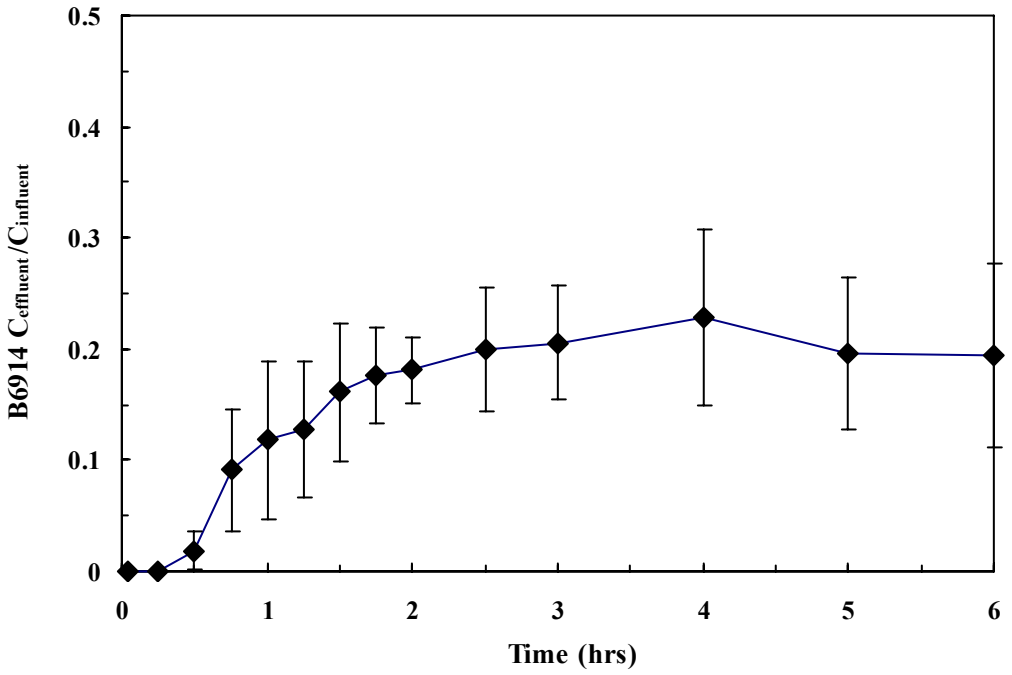


Figure 3.6 Breakthrough curve of strain B6914 in columns packed with CBM. Symbols represent the mean of 4 replicate columns with vertical bars showing ± 1 standard deviation (SD). The tested conditions: Flow rate = 40.0 ± 1.0 mL/hr, $C_{influent}$ = 1.1 ± 0.3 × 10^6 CFU/mL.

In addition, bacterial transport experiments in CBM were repeatedly conducted at various times throughout the nearly three-year project period. With increasing the storage time of the mulch, soil and sand, the removal efficiency of strain B6914 decreased in fresh-packed CBM columns (Figure 3.7). During the first year, the CBM achieved 80% or higher bacterial removal. Subsequently, the bacterial removal efficiency decreased over time, falling to around 60% after two years storage. One

possible explanation for this observation is that while the mulch with a certain moisture was stored at room temperature, chemical and/or biological reactions may proceed in the mulch, which resulted in changes in the surface characteristics and/or microbial diversity and abundance in the mulch. All of these changes may influence the transport and capture of strain B6914.

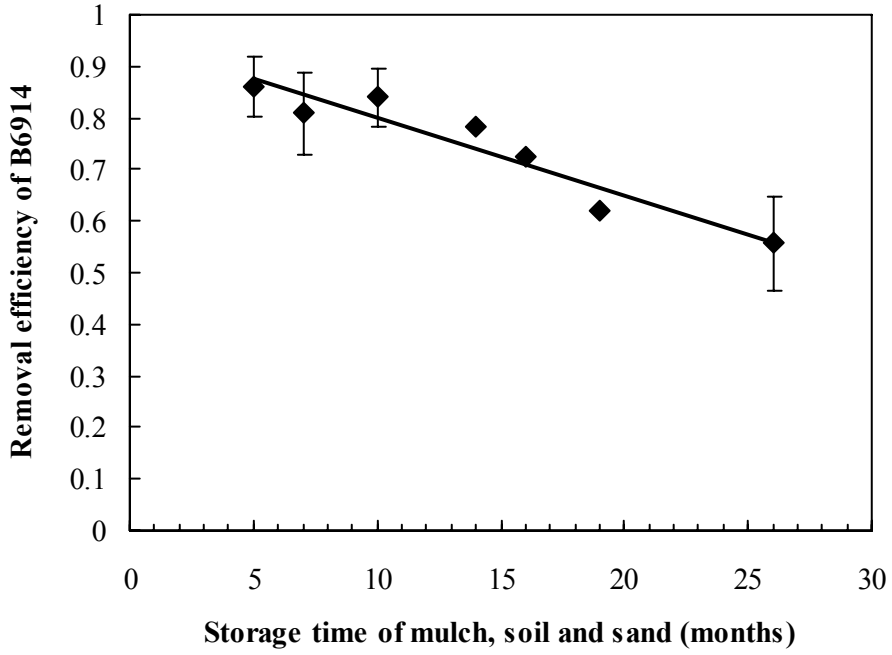


Figure 3.7 The removal efficiency of strain B6914 in fresh-packed CBM with different storage time of mulch, soil and sand during this three-year project. Symbols represent the mean of four replicates, with vertical bars showing ± standard deviation.

As described in Chapter 2, the main mechanisms responsible for the capture of bacteria by porous media are physical straining or filtration, and microbial adsorption to medium surfaces. *E. coli* is a rod-shaped bacterium of 2.0-6.0 μm × 1.1-1.5 μm (Bergey *et al*., 1984), which is less than 5% of the mean diameter (d_{50}) of the CBM

(Table 3.2). Therefore, physical straining by the media probably has a relatively small impact on the retention of strain B6914, based on the theory of Herzig *et al.* (1970), and adsorption may be the main factor controlling the transport of B6914 in CBM.

Bacterial adsorption is a result of hydrophobic, electrostatic and possibly other interactions, with the dominant interaction depending on the characteristics of the bacterial surface and the makeup of the media. *E. coli* O157:H7 strain B6914 is a relatively hydrophilic organism as discussed as in Section 3.3.1 and, as a result, it is expected that it will be more difficult for B6914 cells to partition from the aqueous phase and accumulate on the surface of media compared to hydrophobic cells. Thus, the hydrophobicity of B6914 cells does not correlate well with its high removal by CBM. This finding is consistent with the results of many previous researchers who have concluded that for relatively hydrophilic organisms, the hydrophobicity did not determine the degree of adhesion (Alexander *et al.,* 1991; Gannon *et al.*, 1991; Gilbert *et al.*, 1991). This suggests that electrostatic interaction may be more important. For example, Sharma *et al.* (1985) suggested that electrostatic interactions between bacteria and sand grains are a dominant factor in bacterial adhesion. Similarly, Foppen and Schijven (2006) found an excellent correlation between surface charge of bacteria and bacterial breakthrough. When the zeta potential of *E. coli* ATCC 25922 suspended in various solutions varied from -46.7 to - 17.0 mV, the bacterial efficiency by sands increased from around 30% to 90% (Powelson and Mills, 2001; Foppen and Schijven, 2006). The least negative zeta potential (-17.0 mV) of the bacteria resulted in the best adsorption of bacteria on sands. Therefore,

our observation showing that strain B6914 possesses a weak negative charge (-2.6 ± 0.1 mV) in synthetic runoff, may explain the high removal efficiency of bacteria in CBM.

Attraction of bacteria to the media surface depends on the thickness of the diffuse electric double layer. Increasing ionic strength leads to a decrease in the reduction in electrostatic repulsive force and the compression of the double layer. As a result, the bacterial cells may overcome the repulsion barrier and come close enough to the media surface, thus causing more bacteria to be trapped in the column (Stevik *et al*., 2004). Consistent with this theory, when the ionic strength in the synthetic runoff was increased from 5.8 mM to 50 mM, removal efficiency of B6914 increased from 84% to 97% due to the enhancement of electrostatic interaction (Figure 3.8). Therefore, electrostatic interactions between strain B6914 and CBM grains are a dominant factor in bacterial adhesion.

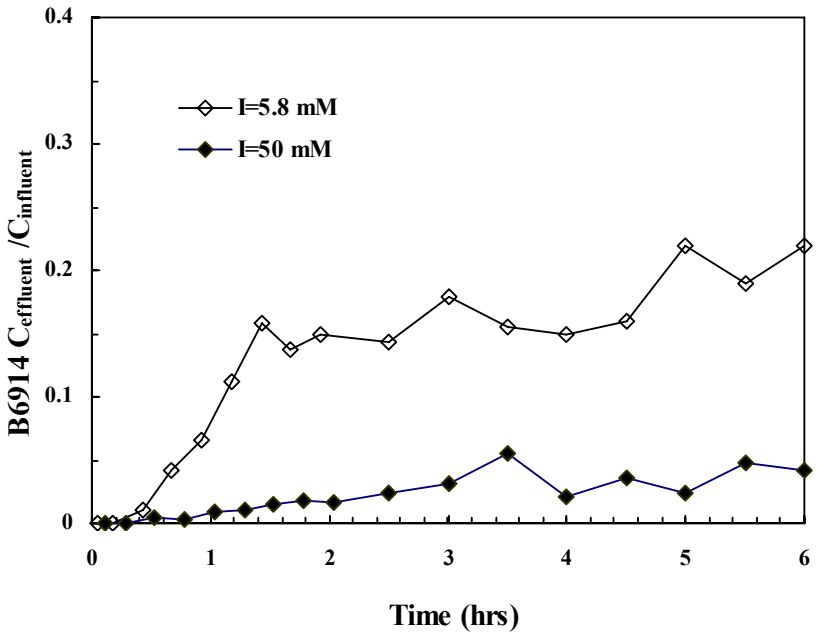


Figure 3.8 Breakthrough curve of strain B6914 in CBM column at the different solution ionic strength.

3.3.3.2 Bacterial transport and capture in IOCS

As described above, electrostatic interactions between *E. coli* O157:H7 strain B6914 and CBM appear to be a dominant factor in the bacterial adhesion. Due to the negative charge of strain B6914 under the test conditions, metal oxides that possess positively charged surfaces have the potential to induce high bacterial adsorption. In this study, two kinds of sands, a coarse sand and a fine sand, were used to evaluate the performance of IOCS on capture and destruction of strain B6914.

The acid-ammonium-oxalate extracted and citrate-dithionite extracted iron data are presented in Table 3.3. The citrate-dithionite extracted iron oxide includes crystalline and amorphous iron oxides, while acid-ammonium-oxalate extracted iron oxide corresponds to amorphous iron oxides. The data clearly show that the synthetic process significantly increased the content of iron oxide in the IOCS compared to the clean sands. Additionally, the data of acid-ammonium-oxalate extracted and citrate-dithionite extracted iron oxide in IOCS and sand demonstrate that nearly 100% of the increase in the content of iron oxide resulting from the coating process was due to creation of amorphous iron oxides. Amorphous iron oxides can contribute significantly to the physical and chemical properties of media, because they may have a high cation-exchange capacity and surface area, which are favorable for the adsorption of bacteria and other contaminants (Jackson *et al.*, 1982).

Table 3.3 The content of iron oxide and surface charge of two sands and two IOCS used in this study.

Media		Citrate-dithionite extracted iron oxide (mg Fe/g dry media)	Acid-ammonium-oxalate extracted iron oxide (mg Fe/g dry media)	Surface charge at pH=7 ($cmol_c$/kg dry media)
Coarse sand	Sand	0.146 ± 0.002	0.046 ± 0.008	-0.29
	IOCS	0.295 ± 0.003	0.188 ± 0.001	-0.18
Fine sand	Sand	0.070 ± 0.007	0.039 ± 0.003	-0.09
	IOCS	0.311 ± 0.009	0.296 ± 0.008	0.20

Back-titration experimental data show that the surface charge of coarse IOCS at a pH of 7 was less negative compared to coarse sand, and fine IOCS possessed a positively charged surface due to higher content of iron oxides (Table 3.3). Iron oxide (pHzpc ≈ 8.5, Stumm and Morgan, 1981) possess a positively charged surface at neutral pH, thus increasing positive charge on the surface of sand coated with iron oxide. Additionally, SEM photographs in Figure 3.9 were taken at 5000X magnifications to observe the surface morphology of the fine sand and the fine IOCS. The fine IOCS had a rougher surface, and the increase of surface area and greater positive surface charge should be favorable for the bacterial adhesion.

Correspondingly, for the transport experiments, IOCS significantly increased the removal of strain B6914 over that of the clean sands as expected due to the high content of iron oxides on the surface (Figure 3.10). Many previous studies have also demonstrated that iron or aluminum hydroxide coated sand can achieve high bacterial removal efficiency (Knapp *et al.*, 1998; Bolster *et al.*, 2001; Abudalo *et al.*, 2005, Foppen and Schijven, 2005). In addition, due to the high content of positively charged iron oxide and more roughness of surfaces, the fine IOCS achieved approximately 99% bacterial removal, which was much higher than observed with the coarse IOCS (around 86%).

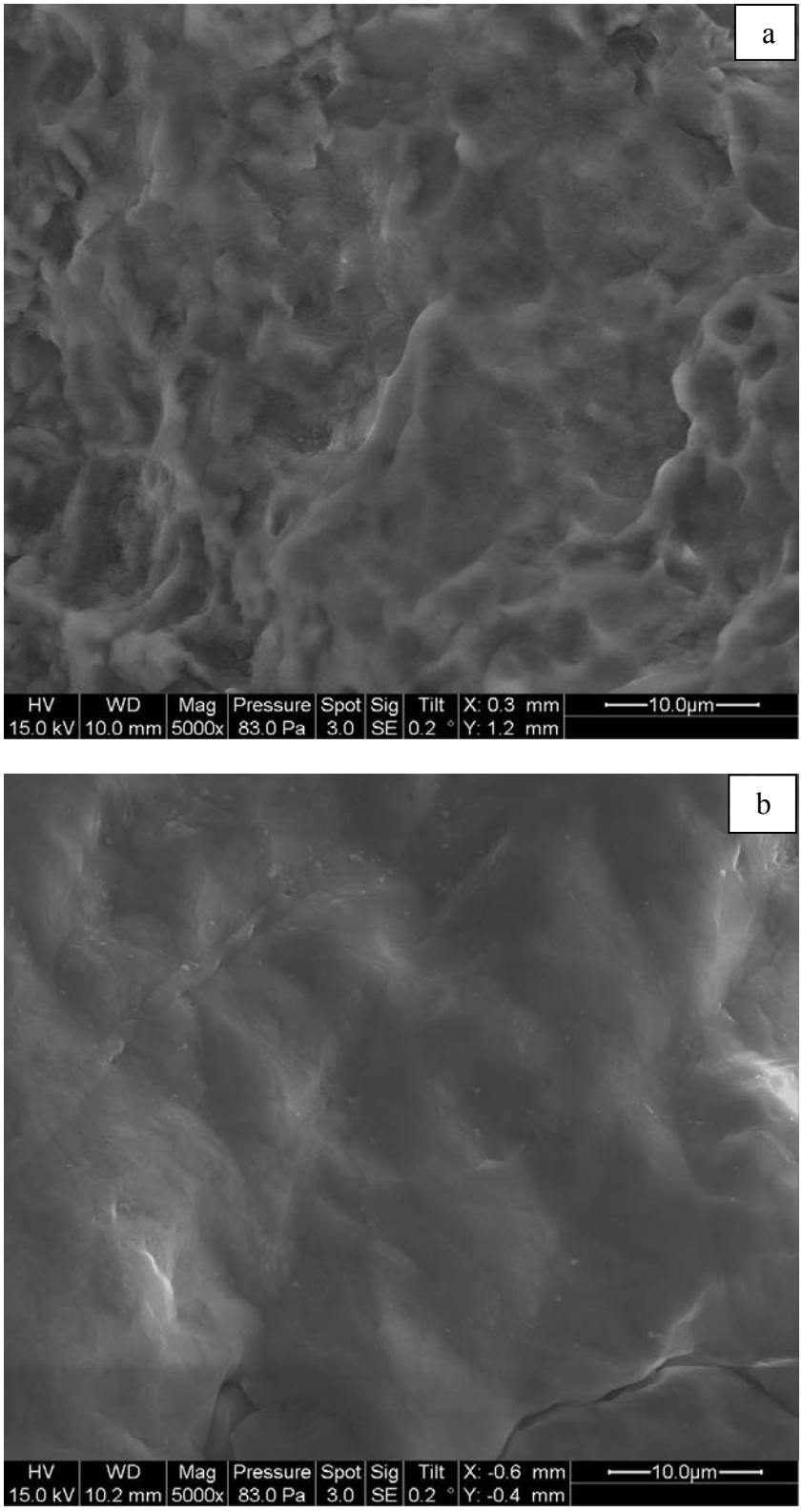


Figure 3.9 SEM micrographs of sample: (a) fine IOCS, (b) fine sand.

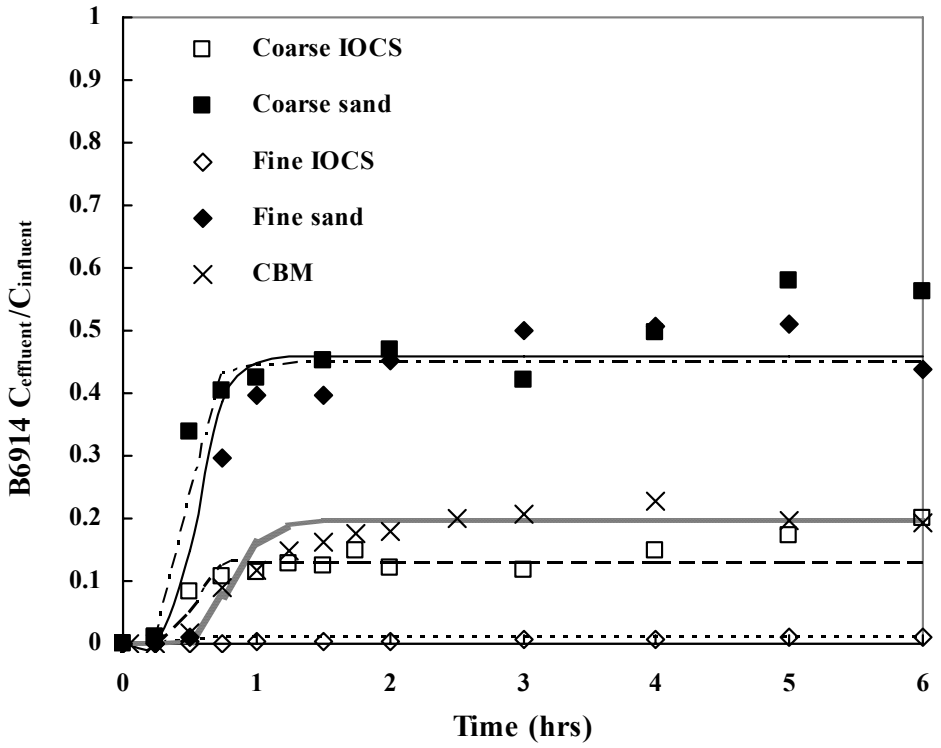


Figure 3.10 Breakthrough curves of strain B6914 and model fit in different media-packed columns. Symbols correspond to experimental data, and lines created by modeling using Equation 2.8. The tested conditions: Flow rate = 40.0 ± 1.0 mL/hr, $C_{influent}$ = 1.1 ± 0.3 × 10^6 CFU/mL.

As described in Section 3.3.3.1, the CBM during the first-year of storage of the media resulted in approximately 80% or higher removal of strain B6914. Although the fine IOCS significantly increased bacterial removal compared to CBM, the bacterial breakthrough point in sand and IOCS appeared earlier than CBM because of the higher permeability of the sands (Figure 3.10).

3.3.3.3 Model simulation of bacterial transport

The bacterial breakthrough curves from these experiments (Figure 3.10) can be described by the one-dimensional advection-dispersion (Equation 2.8), as presented

in Chapter 2. To obtain the advection-dispersion model parameters, the corresponding bromide tracer experiments were conducted. The bromide breakthrough curves for IOCS, sand, and CBM during the first-year of media storage and model fits to the experiments are presented in Figure 3.11. Consistent with bacterial breakthrough curves, the bromide breakthrough point in the CBM appeared later than in both of the sands and the IOCS, and the fine IOCS and sand delayed the appearance of the bromide breakthrough point slightly compared to the coarse IOCS and sand. The best-fit hydrodynamic dispersion coefficient (D) and pore water velocity (v), along with the calculated porosity value are summarized in Table 3.4. For the all media, the advection-dispersion model fits well with the bromide tracer experimental data (Figure 3.11). Best-fit parameters indicated that the coarse IOCS and sand had lower porosity and higher pore water velocity than the fine IOCS and sand, and CBM (Table 3.4). Therefore, when synthetic runoff flowed through the different media-packed columns, the breakthrough of bacteria and bromide occurred the earliest in the columns packed with coarse IOCS and sands.

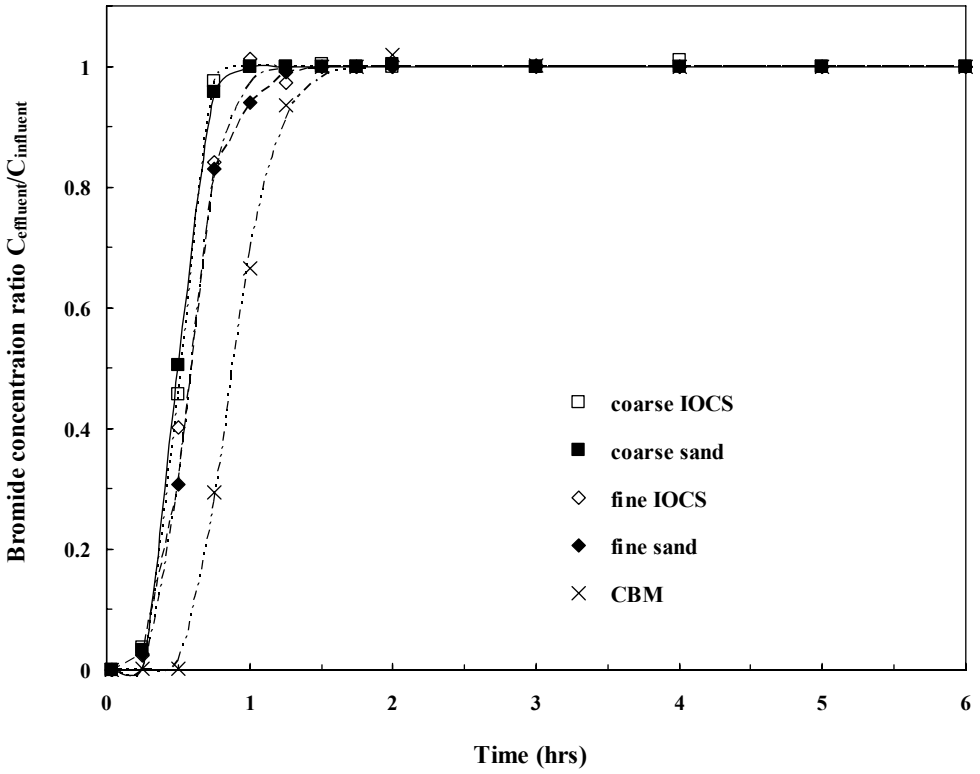


Figure 3.11 Breakthrough curves of bromide tracer in five different media-packed columns. Symbols correspond to experimental data, and lines created by modeling using Equation 2.8. The tested conditions: Flow rate = 40.0 ± 1.0 mL/hr, $C_{influent}$ = 200 mg/mL.

Table 3.4 Experimental conditions and model-fit or calculated parameters of column studies using different media. The data in the rows of fine IOCS and CBM (1-yr aged media, same as in Figures 3.10 and 3.11) represent the mean (n=4) ± 1 standard deviation.

Media		Q (mL/h)	Removal efficiency of B6914 (%)	Best-fit parameters			Calculated parameters		
				D (cm^2/h)	ν (cm/h)	θ	α	η_0	k_a (h^{-1})
Coarse sand	Sand	40.4	68.5	28.2	42.3	0.194	0.066	0.028	1.79
	IOCS	40.7	86.8	17.9	41.7	0.201	0.189	0.027	4.24
Fine sand	Sand	40.1	56.1	34.3	36.0	0.227	0.033	0.031	1.29
	IOCS	39.7 ± 0.9	98.8±0.02	36.2.± 6.59	37.4± 5.14	0.229± 0.062	0.216± 0.038	0.032± 0.007	8.02± 1.83
CBM		40.7± 0.8	83.9± 0.06	18.5±1. 55	23.1± 2.44	0.364± 0.047	0.036± 0.008	0.035± 0.004	1.85± 0.18

The calculated bacterial transport parameters based on the equations 2.9-2.23 are presented in Table 3.4. It is clear that the values of sticking efficiency (α=0.216) and adsorption rate coefficient (k_a = 8.02 hr^{-1}) in the fine IOCS were much higher than that in other media (Table 3.4). Additionally, the results of statistical analyses show that bacterial removal efficiency and calculated bacterial transport data (e.g., sticking efficiency, and adsorption rate coefficient) in the fine IOCS were significantly higher than those in CBM ($P<0.05$, student's t test). This can be explained by the enhanced electrostatic interaction between the positively charged surface of the media and the negatively charged strain B6914.

The bacterial detachment rate has been observed by others to be several orders of magnitude less than the adsorption rate (Hornberger *et al.*, 1992; Tan *et al.*, 1994). Similarly, Foppen and Schijven (2006) also indicated that detachment occurs, but concluded that it is relatively unimportant for simulating the transport of *E. coli*. Therefore, the detachment rate (R_d) was ignored in this research and the breakthrough curves of strain B6914 are simulated using the advection-dispersion-sorption model based on Equations 2.25-2.29. The results show that this filtration model resulted in a generally good fit of the experimental bacterial transport data (Figure 3.10).

3.3.4 Bacterial survival in CBM and IOCS

Based on the experimental transport data in Section 3.3.3, the fine IOCS significantly enhanced bacterial removal from synthetic stormwater runoff compared to CBM. However, direct removal of pathogenic bacteria from stormwater runoff is

not the only important criterion in evaluating BMP performance. Specifically, optimizing subsequent bacterial destruction between storm events is also necessary to make the process sustainable. Therefore, the transport and survival of trapped strain B6914 in fine IOCS and CBM (made using 1-yr aged mulch, soil and sand) was evaluated in four separate column experiments for each media.

The data from the four replicated transport experiments indicated good reproducibility for bacterial transport and capture in the CBM made using the same aged media and fine IOCS, under the same experimental conditions (Figure 3.12). However, the breakthrough curves for the columns packed with CBM were somewhat more variable, which may be a result of heterogeneities in the physical, chemical, or microbial properties of the mulch, sand and soil, or a non-homogenous dry-packing process.

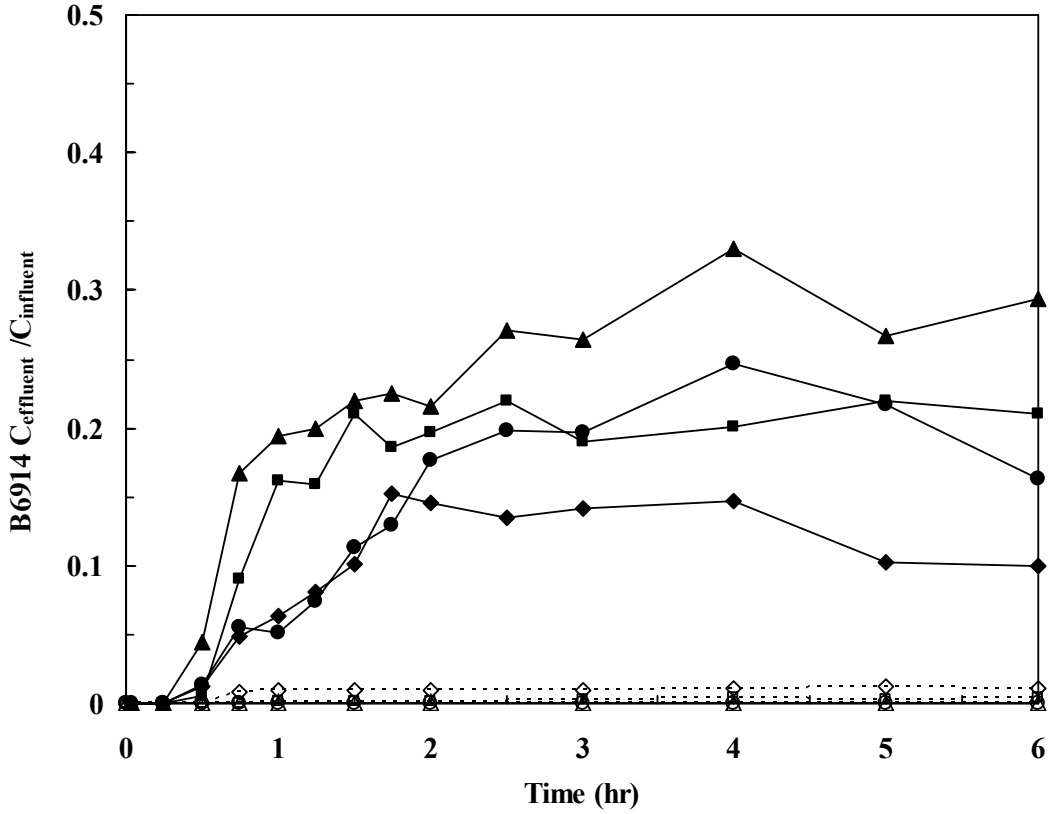


Figure 3.12 Breakthrough curves of strain B6914 in CBM and fine IOCS. Solid points are CBM, and open points are fine IOCS. The tested conditions: Flow rate = 40.0 ± 1.0 mL/hr, $C_{influent}$ = 1.1 ± 0.3 × 10^6 CFU/mL.

After the 6-hr stimulated rainfall application, the CBM columns were gravity drained for 0.5, 1.5, 2.5 or 7 days and the fine IOCS columns were drained for 0.5, 7, 14 or 28 days. After gravity drainage, the longitudinal distribution of strain B6914 attached to the CBM or fine IOCS in the columns was determined, as presented in Figure 3.13. The total number (N) of surviving strain B6914 cells in the column after gravity drainage of a specific day was calculated based on the moisture content, soil weight, and trapped bacterial concentration of each depth. The half-day drainage data are presented as an example of the calculation process (Table 3.5).

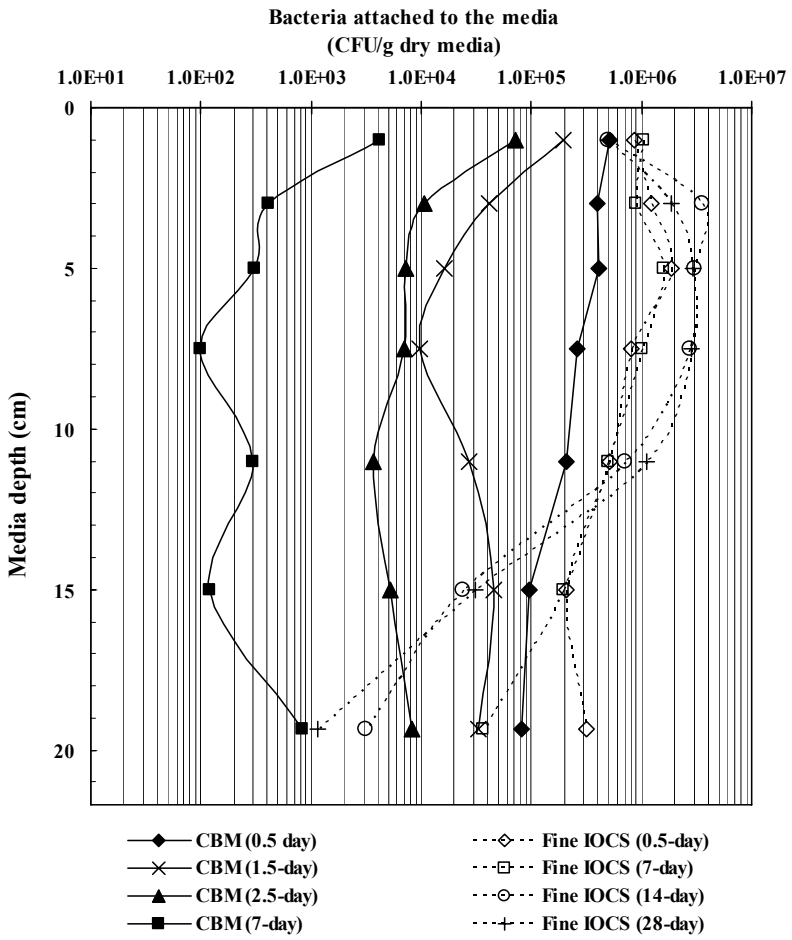


Figure 3.13 Longitudinal distribution of strain B6914 attached to media after gravity drainage for specific times. The bacterial detection limit was 300 CFU/g media).

Table 3.5 Longitudinal distribution of strain B6914 attached to CBM in column after gravity drainage 0.5 day (number of strain B6914 cells trapped in column was estimated to be 2.75×10^8 CFU after 6-hr loading).

Media depth (cm)	Wet media weight of each layer (g)	Moisture (%)	Dry media weight of each layer (g)	Strain B6914 concentration (10^5CFU/g dry media)	Total Strain B6914 amount (10^6CFU/layer)
0-2	17.579	16.901	15.037	13.9	20.9
2-4	17.359	22.546	14.165	3.14	4.45
4-6	15.974	20.510	13.255	1.10	1.46
6-9	22.551	23.668	18.235	0.59	1.07
9-13	32.747	27.782	25.627	0.33	0.83
13-17	34.902	31.329	26.576	0.30	0.98
17-21.7	39.074	31.312	29.757	0.25	0.76
Total (N)					30.3

In the Figure 3.13, it is clear that the number of strain B6914 cells attached to the fine IOCS at each depth of column was significantly higher than that in CBM at the same drainage time. For example, the number of strain B6914 attached to the IOCS at most depths (except the bottom) was 3-4 orders of magnitude higher than that in CBM after one-week gravity drainage. The quick die-off of strain B6914 trapped in CBM may be due to the predation and competition for nutrients from indigenous microorganisms, and strain B6914 cells in IOCS survived better due to lack of predation and competition in fine IOCS.

There was a similar trend of higher numbers of strain B6914 cells attached to media at the top of columns compared to that at the bottom at the same drainage time (Figure 3.13). In addition, trapped strain B6914 at the bottom in IOCS columns died off much faster than at the top. Possible reasons are the likely reduction of dissolved oxygen level and the increase in moisture content with depth. The moisture content in the columns decreased from the top to the bottom after gravity drainage (Table 3.5 and Appendix-3). Generally, bacteria survive better at high soil moisture content. However, moisture content above a certain optimum level has been shown to result in a decrease in microbial cells in natural soil (Postma and Veen, 1990). The moisture and oxygen content at the top media may be more favorable to the survival of strain B6914. Additionally, protozoa are in essence aquatic organisms. The availability of water has been shown to strongly regulate the grazing activity of protozoa. They tend to be more active at higher soil moisture contents, perhaps because increased water contents allow easier movement in soil pores, thus reducing the number of introduced bacteria (Kuikman, 1990).

Based on the concentrations of strain B6914 in the influent and the effluent during bacterial transport experiments (Figure 3.10), the number (N_c) of bacteria captured in the columns during the 6-hr stimulated rainfall application was calculated as follows:

$$N_c = M_{in} - M_{out} = \sum_i C_{influent,i} \cdot \Delta t_i - \sum_j C_{effluent,j} \cdot \Delta t_j \quad (3.4)$$

Based on the calculated values of N and N_c, the total number of remaining strain B6914 attached to the CBM after 0.5-day gravity drainage was approximately 12% of the number of bacteria captured in the columns during the 6-hr simulated rainfall application. That is, approximately 88% of the trapped B6914 cells died off over night (Figure 3.14). The percentage of surviving bacteria attached to the CBM (N/N_c) decreased logarithmically over time, with more than 99.98% of the trapped strain B6914 cells having died-off after one-week of gravity drainage. The number of trapped B6914 cells in the fine IOCS also decreased logarithmically; however, the die-off rate of trapped B6914 cells was much lower than in CBM. Only approximately 52% of the trapped B6914 cells in the fine IOCS died off after gravity drainage for one week, and about 22% cells still survived after four weeks.

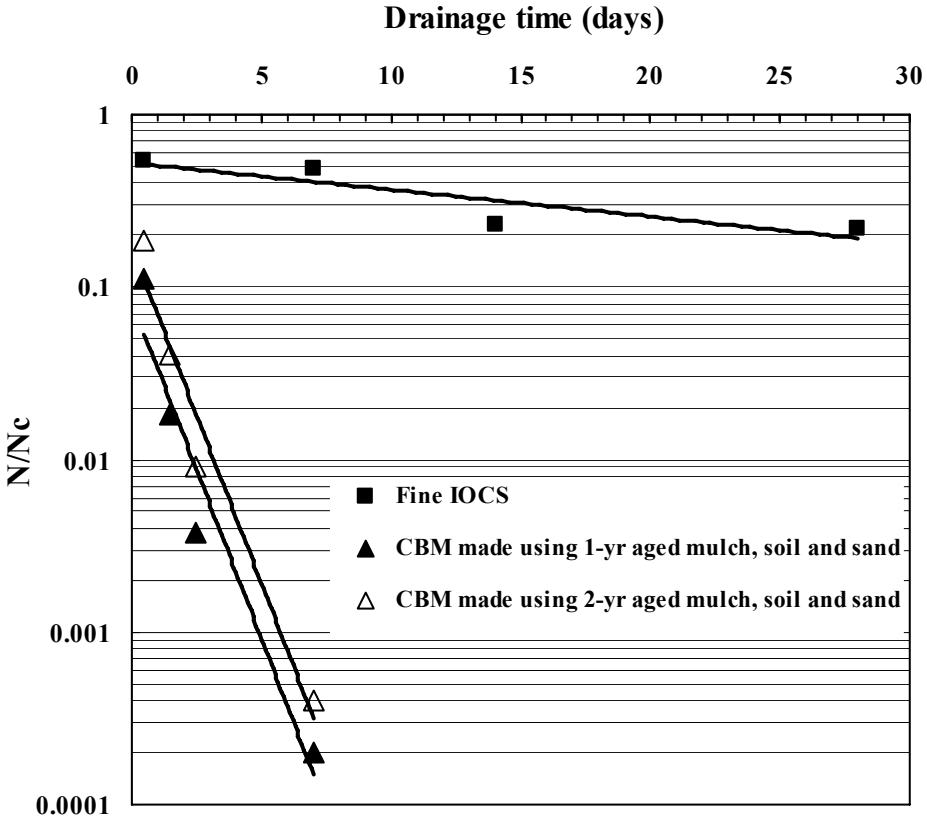


Figure 3.14 The fraction of surviving strain B6914 attached to media with respect to drainage time.

Traditionally, bacterial die-off process has been represented as a simple first-order decay (Biswas *et al.*, 1990):

$$\frac{N}{N_0} = e^{-k_d t} \tag{3.5}$$

where, k_d is the die-off or endogenous decay coefficient (d^{-1}). N_0 is the total number of surviving strain B6914 cells during 6-hr bacterial loading. Captured strain B6914 cells also may decay or die-off during 6-hr bacterial loading. Thus, N_0 will be smaller than N_c. Based on the relatively linear semi-log plots of N/N_c and drainage time in Figure 3.14, the bacterial die-off can be rewritten as following:

$$\frac{N}{N_c} = \frac{N_0}{N_c} e^{-k_d t} \tag{3.6}$$

The best-fits of this first-order decay rate equation were obtained by linear regression, and are presented in Table 3.6. The calculated decay rate coefficients are generally consistent with those found in previous research on the survival of *E. coli* in soil with/without amendment of nutrients (such as manure, feces, slurry), which indicate that the die-off rate of *E. coli* is approximately 0.04-0.30 d^{-1} (Table 2.1). However, the decay rate coefficient for CBM is higher than those observed in soil amended with manure (Table 2.1). This is probably because the mulch, the primary source of organic matter in the CBM, does not provide as much soluble organic nutrients for supporting growth of introduced bacteria as manure or feces.

It is clear that the decay rate (0.90 d^{-1}) of the captured strain B6914 cells in CBM was much higher than that (0.04 d^{-1}) in the fine IOCS. The mulch and soil in the CBM also provide plenty of microorganisms for predation and competition for nutrients. For example, there were approximately 10^7 CFU heterotrophic bacteria and thousands of protozoa per gram CBM. In comparison, the IOCS had little or no background native population of microorganisms. Thus, it is not surprising that the captured B6914 cells in CBM died off faster than that in the fine IOCS, because of the lack of predation or competition in the IOCS as studied. Similarly, the best-fit values of N_0/N_c also indicate that during the 6-hr bacterial loading, captured strain B6914 cells in CBM died off faster than that in the fine IOCS. However, these results are probably not representative of what would happen in the field because an IOCS layer, once installed in the soil, would likely become inoculated with native soil microbes from the surrounding soil.

Compared to fine IOCS and CBM, strain B6914 survived poorly in the synthetic stormwater runoff. The decay rate in the synthetic runoff (2.01 d^{-1}) was much higher than that of trapped strain B6914 in fine IOCS and CBM. Therefore, porous media provided a more suitable environment (nutrients, moisture, etc.) for strain B6914 compared to the synthetic runoff.

Table 3.6 The best-fit first-order decay rates and regression equations of strain B6914 in fine IOCS and CBM.

Media	Decay rate k_d (d^{-1})	N_0/N_c (%)	Decay equations
Fine IOCS	0.036	52.6	$N/N_c=0.53\cdot e^{-0.04t}$ $R^2=0.779$
CBM made using 1-yr aged mulch, soil and sand	0.903	8.38	$N/N_c=0.084\cdot e^{-0.90t}$ $R^2=0.937$
CBM make using 2-yr aged mulch, soil and sand	0.897	17.0	$N/N_c=0.17\cdot e^{-0.90t}$ $R^2=0.960$

As discussed in Section 3.3.3.1, the removal efficiency of strain B6914 in CBM was significantly influenced by the storage time of mulch, soil and sand. Specifically, the removal efficiency decreased from 80% to 60% after two years storage. Interestingly, the survival experimental data show that the storage time of the CBM media had no significant effect on the survival of trapped strain B6914 cells. The decay rate of trapped strain B6914 in the media made using 2-yr aged mulch, soil and sand was only slightly lower than that in the media made using 1-yr aged mulch, soil and sand at the same drainage time (Figure 3.14 and Table 3.6). This result indicates that the destruction of trapped strain B6914 in CBM is very reproducible over time.

3.3.5 The effect of native microorganism populations on bacterial transport and survival

As described above, predation and nutrient competition of native microorganisms may be a major factor resulting in the rapid decline of trapped strain B6914 in CBM. To investigate the impact of native microorganisms on bacterial die-off, studies on the survival of strain B6914 in columns sterilized by γ-irradiation were conducted in parallel to the non-sterile column experiments described above. CBM made using 2-yr aged mulch, soil, and sand media were used in this study. A comparison of the breakthrough curves for B6914 indicates that there was no significant difference between the non-sterile and γ-irradiated CBM (Figure 3.15). Thus, irradiation did not significantly change the transport and capture of strain B6914 through the columns. Additionally, the results of the bromide tracer experiments showed that γ irradiation had no significant effects on the porosity or dispersion coefficient of the media in the column (Table 3.7). Consistent with these data, Lensi *et al.* (1991) found that γ irradiation of dry soil has no significant effect on pore volume distribution. The bromide tracer data for the four replicates also indicate that the hydraulic characteristics in the columns packed with the same media were very repeatable.

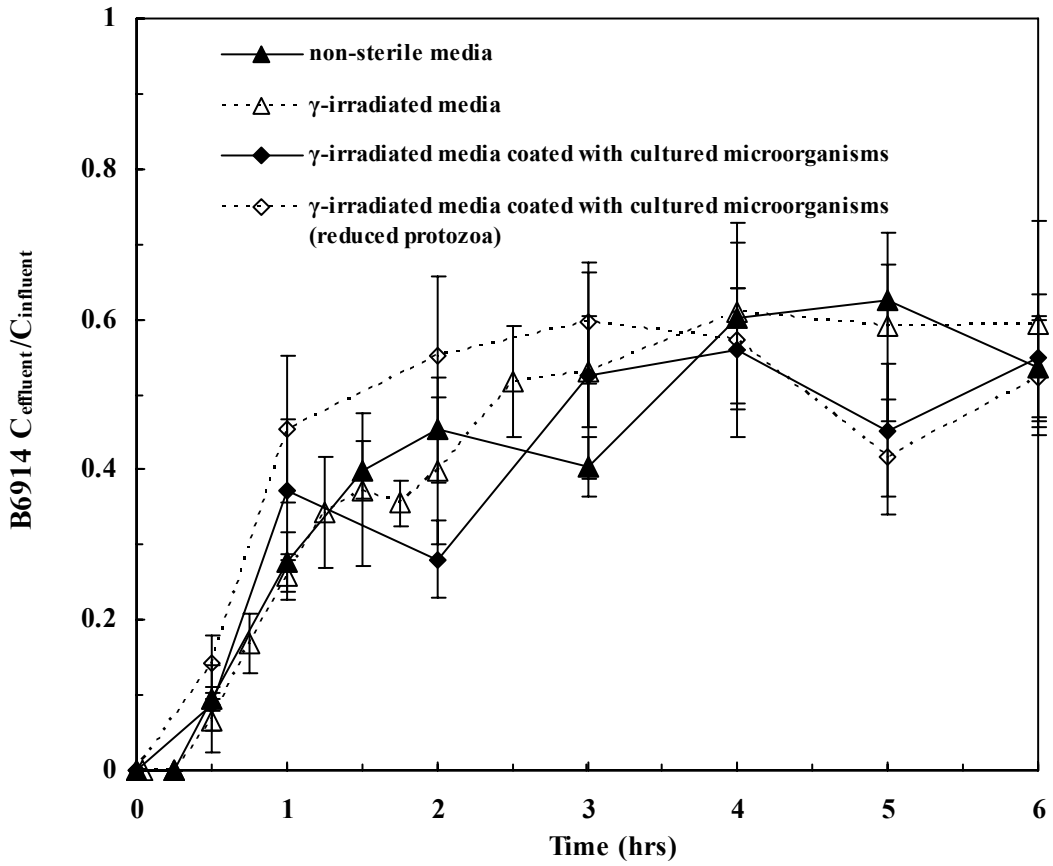


Figure 3.15 Breakthrough curves of strain B6914 in non-sterile CBM, γ-irradiated CBM, and γ-irradiated CBM coated with cultured microorganisms. Value represented by symbols is the mean of four replicate columns, with vertical bars showing ± 1 standard deviation (SD). The tested conditions: Flow rate = 40.0 ± 1.0 mL/hr, $C_{influent}$ = 1.1 ± 0.3 × 10^6 CFU/mL.

Table 3.7 Experimental data and model-fit or calculated parameters for columns packed by different media. The data represent the mean (n=4) ± 1 standard deviation. The parameters within the same column are insignificantly different (Student's t-test, P > 0.05, n = 4).

Media	Flow rate Q (mL/h)	Removal efficiency (%)	Best-fit parameters			Calculated parameters		
			D (cm^2/h)	ν (cm/h)	θ	α	η	k_a (h^{-1})
Non-sterile media	40.5 ± 0.9	55.7 ± 2.5	21.5 ± 7.0	24.9 ± 2.0	0.334 ± 0.025	0.018 ± 0.001	0.034 ± 0.002	1.00 ± 0.14
γ-irradiated media	40.8 ± 0.5	59.0 ± 1.3	15.9 ± 3.3	24.2 ± 0.9	0.344 ± 0.014	0. 017 ± 0.001	0.037 ± 0.002	0.95± 0.11

After 6-hr bacterial loading, the bacterial survival data (Figure 3.16) show that the amount of trapped strain B6914 in the non-sterile media rapidly declines, while the amount of trapped B6914 in the γ-irradiated media greatly increases within one week. After one-week drainage, more than 99.98% of strain B6914 cells trapped in non-sterile CBM died off, while strain B6914 cells trapped in sterile CBM increased approximately 40 times. This trend that sterile media greatly lowered the die-off rate of trapped pathogenic bacteria is in general agreement with other studies of *E. coli* in aquatic, sediment, and manure-amended soil systems (Hood and Ness, 1982; Laliberte and Grimes, 1982; Jiang *et al.*, 2002). A possible reason for the greater bacterial survival in the sterile columns is that that the process of γ-irradiation has upset the ecology. γ-irradiation killed the native microorganisms that can predate on *E. coli* (e.g., protozoa, bacterial predators), or antagonistic the microorganisms that compete with *E. coli* for the scarce nutrients in the media. Although compared to autoclaving, γ irradiation may have less of an affect on the chemical and physical properties of the media (McNamara *et al.*, 2003), γ-irradiation can actually increase the available nutrients in the media, such as soluble organic carbon, ammonium

nitrogen, exchangeable sulfur and exchangeable Mn, etc (Salonius *et al.*, 1967; Lensi *et al.*, 1991; McNamara *et al.*, 2003), which may have also contributed to the enhanced survival and growth of B6914 in the sterilized column.

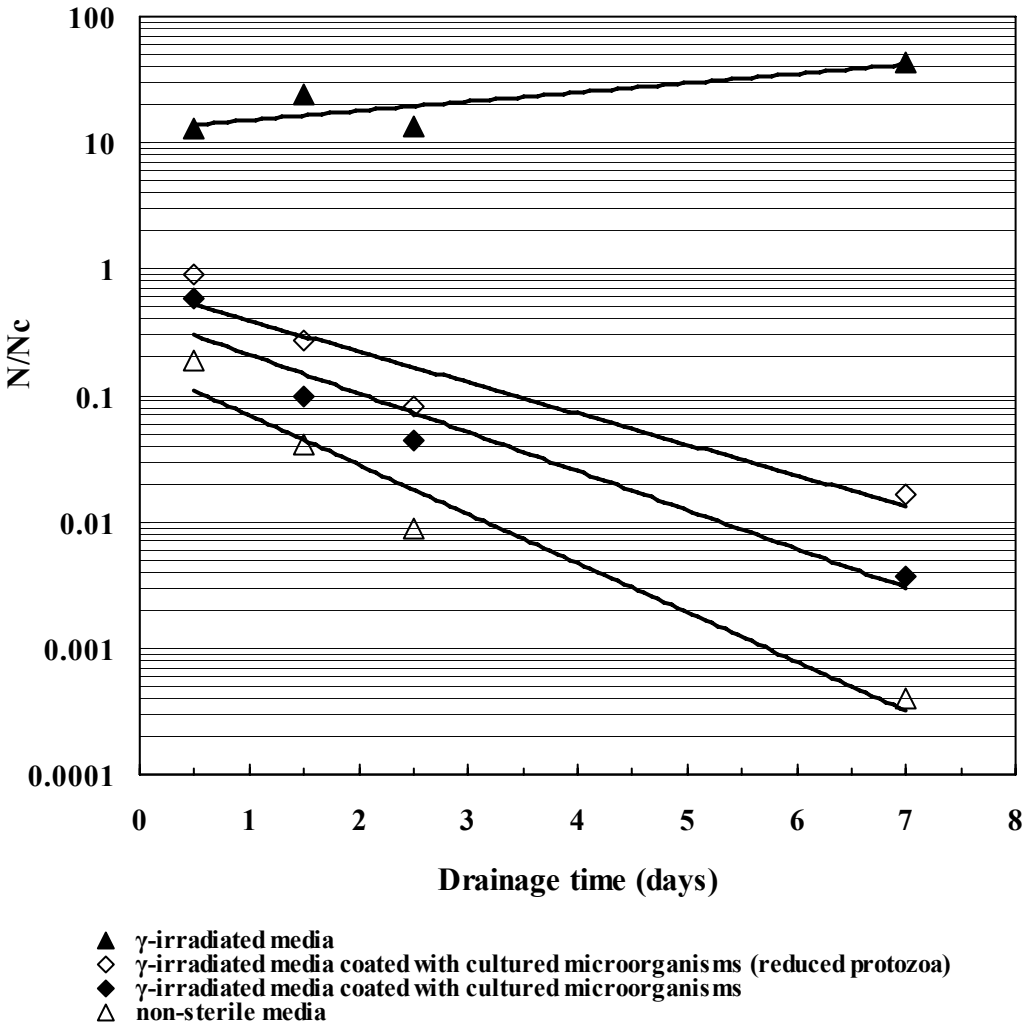


Figure 3.16 The fraction of surviving strain B6914 attached to media with respect to drainage time.

Based on the procedure described in Section 3.2.6, a small test was performed to evaluate the impact of nutrients released during γ irradiation by examining the survival of a concentration of 1.1×10^6 CFU/mL of B6914 in the wash water from the

sterile and non-sterile column. As illustrated in Figure 3.17, the optical density (OD) of the bacterial suspension in the wash water from the non-sterile CBM column decreased quickly, reaching close to that of the OD of the wash water without bacteria (control experiment) after 6 days. However, the OD of the wash water from the γ-irradiated sterile column slightly increased and remained higher than the control. That is, the number of strain B6914 cells in the wash water from the γ-irradiated column increased, while strain B6914 cells in the wash water from the non-sterile column decayed. This further verifies the hypothesis that γ-irradiation increased the soluble nutrients, thus enhancing the survival of strain B6914 in the sterile columns.

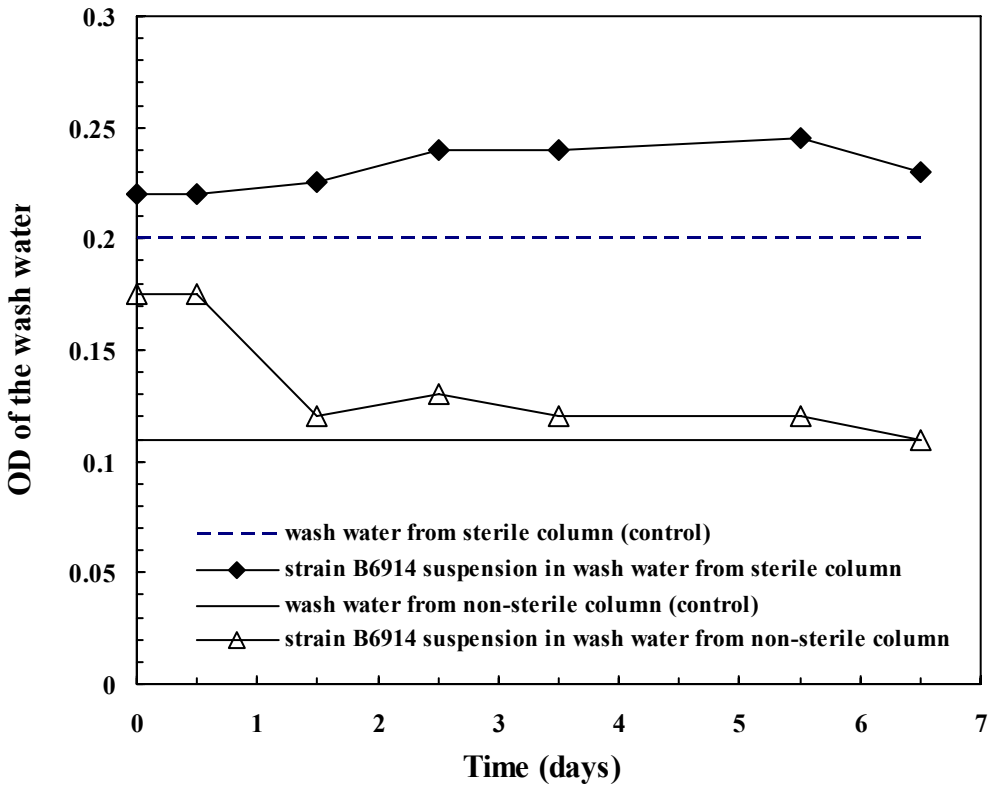


Figure 3.17 OD of the wash water from sterile/non-sterile columns with/without bacteria over time.

These results on the survival of trapped strain B6914 in the sterile and non-sterile media indicate that native microorganisms and nutrients may be the dominant factors controlling the survival of introduced pathogenic bacteria. Therefore, to further evaluate the effects of native microorganisms on the transport and destruction of strain B6914, cultured microorganisms inoculated from CBM were coated onto γ-irradiated sterile media, and then transport and survival experiments were conducted.

The removal efficiency of strain B6914 was not influenced by the addition of cultured microorganisms to the γ-irradiated sterile media (Figure 3.15). However, the addition of cultured microorganisms to γ-irradiated media significantly enhanced the destruction of the trapped strain B6914 (Figure 3.16 and Table 3.8). While the number of strain B6914 in the γ-irradiated media increased, approximately 40% of the trapped strain B6914 cells died off after overnight drainage in the γ-irradiated media coated with cultured microorganism, with the die-off percentage increasing to 99.6% after one week. Therefore, the presence of native microorganisms appears to be a dominant factor contributing to the rapid decline of trapped strain B6914 in the CBM. The specific mechanism appears to be predation or competition for nutrients.

Table 3.8 The best-fit first-order decay rates and regression equations of strain B6914 in the media.

Media	Decay rate k_d (d^{-1})	N_0/N_c (%)	Decay equations
γ-irradiated sterile CBM	-0.183*	1250	$N/N_c^*=12.5\cdot e^{0.18t}$ $R^2=0.698$
CBM make using 2-yr aged mulch, soil and sand	0.897	17.0	$N/N_c=0.17\cdot e^{-0.90t}$ $R^2=0.960$
γ-irradiated CBM coated with cultured microorganism (total 1.90×10^5 MPN protozoa)	0.696	42.0	$N/N_c=0.42\cdot e^{-0.70t}$ $R^2=0.927$
γ-irradiated CBM coated with cultured microorganisms (reduced protozoa, total 2.11×10^4 MPN protozoa)	0.564	68.6	$N/N_c=0.69\cdot e^{-0.56t}$ $R^2=0.900$

* Data represent growth rate and growth equation of trapped strain B6914 in sterile CBM.

Compared to the column packed with non-sterile CBM, the total numbers of protozoa and total heterotrophic bacteria attached to the column of γ-irradiated media coated with cultured microorganisms were higher (Figures 3.18 and 3.19); however, the die-off rate of trapped B6914 (0.70 d^{-1}) was lower than that in non-sterile columns (0.90 d^{-1}), as summarized in Table 3.8. Specifically, during 6-hr bacterial loading, approximately 83% ($1-N_0/N_c$) of the captured strain B6914 cells in the non-sterile CBM died off, while only approximately 58% of the trapped strain B6914 cells died off in the γ-irradiated media coated with cultured microorganisms. One possible reason is that γ irradiation increases nutrients in the γ-irradiated media, which reduces the competition for nutrients between trapped strain B6914 cells and the added microorganisms. Although the percentages of surviving strain B6914 cells in the γ-irradiated media coated with cultured microorganisms at the first 3-day drainage time were higher than those in the non-sterile media, more than 99% of trapped strain

B6914 died off in both media. Therefore, predation may be the major factor causing the high die-off rate of trapped B6914 cells in the γ-irradiated media coated with cultured microorganisms. Predation by protozoa has previously suggested as a regulating factor for population size of pathogenic bacteria introduced to soil environments (Sørensen *et al.*, 1999). For wastewater treatment, it was also found that protozoan grazing plays an import role as an enteric bacteria removal mechanism in sand infiltration system (Stevik *et al.*, 1998; Bomo *et al.*, 2004).

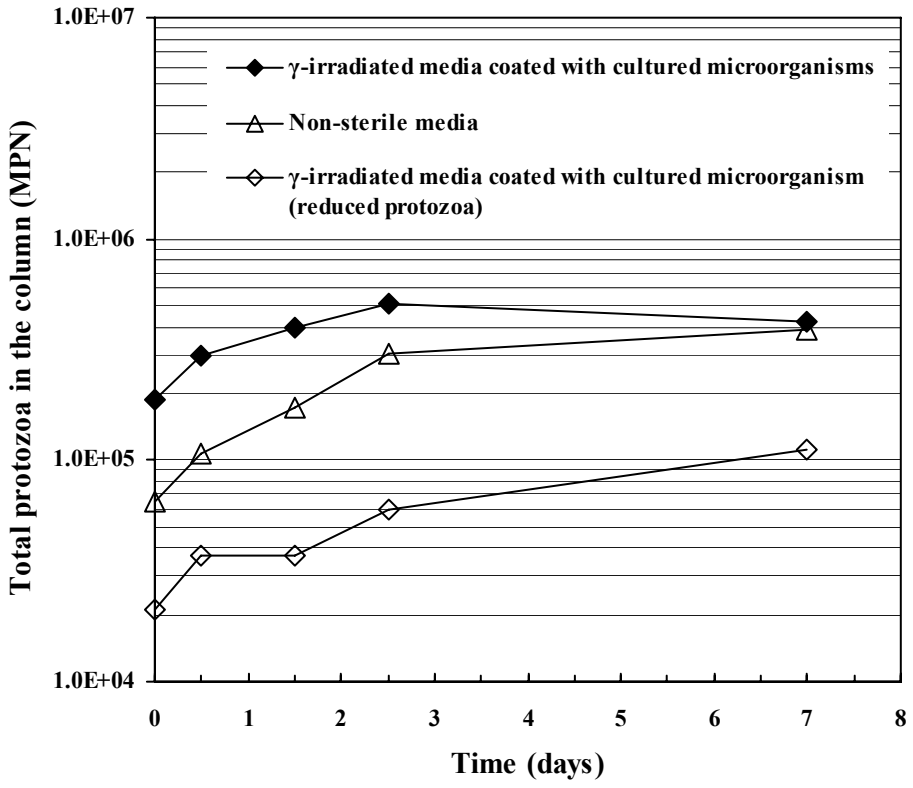


Figure 3.18 Total protozoa attached to non-sterile CBM and γ-irradiated media coated with cultured microorganisms in the columns.

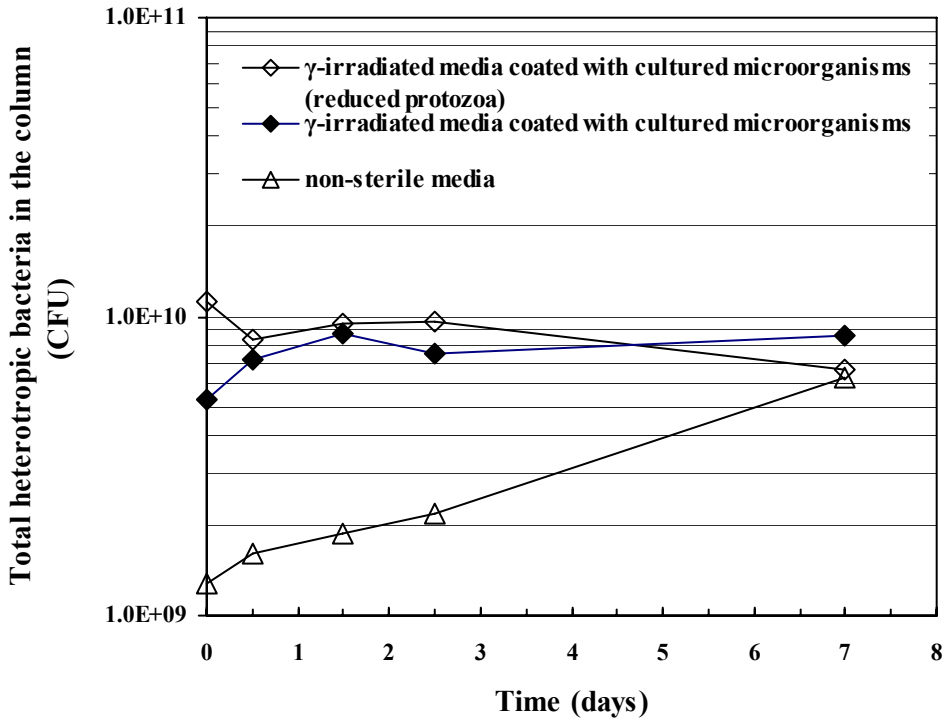


Figure 3.19 Total heterotrophic bacteria attached to non-sterile CBM and γ-irradiated media coated with cultured microorganisms in the columns.

To further investigate the effect of indigenous protozoa on survival of trapped strain B6914, the microbial inocula from CBM were filtered through a 5 μm filter to decrease the concentration of protozoa before coating to γ-irradiated media. As a result, the total number of protozoa in the γ-irradiated media coated with this filtrate was 2.11×10^4 MPN, which was one order of magnitude lower than that (1.90×10^5) in the γ-irradiated media coated with cultured microorganism, as described above (at t=0 day, Figure 3.18).

The results of bacterial survival experiments show that the die-off rate of trapped strain B6914 in the γ-irradiated media coated with reduced concentrations of protozoa was lower than that in the γ-irradiated media coated with the higher

concentrations of protozoa (Table 3.8). After the same gravity drainage time, the die-off percentage in of trapped strain B6914 in the γ-irradiated media coated with reduced concentrations of protozoa was much lower than that in the γ-irradiated media coated with high concentrations of protozoa, especially in the first 2-3 days. For example, approximately 40% of the trapped strain B6914 died off in the γ-irradiated media coated with high concentrations of protozoa after over night drainage, while only approximately 10% of trapped strain B6914 died off in the γ-irradiated media coated with lower concentrations of protozoa. The number of heterotrophic bacteria within the two media was similar (Figure 3.19), therefore, the predation by protozoa may be the primary reason for the die-off of trapped strain B6914. This finding is also consistent with previous studies by Enzinger and Cooper (1976) who studied the relative role of protozoan and bacterial predators on the survival of *E. coli* in estuarine water samples by adding different antibiotics. Their results indicated that predation mainly resulted from protozoa, whereas bacterial predators were not important for coliform destruction. The studies of McCambridge and McMeekin (1980) suggested that interacting microbial predators each played a distinct role in the destruction of fecal bacteria after *E. coli* was introduced into estuarine water, with predacious protozoa exerting their major influence on *E. coli* destruction during first 2 days, which is consistent with our observation.

3.4 Summary and conclusions

Column studies were performed to evaluate the transport and capture of bacteria from synthetic stormwater runoff by CBM and IOCS. The results indicate that CBM

(mixture of sand, sandy loam soil and mulch (leaf compost)) achieved 80% or higher removal of *E. coli* O157:H7 strain B6914 under the test conditions, suggesting that strain B6914 can be effectively reduced by CBM. However, the removal efficiency decreased to around 60% when components of CBM were stored for more than two years at room temperature, which may be attributed to changes in the surface characteristics and/or biological diversity of the media components during the storage time. Coated iron oxides enhanced bacterial adhesion through surface charge modification, increasing bacterial removal to 99.4%. However, the die-off rate (0.90 d^{-1}) of trapped cells was much faster in CBM compared to in the fine IOCS, as studied (0.04 d^{-1}). More than 99.98% of B6914 cells attached to CBM died off within one week. In comparison, only approximately 52% of trapped B6914 cells in the fine IOCS died off after gravity drainage for one week and about 22% cells still survived after four weeks, which may represent a potential threat for receiving waters. Therefore, CBM have a greater potential to render the process sustainable, at least until the IOCS would get colonized by native soil microorganisms.

To investigate the impact of indigenous microorganisms in CBM on bacterial die-off, the survival of strain B6914 in parallel non-sterile media, γ-irradiated sterile media and γ-irradiated sterile media coated with cultured microorganisms were compared. The trapped B6914 in the γ-irradiated media kept growing throughout one week, because γ-irradiation not only killed the native microorganisms, but also may have increased available nutrients. However, once the γ-irradiated media was coated with microorganisms cultured from the CBM, the number of trapped strain B6914 cells quickly decreased over time, although the die-off rate (0.70 d^{-1}) was lower than

that in the non-sterile CBM (0.90 d^{-1}). Additionally, survival experiments using the media coated with different concentrations of protozoa indicate that the die-off of trapped B6914 increased with increasing concentrations of protozoa. Therefore, it is concluded that native microorganisms play a dominant role in the destruction of trapped strain B6914 in conventional bioretention systems.

Chapter 4: Long-term sustainability of bacterial removal in conventional bioretention media

4.1 Introduction

Estuaries, coastal waters and other water bodies are increasingly impaired by microbial contamination from urban stormwater runoff due to the dramatic increase in human and animal populations and their resulting fecal wastes in local watersheds (USEPA, 2001b). Bioretention, a mulch/soil/vegetation-based BMP system, has significant potential to reduce the level of bacteria from urban stormwater runoff by physical filtration, sorption, biological predation and other mechanisms (DER, 2001; USEPA, 2004). As described in Chapter 3, laboratory column studies performed as part of this research demonstrated that conventional bioretention media (CBM) can achieve a mean 70% efficiency for removal of *E. coli* O157:H7 strain B6914 from simulated urban stormwater runoff under tested conditions. Consistent with these results, Rusciano and Obropta (2007) observed a mean reduction coefficient of 91.5% for fecal coliform bacteria in laboratory bioretention columns. However, there are no published data available on the long-term performance of bioretention media for removal of pathogenic bacteria from urban stormwater runoff, and many questions remain. For example, does the bacterial removal capacity of bioretention media improve or degrade over time? Does the survival of the captured bacteria increase or decrease? Do the mechanisms of bacterial capture and destruction change over time?

Several physical, chemical, and biological properties of bioretention media (the mixture of sand, soil and mulch (leaf compost)), are expected to change over time,

and thereby potentially influence bacterial capture and survival. The application of mulch may have significant effects on the organic matter content, soil respiration, microbial diversity and abundance, cation-exchange capacity of soil, water holding capacity and soil aggregation ability (Tiquia *et al.*, 2002; Baldwin and Greenfield, 2005). Mature compost is relatively resistant to further decomposition due to relative high C:N (Baldwin and Greenfield, 2005). However, available organic nitrogen from soil and stormwater runoff may result in further organic decomposition in bioretention media over time. For example, Elshorbagy and Mohamed (2000) found that the organic matter content of a mixture of municipal solid waste compost and a silty sand soil in their column decreased from 51% to 38.4% at a constant water content of 26% after 15 days incubation. The surface charge and hydrophobicity of porous media are controlled to a large extent by the amount of organic matter present (Newby *et al.*, 2000); thus degradation of organic matter in the mulch and sandy loam soil with loading of stormwater runoff over time may result in changes to the media surface, in particular the electrostatic charges and hydrophobicity, which play a significant role in the transport and capture of bacteria through soil.

Additionally, organic amendments such as compost can modify the composition of the bacterial taxa in soil, and increase the level of microbial activity resulting in increased competition and/or antagonism among microbes and finally, decrease the activity of pathogens (Tiquia *et al.*, 2002). As reported in Chapter 3, experiments comparing the survival of strain B6914 in non-sterile media, γ-irradiated media, and γ-irradiated media coated with cultured microbes indicated that the biological activity of the native microbes significantly influences the survival of strain B6914 cells

captured in CBM. Protozoa are the most effective of bacterial feeders because of their feeding and growth characteristics. Protozoa eat first, and then multiply through division with generation times potentially as short as those of bacteria. As a result, protozoa can quickly develop a high grazing pressure (Clarholm, 2002). The long-term introduction of pathogenic and other bacteria from urban stormwater runoff into bioretention media may stimulate the growth of protozoa and other predators, and thereby influence the microbial community composition in the media. Such changes in the microbial community may in turn influence the capture and die-off of newly-introduced bacteria and vise versa.

The goals of the study presented in this chapter were to: (1) evaluate long-term (18-month) removal of *E. coli* O157:H7 strain B6914 from synthetic urban stormwater runoff by CBM using laboratory column tests, (2) observe changes in the distribution and survival of captured bacteria in the aged media over time, and (3) investigate the mechanisms of bacterial capture and destruction and whether these mechanisms evolve temporally. These results provide important information on the potential sustainability and effective lifetime of bioretention media for bacterial removal.

4.2 Materials and methods

4.2.1 Test microorganism

A derivative of *E. coli* O157:H7, strain B6914, which has lost the shiga-toxin genes and been engineered to contain the green fluorescent protein gene, was used as

the test microorganism in this study. Strain B6914 was cultivated following the procedures described in Section 3.2.1.2

4.2.2 Long-term column study setup

The experimental set up for the 18-month long-term sustainability experiment is shown schematically in Figure 4.1. Five glass chromatography columns (Cat. No. 420401-2520, Kontes, Vineland, NJ) were used, the properties of which are presented in Section 3.2.4.1. CBM were used in this study, consisting of a mixture of sandy loam soil, U.S. Silica® Mystic White II pool filter sand, and mulch, as outlined in Section 3.2.2. The media were dry-packed into each column following the procedure described in Section 3.2.4.1.

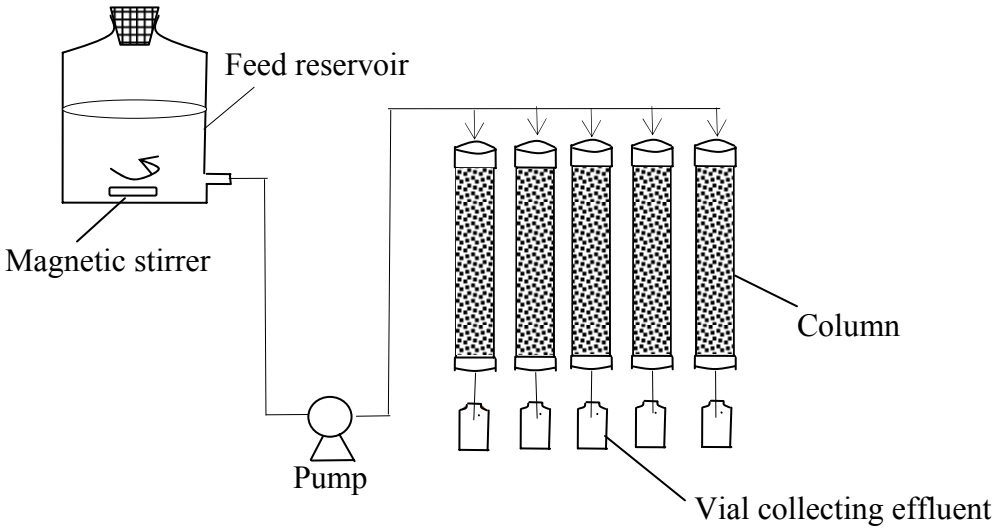


Figure 4.1 Schematic diagram of the long-term column experimental setup.

4.2.3 Bacterial transport and capture experiments

To evaluate the long-term transport and capture of bacteria in CBM, five columns were loaded with a suspension of B6914 in synthetic runoff once every two weeks. Each column was loaded in this manner for a different total duration: 2-, 5-, 9-, 13-, and 18-months. Loading of the 2-, 5-, 9-, 13-, and 18-month columns was initiated on Apr 4, 2007, June 6, 2007, Feb 8, 2007, May 31, 2006 and May 31, 2006, respectively.

The biweekly loadings were performed as described in Section 3.2.4.3 and briefly outlined here. Firstly, prior to initiating each bacterial transport experiment, the columns were flushed with sterile synthetic runoff (Table 3.2), until the pore volume was saturated. The strain B6914 and total heterotrophic bacteria in the wash effluent were enumerated by the methods in Section 3.2.7.1. After saturation, the transport experiments were performed by loading the columns with a suspension of B6914 in synthetic runoff (nominal $C_0 = 1 \times 10^6$ CFU/mL) for 6 hrs at a flow rate of 40 mL/hr (8 cm/hr). During the transport experiment, the column influent and effluent every one hour were monitored for the numbers of strain B6914 by the methods in Section 3.2.7.1. After loading, the columns were allowed to drain by gravity.

4.2.4 Bromide tracer transport experiments

Bromide tracer tests were performed to support the microbial transport and capture studies by providing estimates of the average pore water velocity and hydrodynamic dispersion of water flow in the columns. Specifically, during the

bacterial transport experiments performed after durations of 0, 2, 5, 9, 13 and 18 months, sodium bromide was dissolved in the synthetic runoff feed solution to obtain a bromide concentration of 200 mg/L. Subsequently, the bromide concentration was monitored in the column effluent by the methods in Section 3.2.7.3 to obtain the bromide breakthrough curve. As described in Section 3.2.4.2, bromide breakthrough curves were described by the one-dimensional advection-dispersion equation, and the best-fit parameters (hydrodynamic dispersion coefficient D, and average pore velocity ν were obtained by using a FORTRAN program "trafit1d". Finally, these best-fit parameters were coupled with the bacterial breakthrough data to calculate the parameters (retardation factor R, sticking efficiency α, single collector collision efficiency η_0, and adsorption rate coefficient k_a) using the equations 2.9-2.23 presented in Section 2.4.

4.2.5 Bacterial survival experiments

To evaluate temporal changes in bacterial distribution and survival in the CBM, each column was sacrificed at the end of its loading period. Specifically, after durations of 2, 5, 9, 13 and 18 months, one of the five columns was sacrificed after the last 6 hr stimulated rainfall application and overnight gravity drainage. After drainage, the media was removed and the trapped B6914, heterotrophic bacteria and protozoa in the media were enumerated by following the procedures and methods described in Section 3.2.5 and Section 3.2.7.1. In addition, the change in the surface charge of the media was determined by following the procedure described in Section 3.2.7.4.

4.3 Results and discussion

4.3.1 Long-term bacteria transport and capture

Bacterial transport and capture in CBM was quantified through enumerating B6914 in the influent and effluent of the columns used in the bacterial transport experiments. When the synthetic runoff with the strain B6914 suspension was first loaded into the five fresh-packed columns, the breakthrough curves were somewhat variable, as shown in Figure 4.2. The removal efficiency for strain B6914 was calculated using Equation 3.1, and the result shows that the removal efficiency in the five columns ranged from 52% to 90% with a mean of 72% (standard deviation = 16%). In comparison, Rusciano and Obropta (2007) observed a mean reduction coefficient of 91.5% for fecal coliform bacteria in laboratory bioretention columns which were loaded with bacterial suspensions at a range of 2.3×10^3 to 2.3×10^7 CFU/100 mL. Similarly in other studies of fecal coliform and *E. coli* transport and capture in a bioretention facility, 69% and 71% mean concentration-based removals have been reported (Hunt *et al.*, 2008).

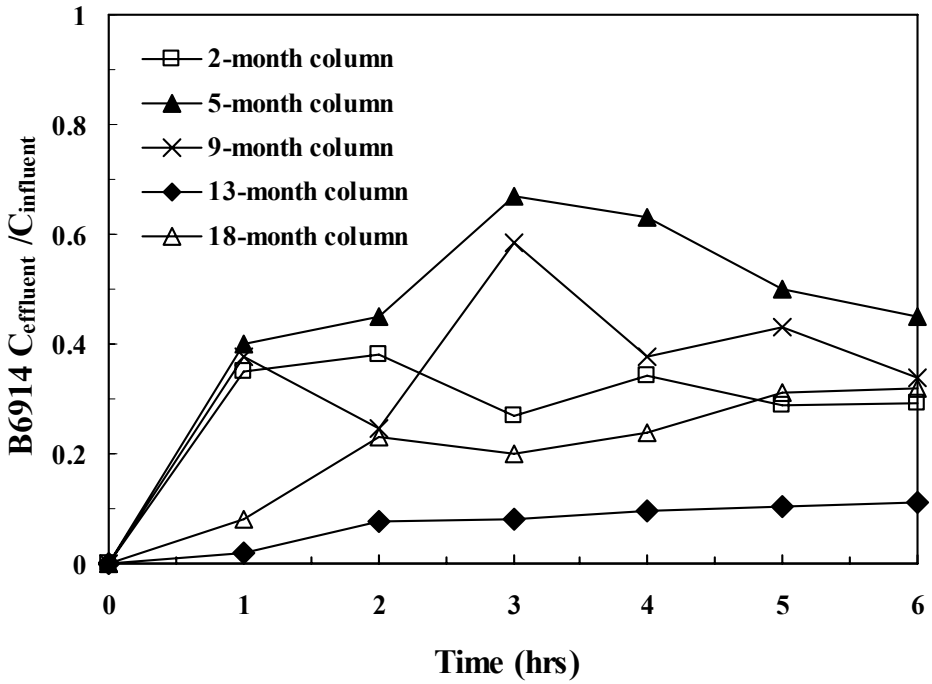


Figure 4.2 Breakthrough curves for strain B6914 in the five CBM columns during the first bacterial loading experiment in each column. The tested conditions: Flow rate = 40 ± 1 mL/hr, $C_{influent}$ = 1.1 ± 0.3 × 10^6 CFU/mL.

As described in Chapter 3, the variability observed in the bacterial removal efficiency among the different columns during the initial loading may be primarily due to changes in surface characteristics and microbial composition of the mulch, soil and sand with storage time. As noted in Section 4.2.4, all five columns studies were started at different times, and as a result, the bioretention media had been stored for variable time. For example, the 13- and 18-month column studies were the first initiated and those had the best initial removal efficiency. Additionally, naturally occurring heterogeneities in the physical, chemical, and/or microbial properties of the CBM mixture and the non-homogenous dry-packing process may also have affected the transport and capture of strain B6914 to some extent.

Interestingly, despite the variability in the initial removal efficiency, the long-term performance of bacterial removal from synthetic runoff in the five columns exhibited the same trend of improving removal efficiency for bacteria over time (Figure 4.3). In fact after 6 months, the removal efficiencies for strain B6914 in CBM were all greater than 97%, and subsequently showed little variability with respect with time. These data suggest that bacterial removal efficiencies may be expected to increase over time. Consistently, the results of bacterial column studies by Rusciano and Obropta (2007) showed that the reduction coefficient for fecal coliform by CBM increased over time in six simulated storm events over a period of six months.

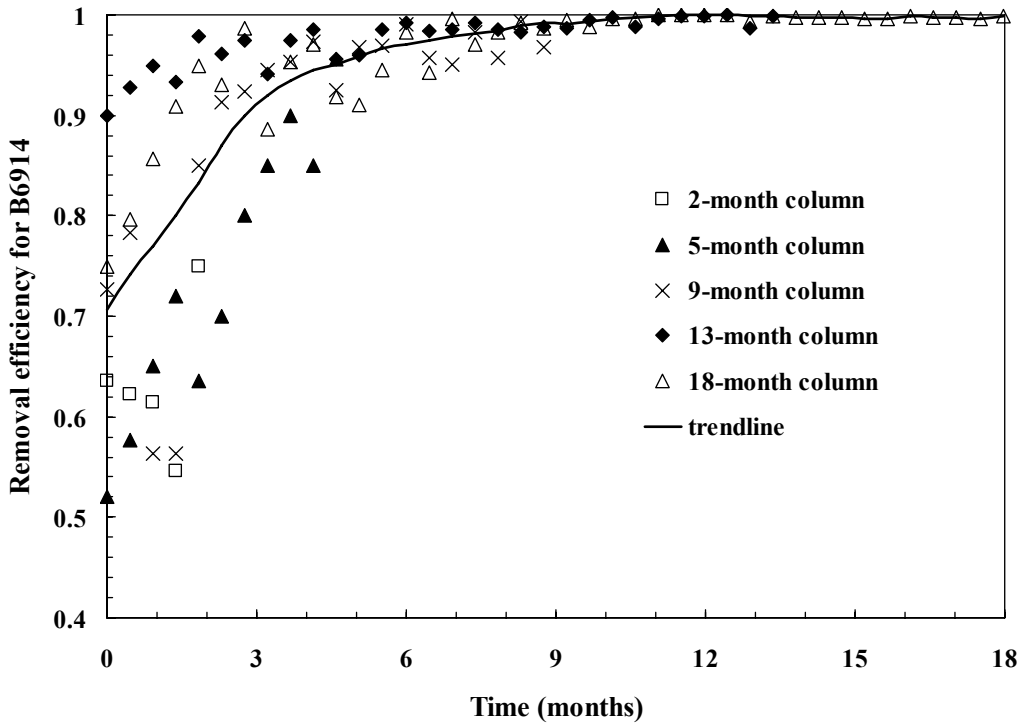


Figure 4.3 Removal efficiencies for strain B6914 by CBM in five columns for long-term experiments. The trendline was obtained by smoothing the mean line and is provided for illustrative purposes.

4.3.2 Bromide tracer tests

After durations of 0, 2, 5, 9, 13, and 18 months, the conservative (bromide) tracer tests in the five columns were performed. The experimental data along with the advection-dispersion model fits are presented in Figure 4.4. The bromide broke through earlier during 5-, 9-, 13, and 18-month experiments than fresh or 2-month media, with the occurrence of the point of saturation ($C_{effluent}= C_{influent}$) slightly delayed in the 5-, 9-, 13, and 18-month media (Figure 4.4). The best-fit parameters (Table 4.1) indicate that the porosity of media decreased, and the average pore water velocities and hydrodynamic dispersion coefficient increased to some extent over time. These trends are favorable for the physical straining/filtration of bacterial cells and microbial adsorption to the media. Consistent with these trends, the decrease of porosity may be caused by the aggregation of porous particles or the formation of biofilm on the porous media over time (Taylor and Jaffé, 1990).

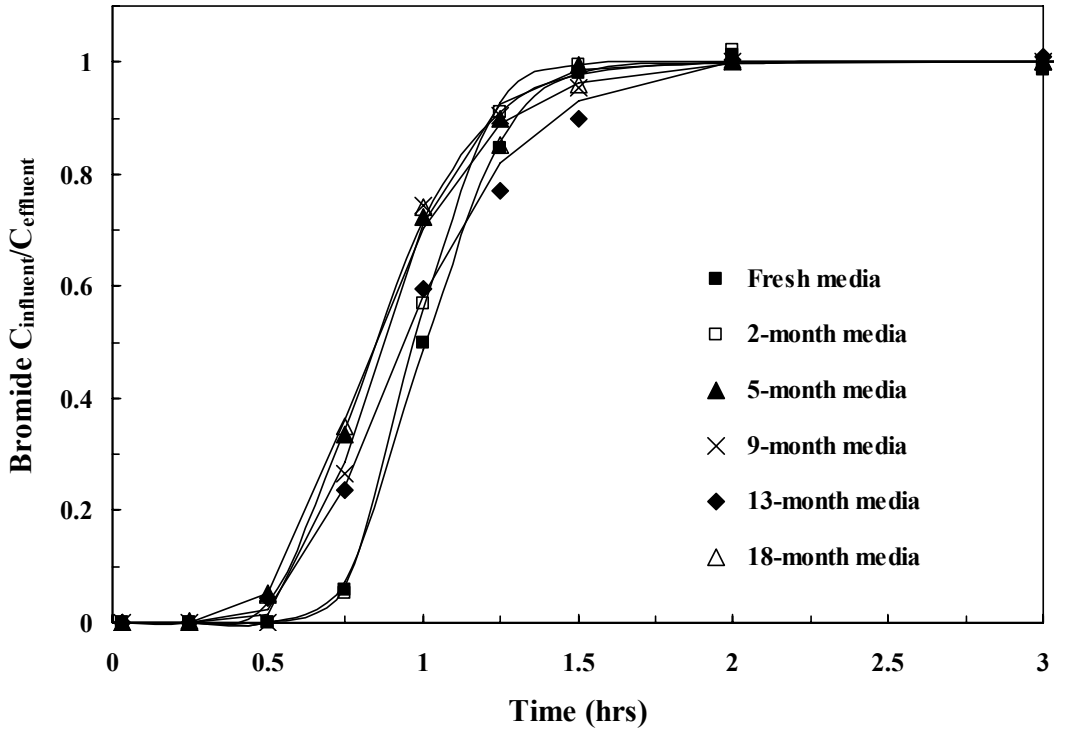


Figure 4.4 Bromide breakthrough curves in the CBM columns at the end of fresh, 2-, 5-, 9-, 13-, and 18-month transport experiments. Symbols correspond to experimental data, and the lines are the best-fit for the advection-dispersion equation.

Table 4.1 Experimental data and model-fit or calculated parameters for column studies at the end of fresh, 2-, 5-, 9-, 13-, and 18-month transport experiments.

Column	Flow rate Q (mL/h)	Removal efficiency (%)	Best-fit parameters			Calculated parameters		
			D (cm^2/h)	ν (cm/h)	θ	α	η	k_a (h^{-1})
2-month	40.7	81.1	7.10	22.0	0.378	0.039	0.030	1.63
5-month	40.4	95.6	13.9	23.0	0.360	0.062	0.032	3.17
9-month	40.7	99.1	17.4	24.3	0.342	0.108	0.033	5.72
13-month	40.3	99.8	24.8	22.1	0.372	0.153	0.031	6.55
18-month	40.1	99.9	28.8	24.5	0.333	0.140	0.034	7.80

Additionally, based on the best-fit parameters from the bromide tracer tests and the bacterial transport experimental data, bacterial transport parameters (sticking efficiency α, single collector collision efficiency η_0, and adsorption rate coefficient k_a) were calculated and presented in Table 4.1. It is clear that the value of the sticking efficiency α in CBM columns employed with periodic runoff application first increased linearly within 13 months, and subsequently showed little variability with respect with time (Figure 4.5). Thus, bacterial removal efficiencies may be expected to increase over time, and approach a stable value after 13-month runoff application.

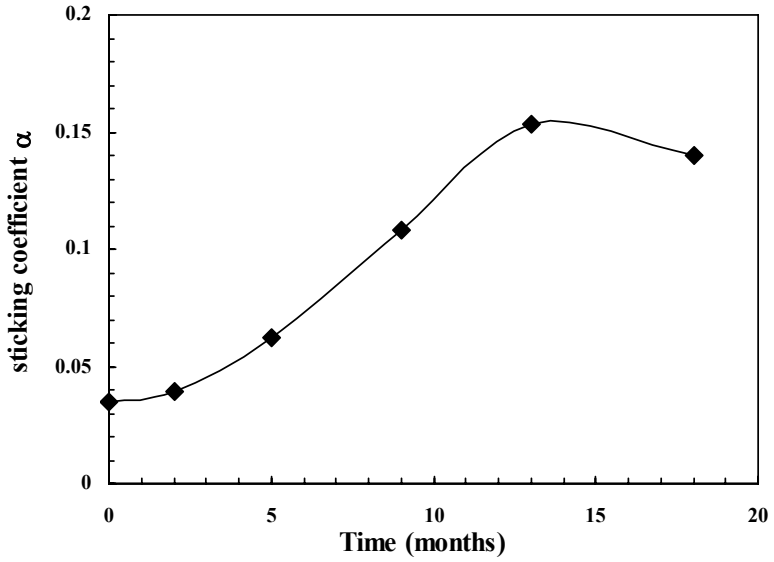


Figure 4.5 The sticking efficient α in long-term CBM column with respect with time.

4.3.3 Long-term bacterial survival in CBM

The transport and capture data have indicated a high removal of strain B6914 by CBM in the columns over the long term. However, the direct removal of bacteria from stormwater runoff is not the only important criterion in evaluating BMP performance. Specifically, optimizing subsequent bacterial destruction between storm events is also necessary to make the process sustainable.

As described above, in the long-term transport experiment, the columns were loaded with a suspension of B6914 in synthetic runoff once every two weeks. In each case, prior to initiating a bacterial transport experiment, the columns were first saturated with sterile synthetic runoff. During the flushing to achieve saturation, numbers of strain B6914 were monitored in the effluent. However, after the two-week gravity drainage period, the concentration of strain B6914 cells in the wash effluent was much lower than the detection limit (30 CFU/mL). These data indicate that the trapped strain B6914 cells had either completely died off, or they had become irreversibly trapped in the bioretention media. This conclusion is supported by the results of the bacterial survival experiment at room temperature that was described in Chapter 3. That study showed that the number of trapped strain B6914 cells logarithmically decreased over time, with more than 99.98% of the trapped B6914 cells having died off after a one-week gravity drainage.

To further investigate the fate of the trapped B6914 cells in aging media, the five columns were sacrificed and analyzed at durations of 2, 5, 9, 13 and 18 months, respectively. One key analysis was to enumerate the trapped B6914 cells at different depths after the final 6-hr simulated rainfall application and overnight gravity

drainage. The experimental results (Figure 4.6) show that, indeed, not all of the strain B6914 cells trapped during the loading and overnight drainage died. However, the number of remaining strain B6914 cells logarithmically decreased with respect to depth from the top of the column. Gagliardi and Karns (2000) reported similar experimental data. *E. coli* O157:H7 strain B6914 suspensions were spread evenly to soil surfaces, followed by simulating rainfall application. The experimental results showed that the number of strain B6914 trapped in the media after overnight drainage also logarithmically decreased with respect to depth (0-10 cm). Jiang *et al.* (2007) also found no significant difference in bacterial removal between columns with the same inner diameter and different lengths. This indicates that most introduced cells were trapped on the top of columns.

Additionally, the decrease in cell numbers with depth matches the observation that the moisture content rapidly increased with depth (Figure 4.7). Moisture content above a certain optimum level has been shown to result in a decrease in microbial cells in natural soil (Postma and Veen, 1990). In the meantime, predators, like protozoa, tend to be more active at higher soil moisture contents, as discussed in Section 3.3.4. Thus, the number of remaining strain B6914 at the bottom of columns was lower than at the top.

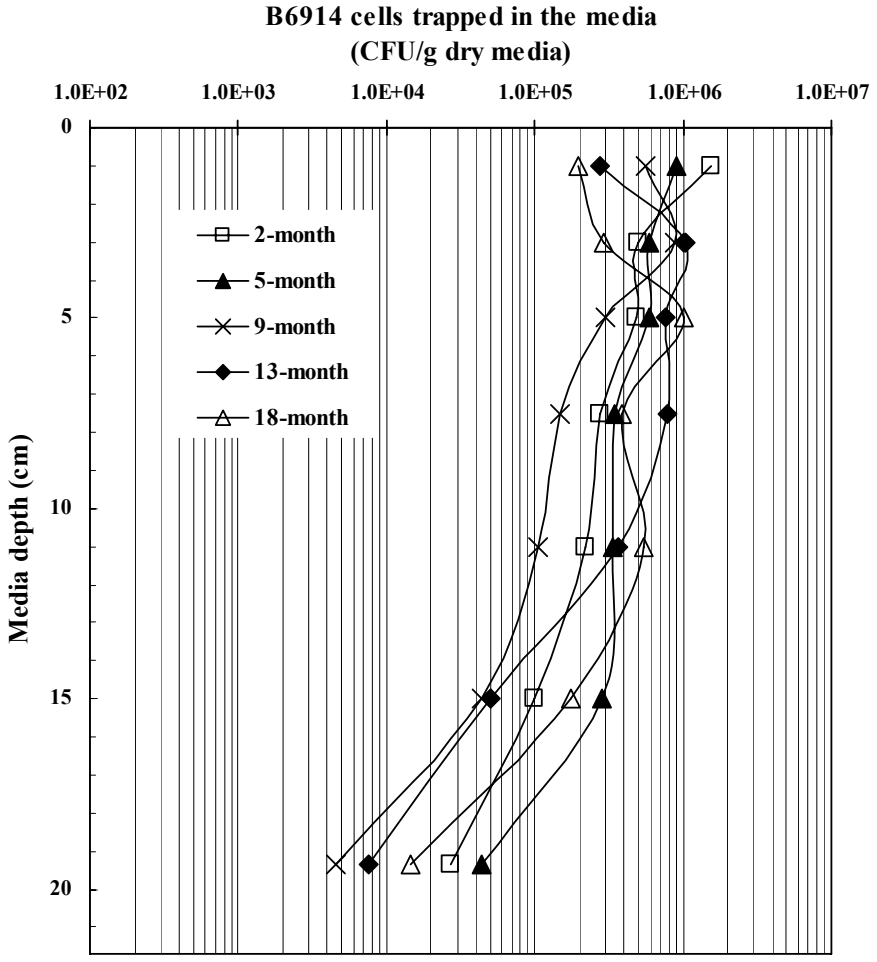


Figure 4.6 Longitudinal distribution of strain B6914 attached to the media in the 2-, 5-, 9-, 13-, and 18-month columns after gravity drainage overnight. Each symbol represents the concentration of remaining strain B6914 cells trapped in the porous media (CFU/g dry media).

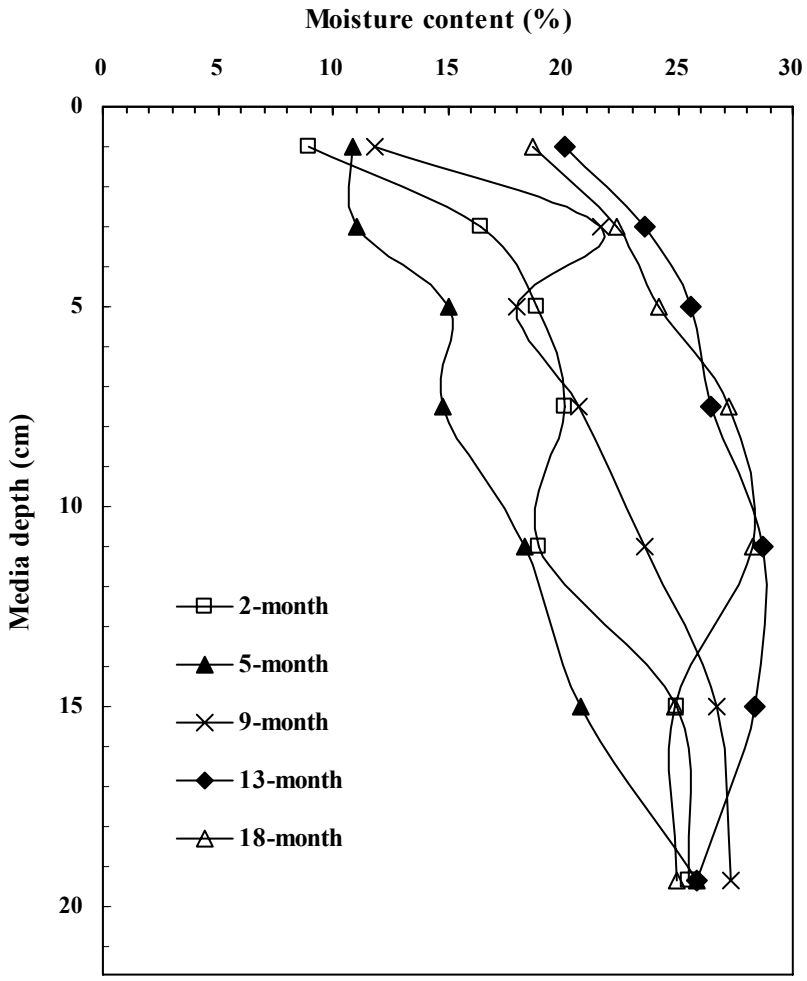


Figure 4.7 Longitudinal water contents of the media in the 2-, 5-, 9-, 13-, and 18-month columns after gravity drainage overnight. Each symbol represents the moisture content of each depth (%).

The concentrations of strain B6914 in the influent and the effluent during bacterial transport experiments (Figure 4.8) were used to calculate the number of bacteria initially trapped in the columns during the 6-hr stimulated rainfall application (N_c) as following equation 3.3. Additionally, the concentrations of strain B6914 as a function of media depth in the column (Figure 4.6), were used with the moisture contents, and soil weight to calculate the total number of remaining cells in the

column after gravity drainage overnight (N). The difference between the calculated values of N and N_c was used to estimate the reduction in the total number of B6914 cells trapped in the porous media over time. Based on these values, the number of strain B6914 cells in 2-, 5-, 9-, 13-, and 18-month columns decreased by 78.2%, 82.0%, 86.0%, 80.6%, and 80.5% after gravity drainage overnight, respectively. Removal efficiency in the columns improved over time (Figure 4.8), as described above, so more bacteria were trapped during the final 6-hr loading in the older media. Therefore, although the fractional decrease was similar in each column, the number of trapped B6914 cells that decayed or died off overnight was much greater in the older columns. Possible explanations for this phenomenon of increased decay and/or die-off of trapped cells related to microbiological and/or physiochemical properties of the media over time were investigated, as discussed below.

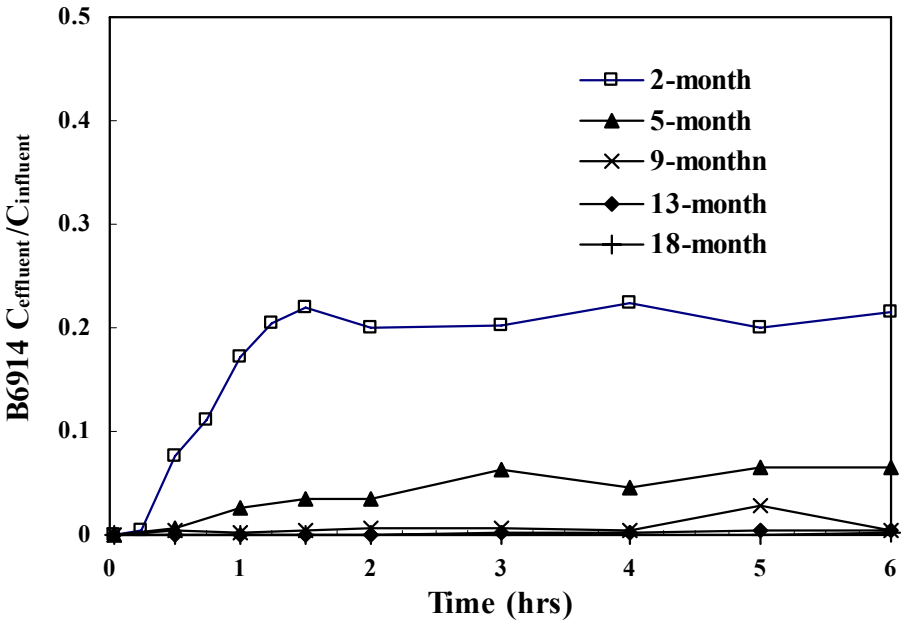


Figure 4.8 Breakthrough curves of strain B6914 in the 2-, 5-. 9-, 13-, and 18-month columns.

In addition, during this long-term experiment, the strain B6914 suspension was loaded into columns once every two weeks. Billions of strain B6914 cells were captured in the long-term CBM. For example, approximately 10^{10} strain B6914 cells were accumulated in the 18-month column, which was ten times higher than the total native heterotrophic bacteria in CBM column. The cumulative die-off fraction of long-term column can be calculated using $\frac{N_i}{\sum_{i=0}^{n} N_{c,i}}$, where N_i is the number of bacteria survived in the column after the i^{th} rainfall application and overnight drainage, $N_{c,i}$ is the number of bacteria trapped in the column during the i^{th} rainfall application; n is the total simulated runoff number). The experimental data demonstrate the cumulative bacterial die-off fraction was more than 99% at the end of the 18-month media test. Therefore, bioretention facilities have tremendous capacity for the capture and attenuation of pathogenic bacteria in the stormwater runoff.

4.3.4 Long-term changes in microbial composition in CBM

E. coli cells introduced into soil have been observed to not be well-adapted to survive (Cools *et al.*, 2001; Oliver *et al.*, 2006). Klein and Casida (1967) suggested that the major death factor for *E. coli* in natural soil was their inability to step down their metabolic rate to meet the low availability of nutrients in soil. Similarly, in water, enteric bacteria appear to be unable to adequately compete with natural microflora for low concentrations of nutrients (Sinclair and Alexander, 1984). Additionally, predation by indigenous protozoa and bacterial predators can also contribute to the decline in pathogenic bacteria introduced to soil environment

(Sørensen *et al*., 1999), which is consistent with the observations discussed in Chapter 3.

To evaluate the impact of predation by indigenous protozoa over time, the concentrations of protozoa were measured at every depth in the sacrificed 2-, 5-, 9, 13-, and 18-month columns, and almost all values were significantly higher than in fresh media (Figure 4.9). In addition, the total number of protozoa in the columns significantly increased over time (Figure 4.10). For example, total protozoa in the 13, and 18-month column were approximately ten times higher than in a column packed with fresh media. Sørensen *et al.* (1999) also found that the number of indigenous soil flagellates and ciliates increased when high numbers of *E. coli* cells were introduced to soils. Furthermore, many researchers have indicated that predation by indigenous protozoa in porous media or estuarine water is a primary factor regulating the population sizes of introduced bacteria (Enzinger and Cooper, 1976; Sørensen *et al.,* 1999; Stevik *et al.*, 2004). Similarly, our observations reported in Chapter 3 indicated that die-off of trapped strain B6914 was more rapid with increasing concentrations of protozoa attached to media. Therefore, these data indicate that the temporal increase in the number of protozoa may have contributed, via predation, to the higher bacterial removal and increased die-off of trapped bacteria that was observed over the long-term study, as described in Sections 4.3.1 and 4.3.3.

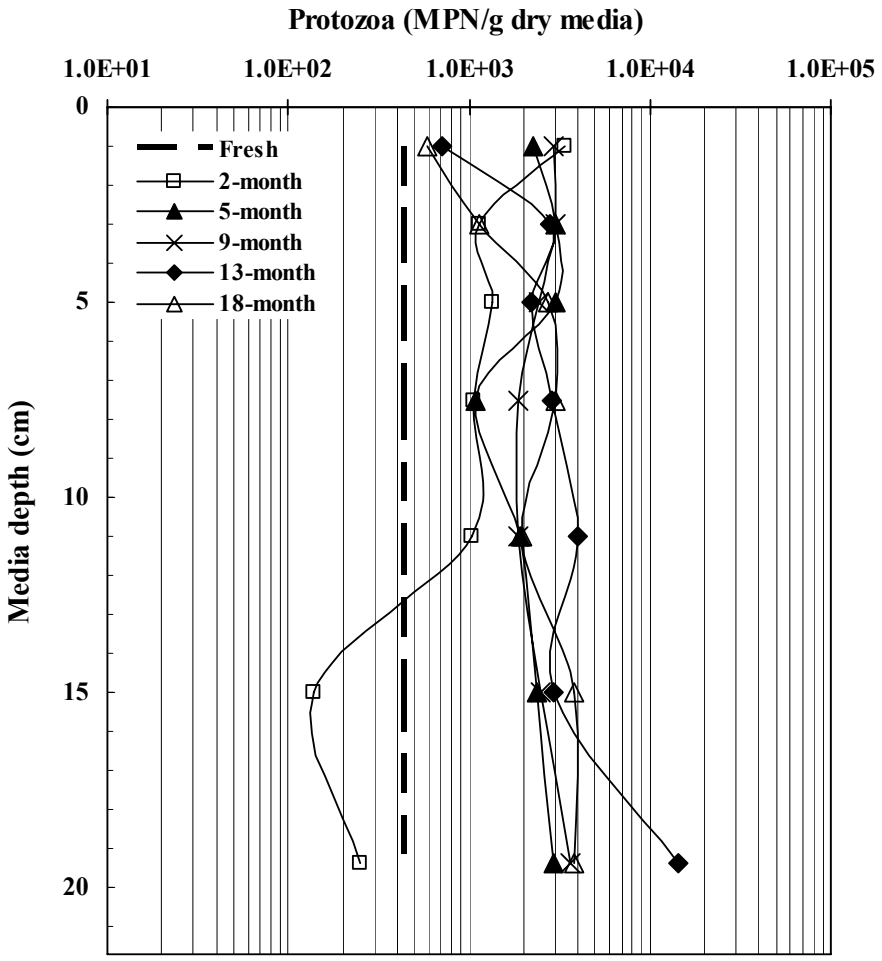


Figure 4.9 Longitudinal distribution of protozoa in the fresh, 2-, 5-, 9-, 13-, and 18-month media.

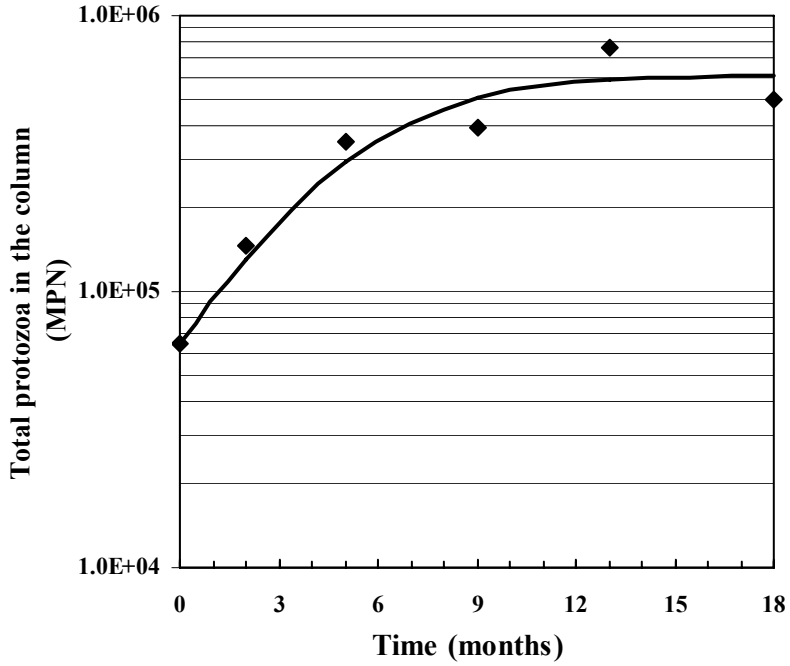


Figure 4.10 Total protozoa attached to the fresh, 2-, 5-, 9-, 13-, and 18-month media in the columns. Line is given by fit of Verhulst equation.

However, the growth rate of protozoa was not a constant. The data were much better fit by the logistic equation of Verhulst (1838).

$$\frac{dN}{dt} = rN(1 - \frac{N}{K}) \tag{4.1}$$

where: N is the number of microorganisms at time t; r is the growth rate constant; and K is the maximum number of microorganism that the given environment can support, i.e., the upper limit of population growth. This logistic equation can be converted to an algebraic form:

$$N = \frac{KM}{M + (K - M) \cdot e^{-rt}} \tag{4.2}$$

where: M is the number of microorganisms at t=0. In this long-term study, protozoa attached to CBM in the columns initially grew exponentially, and then slowed down

to reach a constant level (Figure 4.10), known as the carrying capacity, due to limited environmental conditions (such as food sources). Therefore, the initial first-order growth rate is selected as the value of r. Then, by minimization of the sum of quadratic differences between observed and model-predicted values, the values of best-fit K, M, r are 6.1×10^5 MPN, 6.5×10^4 MPN, and 0.41 $month^{-1}$, respectively. As described above, the number of indigenous soil protozoa increased when strain B6914 cells were introduced to columns every two weeks. However, the food source may become a limiting nutrient with increasing numbers of protozoa. Thus, the total number of protozoa kept growing up to the 13-month bacterial loading, afterwhich it reached a plateau.

In addition to predation by indigenous protozoa, competition with native bacteria for limited nutrients, or predation by other bacterial predators can also reduce the number of introduced pathogenic bacteria. Numbers of bacterial predators were not monitored in this study. However, Enzinger and Cooper (1976) studied the relative role of protozoan and bacterial predators on the survival of *E. coli* in estuarine water samples by adding different antibiotics. Their investigation indicated that predation mainly results from protozoa, whereas bacterial predators were not important for coliform destruction. Therefore, the predation by indigenous bacterial predator may have little effect on the rapid decline of trapped B6914 cells that was observed in the long-term experiment.

Competition for limited nutrients, however, is likely. For example, the trapped strain B6914 cells might compete with native heterotrophic and chemoorganotrophic bacteria for organic substrate. Therefore, heterotrophic bacteria were monitored

using total heterotrophic plate counts. The heterotrophic plate count data show that the number of heterotrophic bacteria associated with the porous media did not change significantly compared to the fresh CBM, either longitudinally along the column (Figure 4.11), or over the course of the study (Figures 4.12). Nevertheless, like the *E. coli* strain B6914 cells introduced to the media once every two weeks, the indigenous heterotrophic bacteria may also be a food source for protozoan predators. So, it is interesting that the total heterotrophic bacterial numbers stayed relatively spatially and temporally constant. One possible explanation is that the heterotrophic bacteria that survived are those which are sorbed to soil particles and within small pore spaces and are, therefore, protected from the larger protozoa (Newby *et al.*, 2000). This hypothesis is supported by the observation that when the columns were flushed prior to initiating each bacterial transport experiment to reach saturation of the pore volume, the concentration of heterotrophic bacteria in the wash effluent exponentially decreased over time (Figure 4.13). These data indicate that though the total heterotrophic bacteria associated with the porous media probably did not decrease over time, presumably because they were protected by their location, the free heterotrophic bacteria did decrease, probably due to the predation of indigenous protozoa. Thus, the attached heterotrophic bacteria stay relatively constant and could be competing with strain B6914.

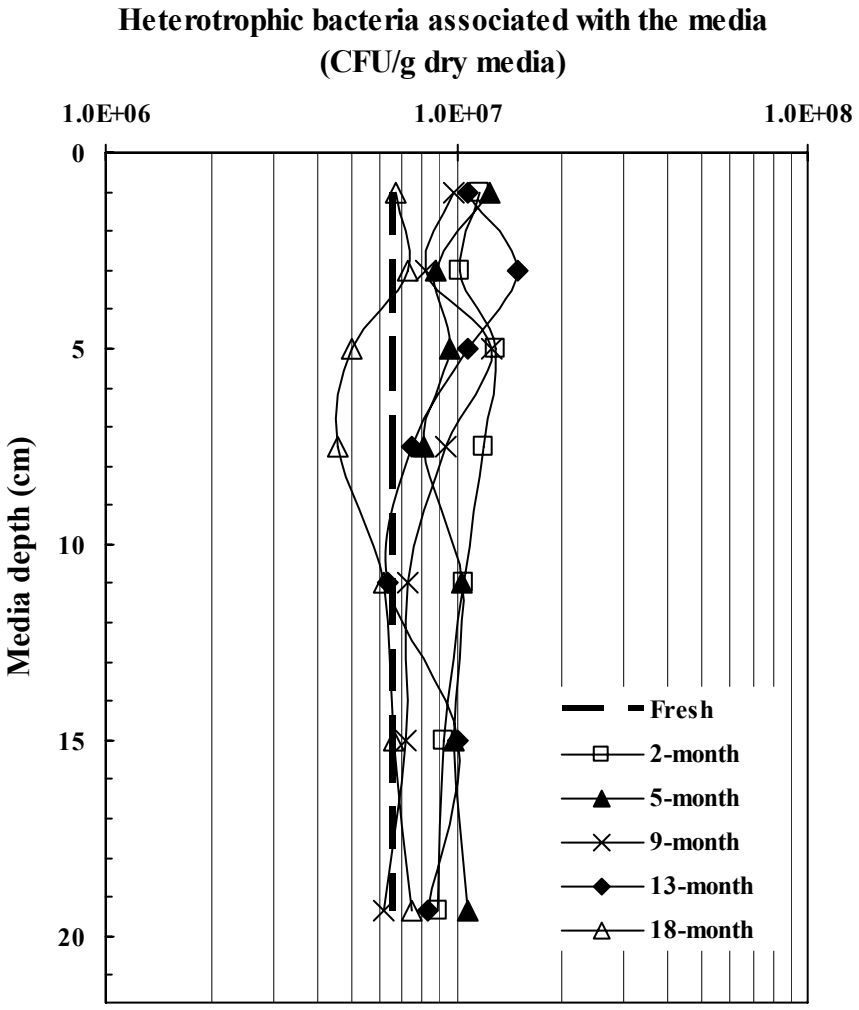


Figure 4.11 Longitudinal distributions of heterotrophic bacteria in the fresh, 2-, 5-, 9-, 13-, and 18-month media.

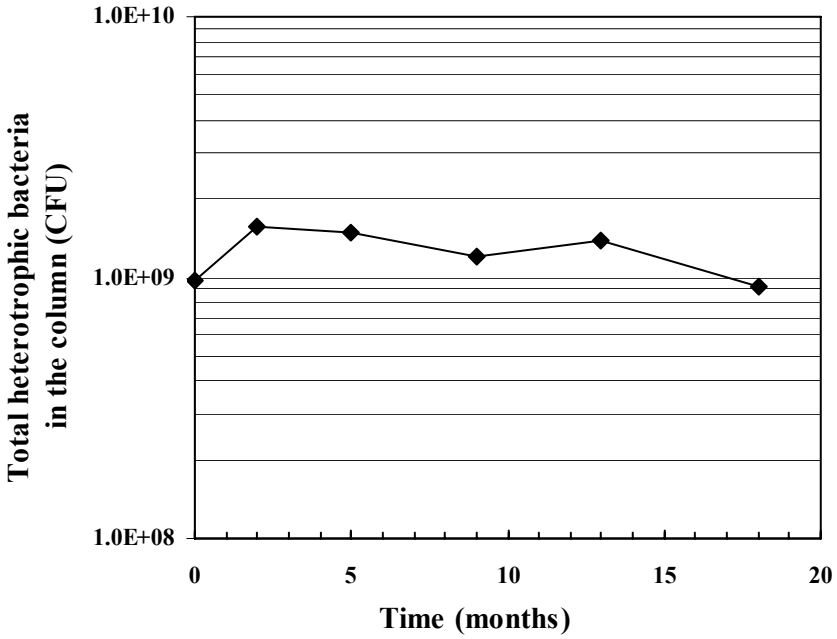


Figure 4.12 Total heterotrophic bacteria attached to fresh, 2-, 5-, 9-, 13-, and 18-month media in the columns.

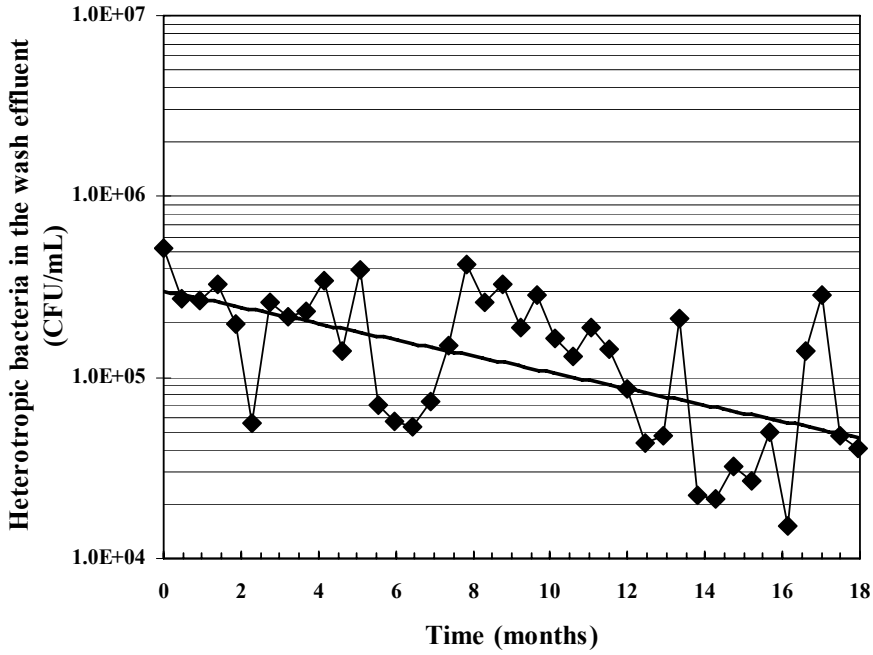


Figure 4.13 Heterotrophic bacteria in the wash effluent collected before the transport experiments once every two weeks. Line is the semi-logarithmic trendline.

4.3.5 Change in physiochemical characteristics in long-term CBM

Surface characteristics (hydrophobicity and electrostatic charge) of bacteria and porous media are important factors that influence the initial adhesion of bacterial cells to porous media (Stevik *et al.*, 2004). Strain B6914 is a relatively hydrophilic organism, as discussed in Chapter 3. As a result, it was expected that it would be more difficult for B6914 cells to partition from the aqueous phase and accumulate on the surface of media compared to hydrophobic cells. Thus, electrostatic interactions the between bacteria and particle grains were expected to potentially be a dominant factor in adhesion of strain B6914 to the bioretention media.

As reported in Chapter 3, *E. coli* O157:H7 strain B6914 used in this study displayed a week negative charge (-2.6 ± 0.1 mV) in synthetic runoff. The fresh media had a small negative charge at pH 7, the pH of CBM (Figure 4.14). However, over the course of the experiment, the overall surface charge of the media in the five columns became slightly more negative compared to the fresh media. This trend is unfavorable for initial bacterial adhension via electrostatic interaction because of the negative charge on the surface of the bacteria, resulting in repulsion. This is a common situation, as both the porous media and microbial cells tend to be negatively charged (Newby *et al.*, 2000). Thus, the change in the media surface charge may not be a key factor controlling the improved bacterial removal in the long-term experiments.

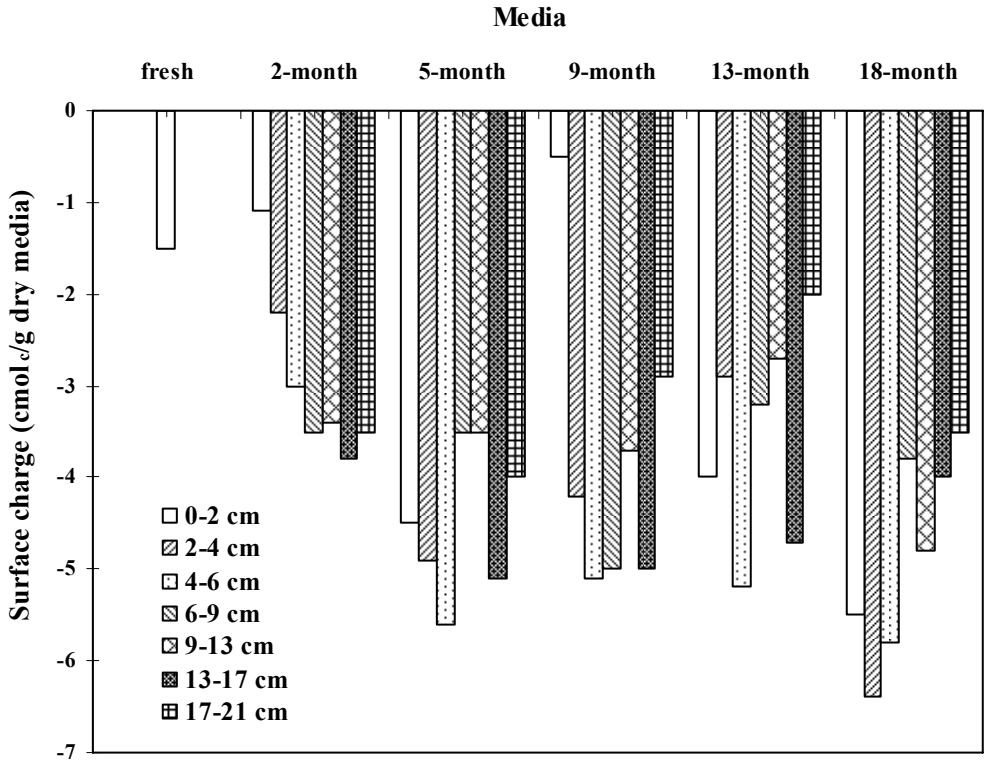


Figure 4.14 Longitudinal distribution of media surface charge (at pH = 7) in the long-term columns.

However, it may be indicative of increased biofilm formation in the media. As described in Chapter 2, *E. coli* is a gram-negative bacterium containing an outer membrane composed by phospholipids and lipopolysaccharides (LPS). The presence of extracellular polymer substance (polysaccharides) can induce bacterial cell-to-cell contact, thus generating biofilm in the media by hydrogen bonding and polymer bridging. Therefore, with the periodical application of the synthetic runoff to the long-term columns, more and more strain B6914 cells were trapped in CBM. Some of them may serve as a food source for predators, and some of them and/or native bacteria may generate biofilm on the media surface. The formation of biofilm may

contribute to the enhancement of bacterial adhesion via increasing polymeric interactions between the biofilm and strain B6914 cells surface, increasing media surface roughness, reducing hydraulic conductivity and increasing the dispersion (Vandevivere and Baveye, 1992; Liu and Li; 2008). Thus, biofilm formation may be an important reason for the improving bacterial removal in aged CBM that was observed over the long-term study. However, due to the high content of organic matter of mulch (>30%), it is a challenge to determine the change in the content of biofilm attached to the CBM during the long-term study.

4.4 Summary and conclusions

This study investigated the long-term performance of bacterial removal from synthetic urban stormwater runoff in CBM columns using 18-month long-term tests. The experimental data exhibited a high removal efficiency for strain B6914 by CBM in the columns, initially ranging from 52% to 90%. Furthermore, the bacterial removal efficiency increased over time with removal efficiency of greater than 97% after 6 months. Additionally, large numbers of the trapped B6914 cells died off very quickly between runoff application events, which is important for the process to be sustainable. Specifically, more than 80% of trapped B6914 cells consistently died off after overnight gravity drainage, when the columns were sacrificed after 2-, 5-, 9-, 13-, and 18-months of simulated stormwater loading. Although the percentage decrease in the number of strain B6914 cells was similar over time, the absolute number of cells that decayed or died overnight increased due to the improved capture of strain B6914 over time.

Several mechanisms for explaining the improved performance for bacterial capture and destruction in the long-term experiments were investigated. Porosity was observed to decrease, and hydrodynamic dispersion increased over time, of which both trends are favorable for improving the physical straining of cells and bacterial adhesion, thus enhancing bacterial removal. Predation, mainly from protozoa, and low available nutrients were other important factors potentially contributing to die-off of trapped strain B6914 cells in CBM that were investigated. The spatial and temporal decline in the numbers of introduced strain B6914 coincided with an increase in the number of protozoa. These data indicate that protozoan predators likely play a major role with respect to survival of the introduced *E. coli*. On the other hand, competition with indigenous heterotrophic bacteria apparently had little effect on bacterial destruction as the number of attached heterotrophic bacteria stayed relatively constant with time. These attached bacteria were presumably protected from the protozoan predators. However, the heterotrophic bacteria in solution in the column effluent did decrease with time and may contribute to the sustainability of bioretention system by serving as food sources for protozoa. Finally, the charge on CBM was observed to become more negative over time, which is unfavorable for capture of negatively charged strain B6914 via electrostatic interactions. This may, however, be indicative of increased biofilm formation in the media over time, which may contribute to improved bacterial removal.

Chapter 5: Effect of temperature on transport and destruction of indicator bacteria in conventional bioretention media: Field and laboratory evaluations

5.1 Introduction

Stormwater runoff contributes a large fraction of the total microbial loading to receiving waters and, therefore, has the potential to adversely impact contact recreation areas and drinking water sources (Stoner and Dorfman, 2006). Microbial pollutants in urban stormwater runoff mainly originate from the fecal matter of pets (e.g., dogs, cats), wild birds and animals (e.g., geese, pigeons, ducks, deer, etc.), as well as humans (Mallin *et al.*, 2000). Environmental conditions, such as the season and associated weather condition (e.g., temperature), significantly influence the density and activity of domestic and wild animals and the associated fecal loading to stormwater runoff (Frenzel and Couvillion, 2002).

Various environmental factors also affect the survival of microbial pollutants once they are introduced into the environment. For example, temperature plays an important role in controlling microorganism growth, die-off and inactivation, and has often been cited as the most important environmental factor impacting the survival of microbial pollutants (USEPA, 2006). In general, a lower temperature is more favorable for the survival of bacteria, possibly due to a low decay rate and slowed predation by indigenous protozoa and bacterial predators (Wang *et al.*, 1996; Sjogren, 1994; Sørensen *et al.*, 1999; Cools *et al.* 2001; Jiang *et al.*, 2002). Therefore, temperature has the potential to impact the loadings of pathogenic bacteria into urban stormwater runoff, as well as the survival and destruction of the introduced bacteria in

runoff or the soil and water environment. Importantly, these impacts are expected to trend in opposite directions: higher temperature should increase the loading of microbial pollutants, and lower temperature should increase the survival of microorganisms loaded into the soil.

In Chapter 3, laboratory column studies at room temperature (25 ± 3ºC) were described, which demonstrated that fresh conventional bioretention media (CBM) achieved a mean removal efficiency of 70% for *E. coli* O157:H7 strain B6914 from simulated urban stormwater runoff under the tested conditions. In addition, more than 99.98% of the trapped strain B6914 cells in the media died off within one week. However, in field application we are interested in a wide range of climate temperatures. For example, it is reported that Maryland annual mean temperature, winter seasonal mean (December, January, and February), and summer seasonal mean (June, July, and August) are approximately 12 and 23ºC, respectively (Collins, 2008). However, the performance of bioretention media for bacteria removal under different temperatures is unknown. Limited published data from field studies to date have reported on the ability of bioretention to remove pathogenic bacteria during different seasons (Hunt *et al.*, 2008).

Given the lack of information available about effects of temperature on microbial removal and survival in bioretention, the goals of this study were two fold: (1) to evaluate the removal of indicator bacteria (fecal coliform bacteria and *E. coli*) from urban stormwater runoff in field bioretention facilities during different seasons of the year, and (2) to investigate the effect of temperature on the capture and survival of bacteria (*E. coli* O157:H7 strain B6914) in CBM using laboratory column studies.

Because the experimental results reported in Chapter 3 indicated that the activities of native microbes significantly influence the survival of *E. coli* O157:H7 strain B6914 in CBM, it was expected that temperature would have a major impact on the proliferation and die-off of native and exogenous microbes. Therefore, a major component of the laboratory studies reported here was to investigate the effects of native microbial predators and competitors (e.g., protozoa and heterotrophic bacteria) in CBM on the survival of trapped strain B6914 cells at different temperatures. Additionally, the levels of indigenous protozoa and heterotrophic bacteria in the field media were investigated to provide information on microbial community composition in bioretention facilities.

5.2 Materials and methods

5.2.1 Field tests

5.2.1.1 Site description

In these studies, bacterial removal from stormwater runoff during storm events was investigated in two existing bioretention facilities: one near a parking lot on the campus of the University of Maryland, College Park (called cell CP) and another one in Silver Spring, MD (called cell SS). Photographs of the two sites are presented in Figures 5.1 and 5.2. Cell CP was constructed in the spring of 2004, and serves a drainage area of approximately 0.28 ha at a cell surface area to drainage area ratio of 6%. The drainage area is highly-used land consisting of 90% imperious surfaces, such as asphalt parking lots, roads and concrete sidewalks for commuter students and athletic event visitors. Cell SS was completed in March 2006 and serves as a runoff

drainage retrofit of a health services facility complex. It has a drainage area of 0.45 ha at a cell surface area to drainage area ratio of 2%. However, the actual ratio is probably larger, based on field observations during rainfall events, which indicates that runoff flows are often directed to peripheral lawns and, thus, decrease the cell inflow (Li, 2007). The drainage area consists of 90% impervious surfaces, such as asphalt parking lots and driveways for inpatients, working commuters, and visitors.

Figure 5.1 Cell CP at the University of Maryland, College park campus (March, 2007)

Figure 5.2 Cell SS at Silver Spring, MD (August, 2006)

Characteristics of the media collected from the two bioretention cells were determined by the University of Delaware Soil Testing Program, and are presented in Table 5.1. Based on the U.S. Department of Agriculture textural classification system, the texture of a media in cells CP and SS is a sandy loam and a sandy clay loam, respectively.

Table 5.1 Media characteristics of the bioretention cell CP and cell SS (Li, 2007).

Media	Soil texture			pH	Organic matter (%)
	sand (%)	silt (%)	clay (%)		
Cell CP	80	13	7	7.3	5.7
Cell SS	54	26	20	7.7	12.2

5.2.1.2 Stormwater sampling and analysis

A total of 13 stormwater events were monitored at the two bioretention facilities between April, 2006 and July, 2007. Flow-weighted composite sampling was employed using two ISCO Model 6712FR auto-samplers (Teledyne IOCS, Inc., Lincoln, NE, U.S.A.) for the input and output flows from the facilities during storm events. Every 2 min, the auto-samplers recorded water level H (ft), and converted the value to the runoff flow rate Q (L/s) using the discharge equations (Table 5.2), which were programmed into auto-samplers before each storm event. The accumulated volume of flow V (L) was then calculated according to a simple numerical integration of flow measurements over time:

$$V = \sum_{i=0} Q(t)_i \cdot \Delta t_i \tag{5.1}$$

Each auto-sampler contained two glass sampling containers (20 L), which were autoclaved before each rainfall event. During the storm event, the auto-samplers, which were set at a temperature of 4ºC, collected stormwater sample in the glass container based on a selected ratio of the sample volume to accumulated stormwater flow volume (e.g., 5 mL : 5 L). The samples were delivered to the lab and analyzed for the event mean concentration (EMC) of fecal coliform bacteria and *E. coli* within 24 hr after sampling. Fecal coliform and *E. coli* were selected for analysis because they are two of the most common indicator organisms used worldwide. Both concentration-based and total organisms-based removals were determined by coupling organism event mean concentration with flow data.

Table 5.2 Discharge equations and weir resolutions for flow measurement devices.

		Device	Discharge equation	Weir resolution	Reference
Cell CP	Input	20-cm Cutthroat flume	$Q = 80.4\ H^{1.83}$	H = 0.06-1.00	Merkley, 2004
	Output	20-cm Thel-Mar weir	$Q = 129\ H^{2.63}$	H = 0.026-0.431	Manual from manufacturer
Cell SS	Input	23-cm Parshall flume	$Q = 86.9\ H^{1.53}$	H = 0.06-2.00	Grant and Dawson, 2001; Blaisdell, 1994
	output	15-cm Thel-Mar weir	$Q = 101\ H^{2.57}$	H = 0.022-0.293	Manual from manufacturer

The enumeration of fecal coliforms (FCs) was accomplished by using the membrane filter procedure, following Standard Method 9222D (APHA *et al.,* 2005). Due to the seasonal variability in FC concentration for the influent and effluent samples from the selected bioretention facilities, a series of dilutions were used. Specifically, based on experience, a range of 0.1 to 100 mL samples were diluted in sterile deionized water to a final volume of 100 mL. Then, each 100 mL sample was aseptically filtered through a 47 mm diameter, 0.45 μm pore size filter of (Cat. No. HAWG047S6, Millipore Corporation, MA, U.S.A.). Subsequently, the filter was transferred using sterile forceps onto a M-FC (EDM, Cat. No. 1.11278.0500) with 1% rosolic acid agar plate and incubated at 44.5 ± 0.2 ºC for 24 ± 2 h. After incubation the colonies with blue color on the M-FC agar plates were counted as fecal coliforms. Duplicate analyses were conducted at each dilution sample. The mean concentration was calculated by arithmetic average.

Subsequently, *E. coli* (EC) were analyzed by following Standard Method 9222 G (APHA *et al.*, 2005). This required transferring the membrane from a positive fecal coliform sample to a plate with an EC medium with 4-methylumbelliferyl-B-D-glucuronide (MUG) (EMD, Cat. No. A.05101.0500). The plates were then incubated

at 44.5 ± 0.2ºC for 24 ± 2 h, after which the colonies with blue fluorescent periphery were counted under a long-wavelength (366 nm) UV light.

5.2.1.3 Soil sampling and analysis

The daily temperatures during the storm events on January 13, 2008; April 1, 2008; June 1, 2008; and June 10, 2008 were 3 ± 3ºC, 16 ± 5ºC , 25 ± 5ºC, and 31 ± 4ºC, respectively. After each rainfall and over night drainage, soil cores were collected at a point (5m away from the entrance) in the bioretention cell CP (Figure 3.3). After the surface plants in the sampling area were removed, soil samples at different depths (0-5, 5-10, 10-20, 20-30 cm) were collected using a core sampler. The soil samples were delivered to the lab and analyzed for the concentrations of protozoa and heterotrophic bacteria within 8 hrs after sampling. Protozoa and heterotrophic bacteria in soil samples were extracted and enumerated using the methods as described in Sections 3.2.5 and 3.2.7.1.

Figure 3.3 Soil sampling from the bioretention cell CP.

5.2.2 Laboratory column tests of temperature impacts on bacterial capture and destruction

5.2.2.1 Test microorganism

A derivative of *E. coli* O157:H7 strain B6914, described in Section 3.2.1.1, was used as the test microorganism in the laboratory tests. Strain B6914 cells were cultivated and harvested following the procedures described in Section 3.2.1.2, and then diluted to a nominal concentration of 1×10^6 CFU/mL in synthetic stormwater runoff (see Table 3.2). This was used as the influent solution in the column transport experiments described below.

5.2.2.2 Bacterial transport experiments

CBM were used in this study, consisting of a mixture of sandy loam soil, U.S. Silica® Mystic White II pool filter sand, and mulch (leaf compost), as described in Section 3.2.2. The media were dry-packed into glass chromatography columns (Cat. No. 420401-2520, Kontes, Vineland, NJ), by following the procedure outlined in Section 3.2.4.1. The column experimental set up was the same as shown schematically in Figure 3.1. To evaluate the effect of temperature on the transport and capture of strain B6914 in CBM, sets of four columns each were placed in an incubator at temperatures of 5, 15, 25, and 37ºC (± 1ºC), respectively. Then, the columns were loaded with a suspension of B6914 in synthetic runoff for 6 hrs at the different temperatures. The loading procedure and subsequent bacterial enumeration were conducted following the same procedures, as described in Section 3.2.4.3.

Additionally, the bromide tracer test was also conducted by following the procedure described in Section 3.2.4.2. The resulting bromide breakthrough curve was fit to the one-dimensional non-reactive solute transport equation to obtain the best-fit parameters (porosity, pore water velocity and hydrodynamic dispersion coefficient), following the method as described in Section 3.2.4.2. Finally, these best-fit parameters were coupled with the bacterial breakthrough data to calculate the parameters (retardation factor R, sticking efficiency α, single collector collision efficiency η_0, and adsorption rate coefficient k_a) using the equations presented in Section 2.4.

5.2.2.3 Bacterial survival experiments

After the 6 hr simulated rainfall application, the columns remained in the incubator at the prescribed temperature and were allowed to gravity drain for variable time periods. Specifically, columns at 5°C were sacrificed after 0.5-, 7-, 14-, and 21-day gravity drainage, columns at 15°C were sacrificed after 0.5-, 3.5-, 7-, and 14-day gravity drainage, and columns at 25°C and 37°C were sacrificed after 0.5-, 1.5-, 2.5-, and 7-day gravity drainage. At the end of each selected drainage period, a column was sacrificed, and strain B6914, heterotrophic bacteria, and protozoa attached to media from seven different depths in the column were enumerated following the methods described in Section 3.2.7.1.

5.2.3 Statistical analyses

Data were analyzed using SAS version 8.01 (SAS Institute, Cary, NC, USA). Temperature effects on bacterial removal efficiency and hydraulic parameters (such as porosity and dispersion coefficient) were evaluated using an ANOVA followed by a Fisher's least significant difference (LSD) test for mean separation. A significance level of $P < 0.05$ was selected for the determination of statistical difference.

5.3 Results and discussion

5.3.1 Field studies

The levels of the two kinds of indicator bacteria (fecal coliform bacteria and *E. coli*) were monitored in the urban stormwater runoff input and the output of two bioretention cells (CP and SS) for thirteen storm events between April 6, 2006 and July 10, 2007. The data collected during these storm events are summarized in Table 5.3.

Several key trends can be observed in these data. For example, as illustrated in Figure 5.4, the data show that the two indicator bacteria levels in the input were highly variable, with a range of 0 (< detection limit) to 24000/100 mL. Of particular interest to this study is the finding that the input bacterial concentrations generally increased with increasing daily average ambient temperature at both bioretention cells CP and SS, especially when the temperature was higher than 20ºC. One possible explanation for this is that high temperature may have increased the density and activities of domestic and wild animals in the impervious area, thereby increasing loading of pathogenic bacteria to stormwater runoff.

Table 5.3 Results of monitoring fecal coliform and *E. coli* in input and output runoff for 13 storm events at bioretention facilities CP and SS (Data of the first two storm events from Li (2007)).

Storm Event date	Cell	Avg. Temp. (ºC)	Input volume (L)	Output volume (L)	Fecal coliform				*Escherichia coli*			
					In (CFU/100mL)	Out	EMC removal (%)	Mass removal (%)	In (CFU/100mL)	Out	EMC removal (%)	Mass removal (%)
04/03/06	CP	16	10358	7097	110	4	91	93	43	4	91	93
	SS		12354	1431	2	2	0	88	2	1	50	94
06/24/06	CP	26	2900	9589	900	1600	-78	-488	307.6	1145	-272	-1131
	SS		22862	23240	>1600	1600	>0	---*	> 2420	5475	> -126	---
10/05/06	CP	18	18310	12827	140	52	63	74	28	46	-64	-15
	SS		78250	34951	180	440	-144	-8.8	40	320	-700	-257
12/22/06	CP	11	20794	2714	170	70	59	95	44	22	50	93
	SS		11432	3428	13	70	-438	-61	5.5	50	-809	-173
02/25/07	CP	1	123626	22875	80	4	95	99	139	1	99	100
	SS		35397	12231	<2	<2	---	---	< 1	< 1	---	---
04/04/07	CP	10	18317	11389	12	219	-1725	-1035	4	120	-2900	-1765
	SS		8885	0	57	----	---	100	26	---	---	100
04/11/07	CP	7	13076	25650	91	91	0	-96	73	80	-9.6	-115
	SS		19933	0	7	----	---	100	6	----	---	100
05/12/07	CP	21	10387	3915	6500	605	91	96	6500	330	95	98
	SS		17678	0	10000	---	---	100	4500	---	---	100
05/16/07	CP	22	664	0	4000	---	---	100	2100	---	---	100
	SS		0	0	---	---	---	---	---	---	---	---
06/13/07	CP	22	2144	0	>16000	---	---	100	2046	---	---	100
	SS		65583	34561	>16000	9000	>44	>70	>24196	12997	>46	>72
06/26/07	CP	28	9114	4961	24000	14300	40	68	15000	12000	20	56
	SS		1648	0	15400	---	---	100	1500	---	---	100
07/05/07	CP	25	1190	0	2400	---	---	100	691	---	---	100
	SS		1540	0	>16000	---	---	100	228	---	---	100
07/10/07	CP	30	13329	8121	1100	7150	-550	-296	1100	6700	-509	-271
	SS		2167	0	4850	---	100	100	7500	---	---	100

*no data obtained

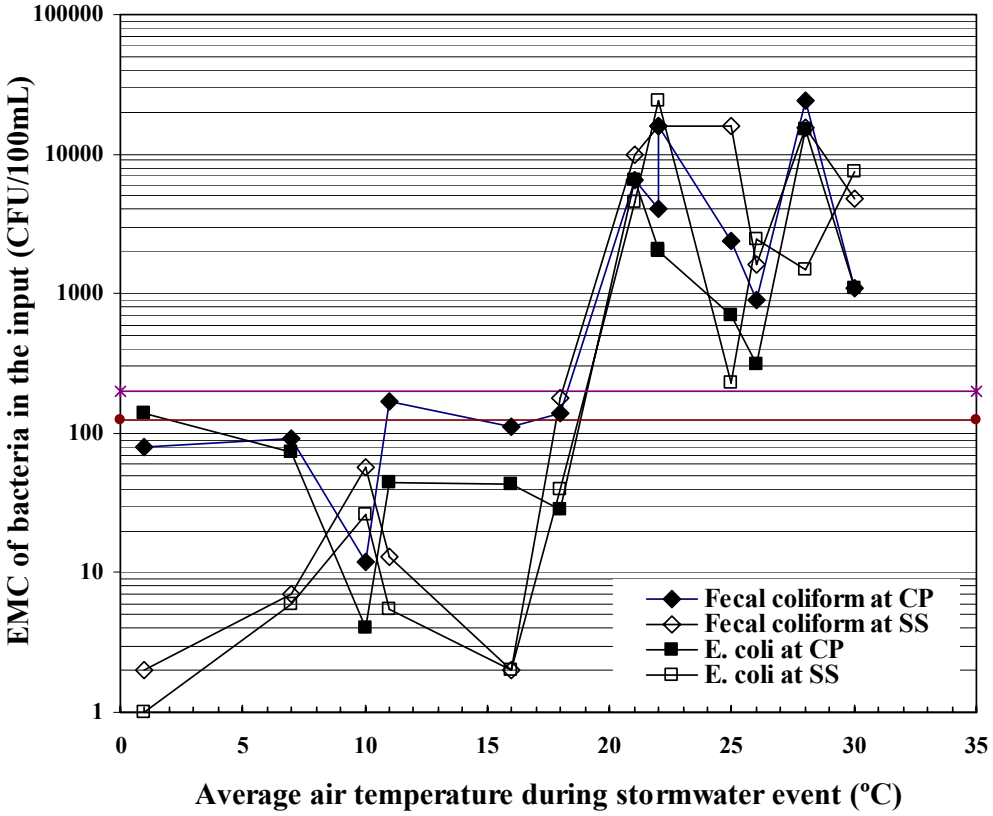


Figure 5.4 The event mean concentration (EMC) of indicator bacteria (fecal coliform and *E. coli*) in the input at bioretention cells CP and SS in thirteen storm events.

Generally, the two bioretention cells reduced the runoff volume through infiltration during the 13 storm events (Table 5.3). However, the output volume was higher than input volume in two storm events at cell CP. This was because the creek near cell CP elevated the groundwater level during intense or long duration events, thus compounding the output volume (Li, 2007). The average reduction of runoff volume in cell CP was 30% (standard deviation is 94%). At cell SS, the output volume was slightly higher than the input volume in only one storm event. The average reduction of runoff volume in cell SS was 75% (standard deviation is 33%).

The volume reduction resulted in the total organism-based removals (mass removals) generally being higher than the concentration-based removals (EMC removals) at both facilities, which promotes water quality improvements. Specifically, in some storm events when the precipitation volume was low, or there was a long dry period since the previous storm event, no measurable flow output was detected. This indicates that the entire runoff volume was stored through infiltration and attenuation by the bioretention media. Therefore, the discharge of bacterial pollutants was zero during these storm events, and the bioretention cells achieved 100% removal efficiencies. Of course in those cases, it was not possible to calculate an EMC removal efficiency.

Similar to the relationship of input bacterial concentration and ambient temperature, the concentrations of indicator bacteria in the output of the bioretention cells generally increased with increasing daily average ambient temperature in the storm events with measurable out flows (Figure 5.5). The concentrations of bacteria in the output were generally lower than those in the output. However, there is no clear trend in EMC removal or mass removal for the two indicator bacteria at different temperatures. In most storm events, the indicator bacterial EMCs in the output were lower than those in the input (Table 5.3), with fecal coliform EMC removal ranging from 40 to 95%, and *E. coli* EMC removal ranging from 20 to 99%, in storm events with measurable outflows and positive removals. Hunt *et al.* (2008) monitored 14 storm events that occurred from Feb. 2004 to March 2006 in the Hal Marshal bioretention cell in urban Charlotte, NC. They reported 69% and 71% mean concentration-based removals for fecal coliform and *E. coli*, which is consistent with

our data with positive bacterial removal. However, during some storm events, the concentrations of fecal coliform and *E. coli* in the output were higher than those in the input, which may be the result of a release of fecal coliform bacteria and *E. coli* from the bioretention media.

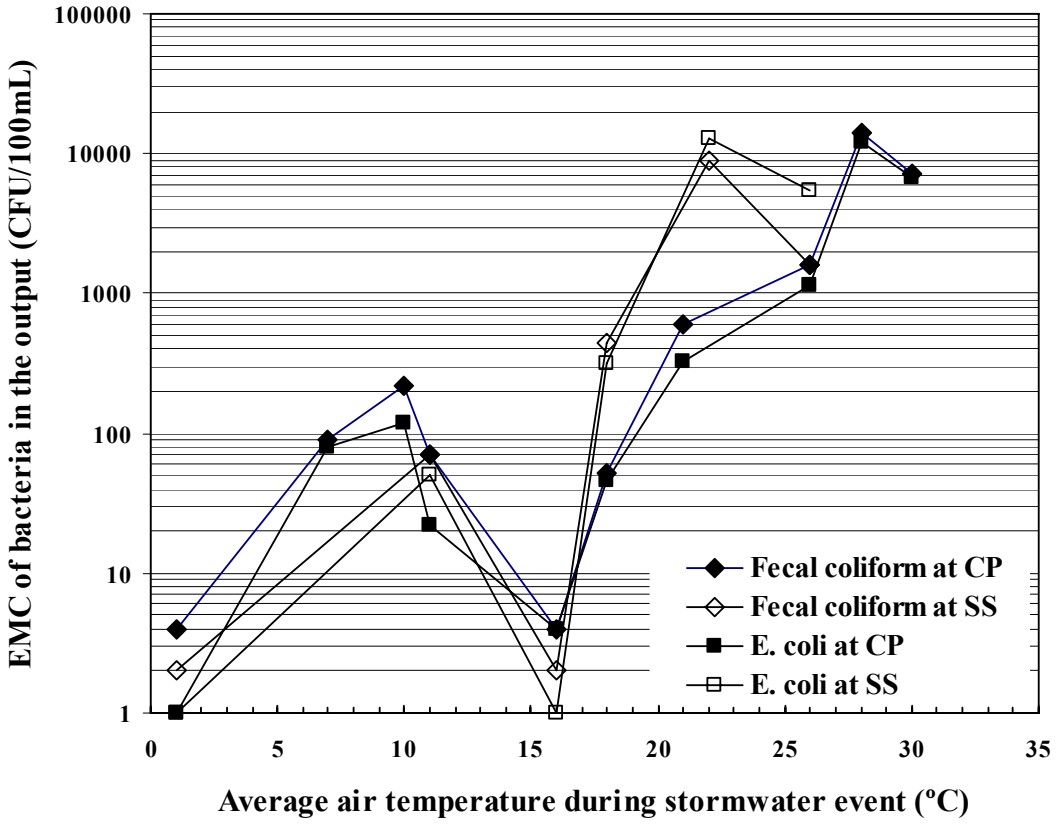


Figure 5.5 The EMC of indicator bacteria (fecal coliform and *E. coli*) in the output at bioretention cells CP and SS in storm events with measurable outflows.

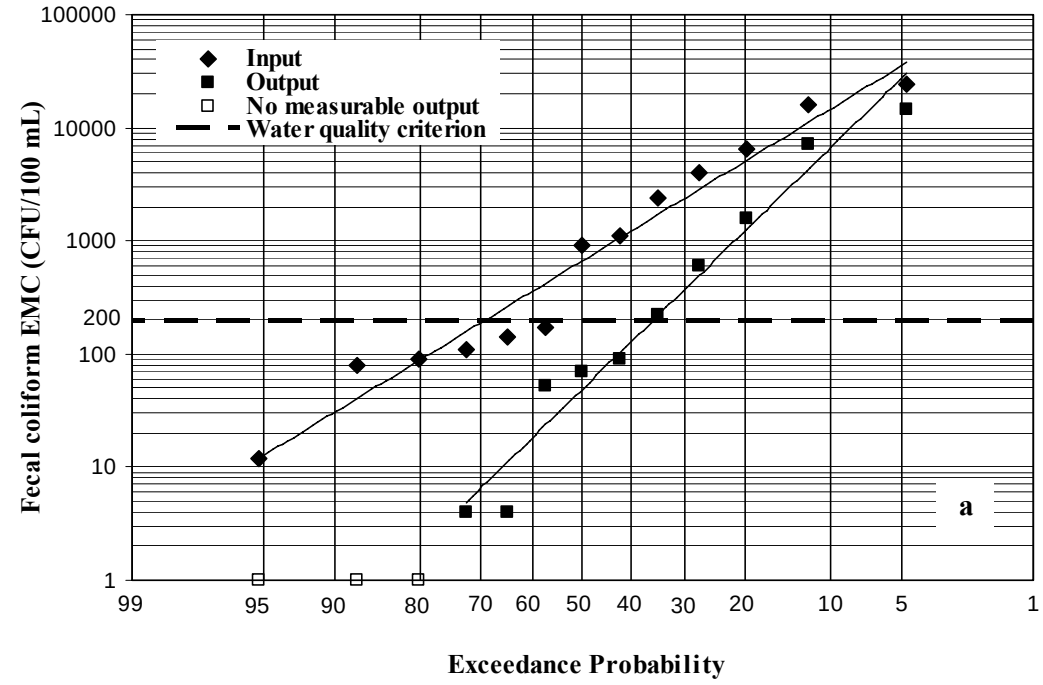
To statistically evaluate these field data, probability plots for the concentrations of the two indicator bacteria in the input and output of cells CP and SS were created by ranking the measured values as done by Davis (2007). The plotting position for each value on the probability scale p was calculated from:

$$p = \frac{i - \alpha}{(n - i + 2\alpha)} \tag{5.2}$$

where i is the i th smallest number among a sample size n, and α represents a constant that describes the plotting position function. Based on the studies of Davis (2007) and Li (2007), a value of α = 3/8 was generally employed to describe the pollutants in bioretention facilities. The input and output data for fecal coliforms and *E. coli* at bioretention cells CP and SS are plotted on a log scale as a function of p in Figures 5.6 and 5.7, respectively. In both cases the data are well described by a straight line (with some deviations at the extremes), implying that the data have a log-normal distribution.

For primary contact water, the U.S. EPA maximum geometric mean criterion is 200 fecal coliforms per 100 mL, and for designated freshwater beaches, the U.S. EPA steady-state geometric mean criterion is 126 *E. coli* per 100 mL (USEPA, 1986). Examining Figures 5.6(a) and 5.7(a), for fecal coliforms, approximately 70% (cell CP) and 50% (cell SS) of the input EMCs are higher than the criterion level, while about 65% (cell CP) and 82% (cell SS) of the output EMCs are expected to meet the water quality criterion.

Similarly examining Figures 5.6(b) and 5.7(b), for *E. coli*, approximately 70% (cell CP) and 45% (cell SS) of the input EMCs are higher than the criterion level, while approximately 68% (cell CP) and 80% (cell SS) of the output EMCs can meet the water quality criterion. Therefore, based on these data for the removal of indicator bacteria, bioretention facilities have the potential to significantly improve the water quality of stormwater runoff with respect to microbial pollutants.



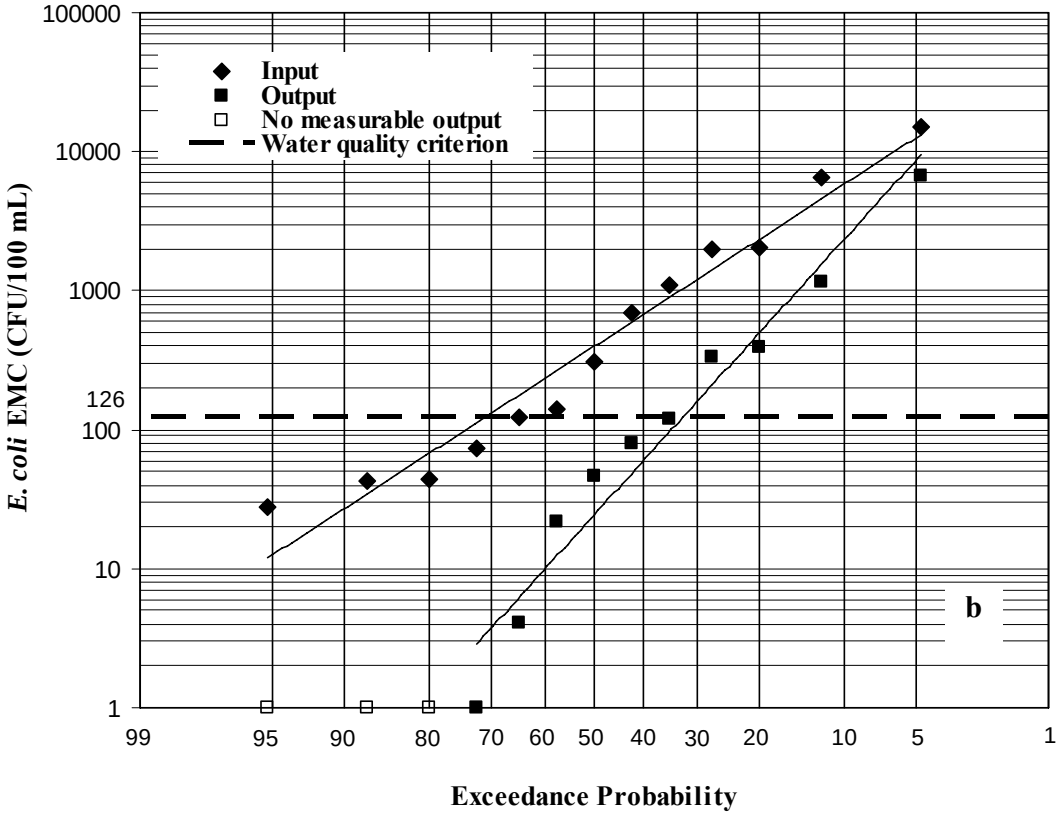
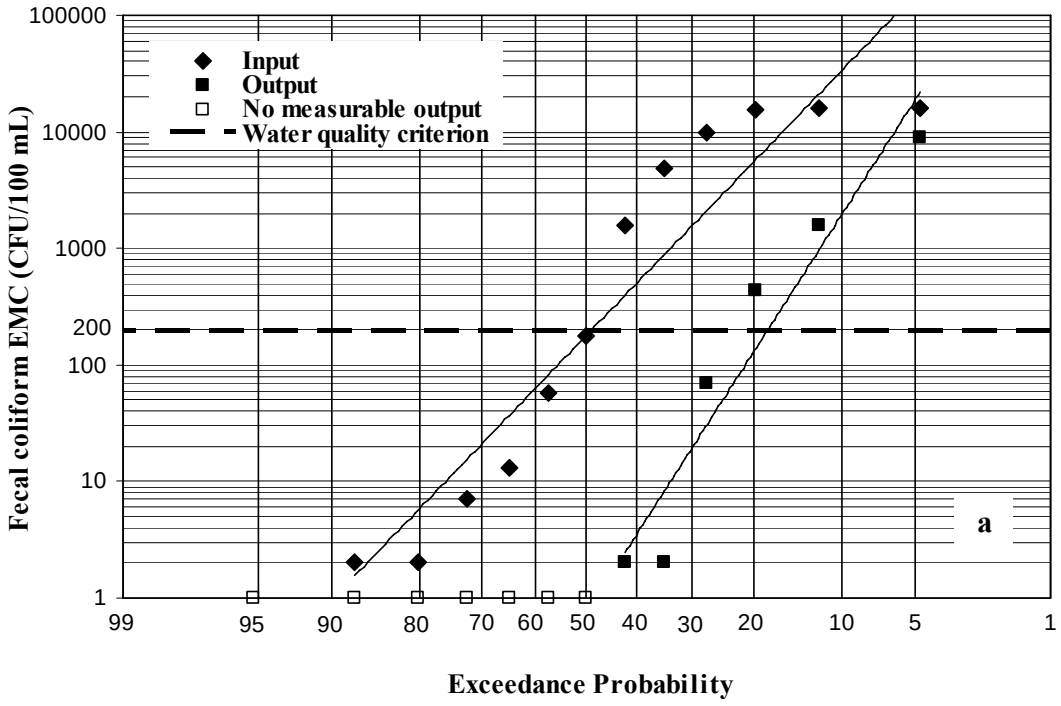


Figure 5.6 Exceedance probability plots for: (a) fecal coliform, and (b) *E. coli* in cell CP.



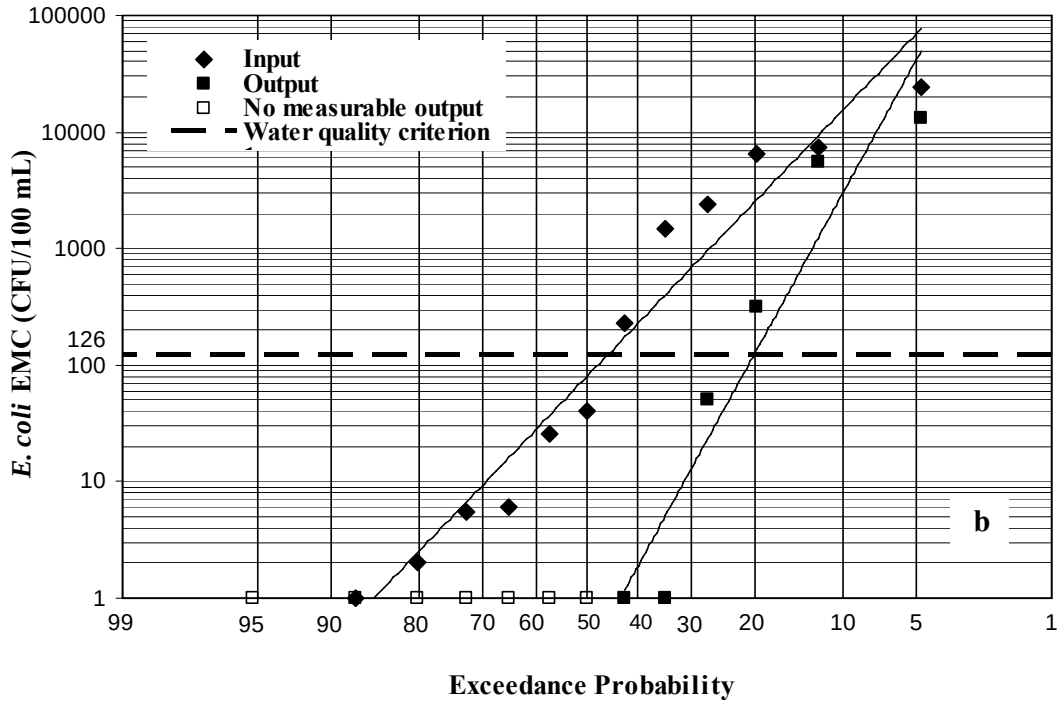


Figure 5.7 Exceedance probability plots for: (a) fecal coliform, and (b) *E. coli* in cell SS.

5.3.2 Laboratory experiments examining the effects of temperature on capture and destruction of strain B6914

5.3.2.1 Transport and capture of pathogenic bacteria

The effect of temperature on the transport and capture of *E. coli* O157:H7 strain B6914 in CBM was evaluated at four temperatures: 5, 15, 25 and 37ºC. The bacterial breakthrough curves are presented in Figure 5.8. Based on these experimental data, removal efficiency for strain B6914 was calculated using Equation 3.1, and the result shows that the bacterial removal efficiency was at 5ºC was slightly lower than at other higher temperatures, possibly due to the lower bacterial decay rate at 5ºC, as discussed below (Table 5.4). Additionally, there was no significant difference in bacterial removal at the other temperatures ($P>0.05$).

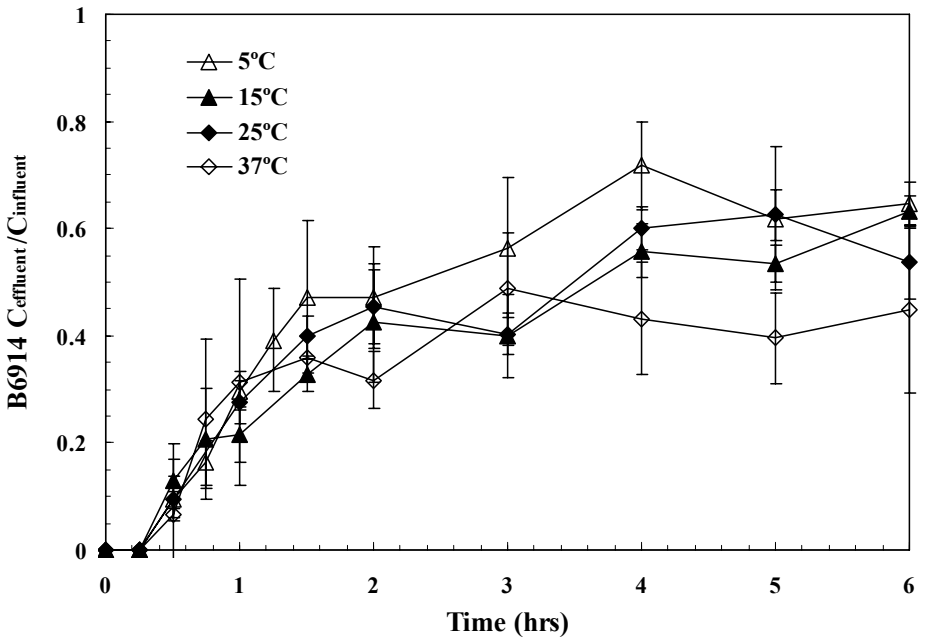


Figure 5.8 Breakthrough curves for strain B6914 in CBM at different temperatures. Symbols represent the mean of 4 replicates with vertical bars showing ± 1 standard deviation. The tested conditions: Flow rate = 40.0 ± 1.0 mL/hr, $C_{influent} = 1.1 \pm 0.3 \times 10^6$ CFU/mL.

The experimental bromide breakthrough data was fit to the one-dimensional non-reactive solute transport equation to obtain the best-fit parameters (porosity, pore water velocity and hydrodynamic dispersion coefficient). The values of the best-fit parameters are summarized in Table 5.4. Consistent with the bacterial transport data (Figure 5.8), the best fit parameters indicate that temperature did not significantly influence media porosity or hydrodynamic dispersion coefficient.

Table 5.4 Experimental conditions and model-fit or calculated parameters for the column studies of temperature impacts on transport and capture of strain B6914. The data represent the mean (n=4) ± 1 standard deviation. Superscripts within the same column (removal efficiency α, porosity θ, or dispersion coefficient D) marked by different letters are significantly different based on LSD ($P < 0.05$).

T (°C)	Flow rate (mL/hr)	Influent *E. coli* (CFU/mL)	Removal efficiency (%)	Calculated parameters			Best-fit parameters		
				α	η	k_a (hr^{-1})	ν (cm/hr)	θ	D (cm^2/hr)
5	39.7 ± 0.8	$1.3 \pm 0.3 \times 10^6$	49.4^a ± 3.6	0.016 ± 0.001	0.034 ± 0.001	0.87 ± 0.08	24.4 ± 0.6	0.332^a ± 0.013	23.9^a ± 6.5
15	40.7 ± 0.9	$1.3 \pm 0.3 \times 10^6$	59.1^b ± 1.3	0.018 ± 0.001	0.037 ± 0.002	1.29 ± 0.18	28.0 ± 2.3	0.299^a ± 0.020	37.4^b ± 2.6
25	40.5 ± 0.9	$1.3 \pm 0.3 \times 10^6$	55.7^b ± 2.5	0.018 ± 0.001	0.034 ± 0.002	1.00 ± 0.14	24.9 ± 2.0	0.334^a ± 0.025	21.5^a ± 7.0
37	40.4 ± 0.2	$1.2 \pm 0.3 \times 10^6$	64.8^b ± 5.9	0.020 ± 0.004	0.038 ± 0.005	1.36 ± 0.29	27.3 ± 3.9	0.311^a ± 0.045	31.1^{ab} ± 12.6

5.3.2.2 Survival of trapped pathogenic bacteria

After the 6-hr simulated rainfall event, the columns were sacrificed after variable periods of gravity drainage. The number of bacteria captured in the columns during the 6-hr simulated rainfall application (N_c) and the total number of remaining

cells in the column after gravity drainage for a specific time (N) were calculated by following the methods presented in Sections 3.3.4. It is clear that strain B6914 cells captured in the CBM survived longer at the lower temperature (Figure 5.9). After one-week gravity drainage, 20.8%, 60.6%, 99.95%, and 99.99% of trapped strain B6914 cells died off at 5, 15, 25, and 37ºC, respectively. This is consistent with observations of other researchers, which indicate that a lower temperature favors the survival of *E. coli* (Sjogren, 1994; Sørensen *et al.*, 1999; Cools *et al.* 2001; Jiang *et al.*, 2002). For example, Wang *et al.* (1996) found that *E. coli* O157:H7 in bovine feces survived for 42 to 49 days at 37ºC, for 49 to 56 days at 22ºC, for 63 to 70 days at 5ºC.

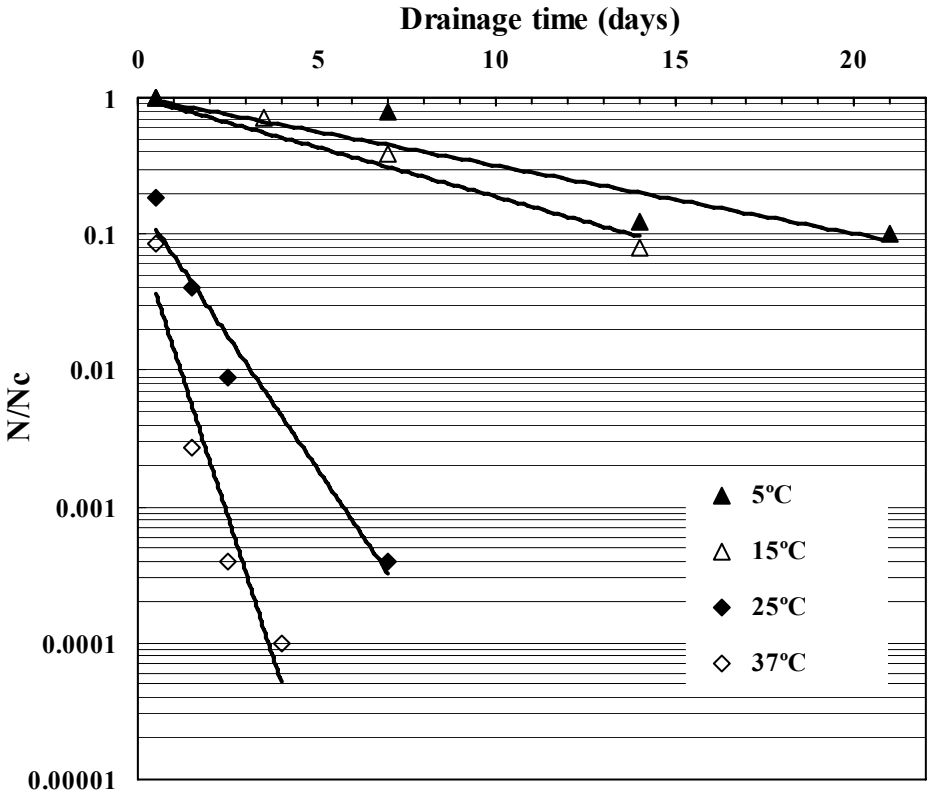


Figure 5.9 The fraction of strain B6914 attached to CBM with respect to drainage time at the different temperatures.

Additionally, the results of the bacterial survival experiments at different temperatures indicate that the total number of trapped B6914 cells in CBM decreased logarithmically with drainage time at each temperature (Figure 5.9). As discussed in Section 3.3.4, the bacterial die-off can be modeled as a first-order decay (Equation 3.5). The best-fits of the regression equations and first order decay coefficients were obtained via linear regression, and are presented in Table 5.5. The best fit die-off decay coefficient increased with increasing temperature, which is generally consistent with that found in previous research on the survival of *E. coli* in soil amended with manure (Cools *et al.*, 2001; Jiang *et al.*, 2002). For example, the die-off decay coefficients of *E. coli* at 5, 15, 21ºC were approximately 0.06, 0.15, and 0.21 d^{-1}, respectively (Jiang *et al.*, 2002).

Table 5.5 The best-fit of regression equations and first-order decay coefficients of strain B6914 in CBM at different temperatures.

Temperature (ºC)	Decay coefficient k_d (d^{-1})	N_0/N_c^* (%)	Decay equations	
5	0.11	1	$N/N_c=e^{-0.11t}$,	$R^2=0.868$
15	0.17	1	$N/N_c=e^{-0.17t}$,	$R^2=0.959$
25	0.90	0.17	$N/N_c=0.17e^{-0.90t}$,	$R^2=0.960$
37	1.87	0.09	$N/N_c=0.09e^{-1.87t}$,	$R^2=0.911$

*N_0 (CFU/g dry CBM) is the total number of surviving strain B6914 cells during 6-hr bacterial loading, as described in Section 3.3.4.

The resulting bacterial decay coefficients can be adjusted for temperature by using the following simplified form of the Arrhenius equation (Tchobanoglous and Schroeder, 1985):

$$k_T = k_{20}\theta^{T-20} \tag{5.2}$$

where: θ is the temperature coefficient, k_T is the bacterial decay coefficient at temperature T(ºC), and k_{20} is the bacterial decay coefficient at 20ºC. Values of k_{20} and θ are determined from the experimental data by performing a linear regression of the observed log k_d values versus temperature. The calculated values of k_{20} and θ are 0.41 d^{-1} and 1.10, respectively (Figure 5.10). Thus, these decay coefficients of trapped strain B6914 cells in the CBM fit well with the first-order decay equation, and k_T can be expressed as: $k_T = 0.41 \bullet 1.10^{T-20}$.

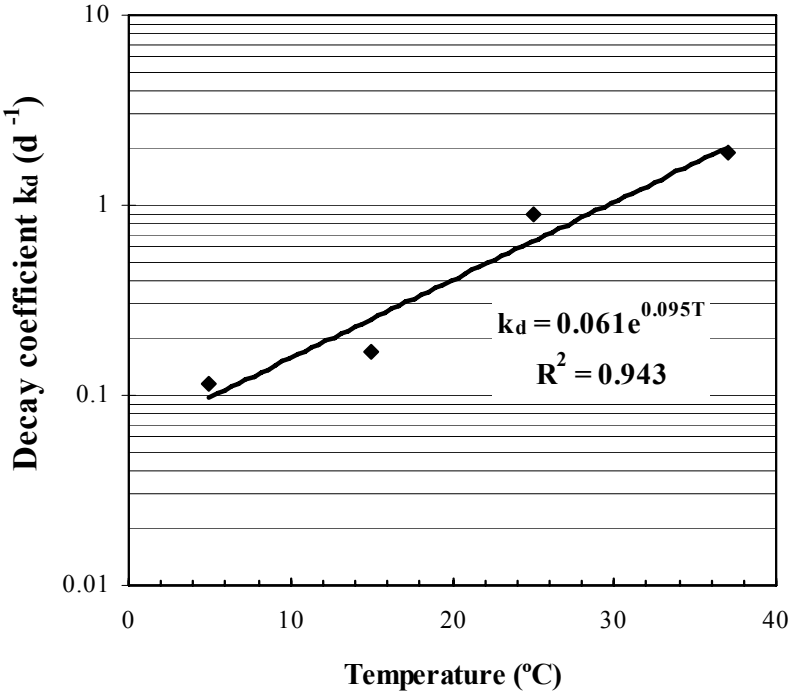


Figure 5.10 The relationship between temperature and die-off rate of strain B6914 in CBM.

5.3.2.3 The effect of native microbes on survival of trapped pathogenic bacteria

As noted above, the decay coefficients of strain B6914 in CBM incorporate several possible phenomena, including losses by predation and death. Clearly, these composite phenomena are a function of temperature (Figures 5.9 and 5.10). As described in Chapter 3, native microbes in CBM significantly accelerate the die-off of trapped strain B6914 cells due to the predation (e.g., by protozoa) and nutrient competition by native microbes (e.g., for carbon). Therefore, the numbers of protozoa and heterotrophic bacteria associated with CBM at the different experimental temperatures were analyzed to explain the phenomena of the variable survival of strain B6914 at the different temperatures.

Experimental results indicate that after 6-hr bacterial loading and overnight drainage, the total number of protozoa in the column increased with increasing temperatures. The number of total protozoa at 37ºC was almost one order of magnitude higher than that at 5ºC (Figure 5.11). This may be because most protozoa in soil are inactive (encysted) for much of time (Foissner, 1987), and high temperature may be favorable for the activation of protozoa.

With increased drainage time, the total number of protozoa in the columns increased exponentially for 21 days at 5ºC. However, the total numbers of protozoa in the columns initially increased exponentially at 15, 25 and 37ºC, after which it leveled off or started to fall. The introduced strain B6914 cells served as the food source contributing to the proliferation of protozoa in the CBM. However, with increasing numbers of protozoa, the food source may become a limiting nutrient, thus, the growth of protozoa starts to fall. Based on the experimental data (Figure

5.11), the growth of protozoa at 15, 25 and 37ºC were much better fit by the logistic (Verhulst) growth model, as described in Section 4.3.4.

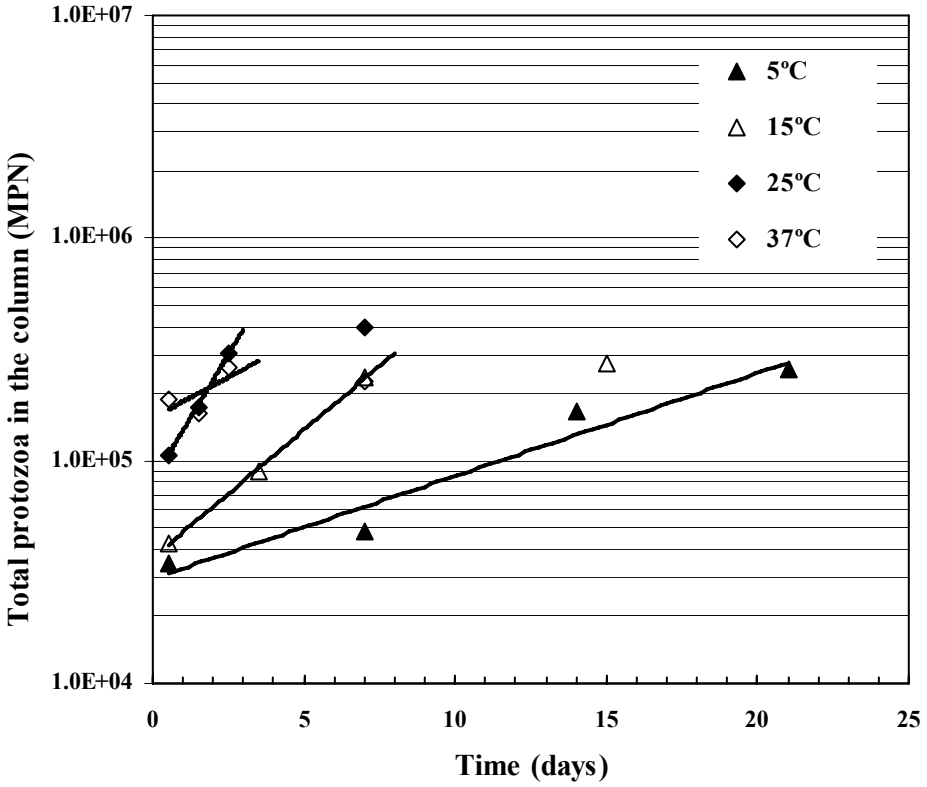


Figure 5.11 Total protozoa attached to CBM in the columns at different temperatures after 6-hr runoff loading.

The initial exponential protozoa growth rate increased with increasing temperature over a range of 5 to 25ºC. However, the growth rate of protozoa at 37ºC was lower than 15 or 25ºC (Figure 5.12). Consistent with these results, Sleigh (1973) reported that the growth and reproduction of protozoa increase with increasing temperature to a point, then stop due to encystment. Lalicker (1948) indicates that the optimal temperature for growth of most protozoa is between 16 and 25ºC, and

protozoa began to encyst at 37ºC (Darby *et al*., 2006). Thus, evidence suggests that the increasing decay coefficient of trapped strain B6914 as the temperature increased from 5ºC to 25ºC, was at least in part due to the greater members of protozoa and faster growth rate of protozoa in the CBM. However, except after the overnight drainage time, the total numbers of protozoa in CBM at 25ºC and 37ºC were similar at other drainage times, while the decay coefficient of trapped strain B6914 at 37ºC was faster than at 25ºC. Thus, as described in Chapters 3 and 4, it appears that predation by protozoa is the major cause for the quick decline in trapped B6914 cells for temperature 5-25ºC, with possibly other factors coming into play at 37ºC. One possible explanation is the higher metabolic and decay rate of strain B6914 at 37 ºC. Faster metabolism and growth tend to result in higher decay rates when substrates is lacking.

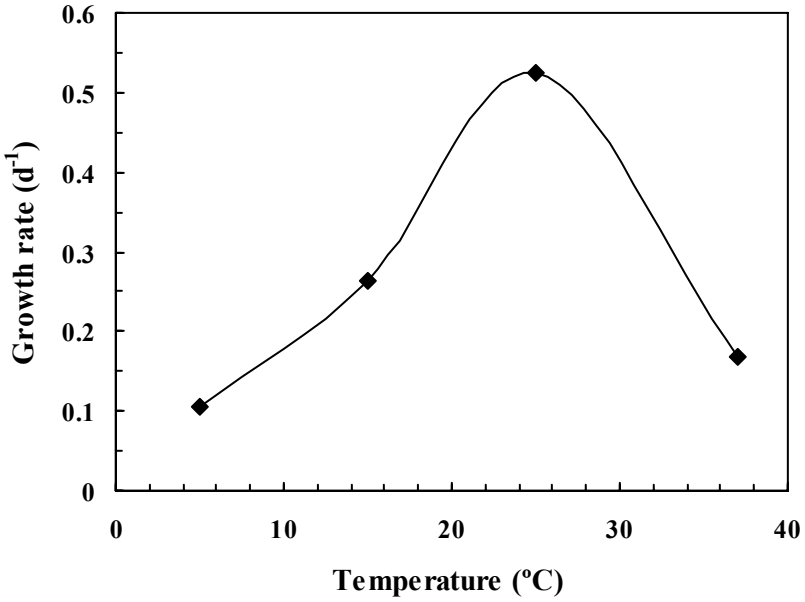


Figure 5.12 The relationship between temperature and growth rate of protozoa in the CBM columns after 6-hr runoff application.

In addition to indigenous protozoa, the soil heterotrophic bacteria were enumerated to evaluate the effect of native competitors on survival of trapped pathogenic bacteria at different temperatures. Heterotrophic plate count data show that total heterotrophic bacteria associated with CBM at 5, 15 and 25ºC increase over time, while the total number of heterotrophic bacteria at 25ºC was significantly higher than at 5 and 15ºC (Figure 5.13). Thus, the increasing numbers of native heterotrophic bacteria associated with the porous media as the temperature increases from 5 to 25ºC may contribute to faster die-off of trapped strain B6914 at via competition with native bacteria for limited nutrients (e.g., organic carbon), or by their serving as substrates contributing to the increases in indigenous protozoa.

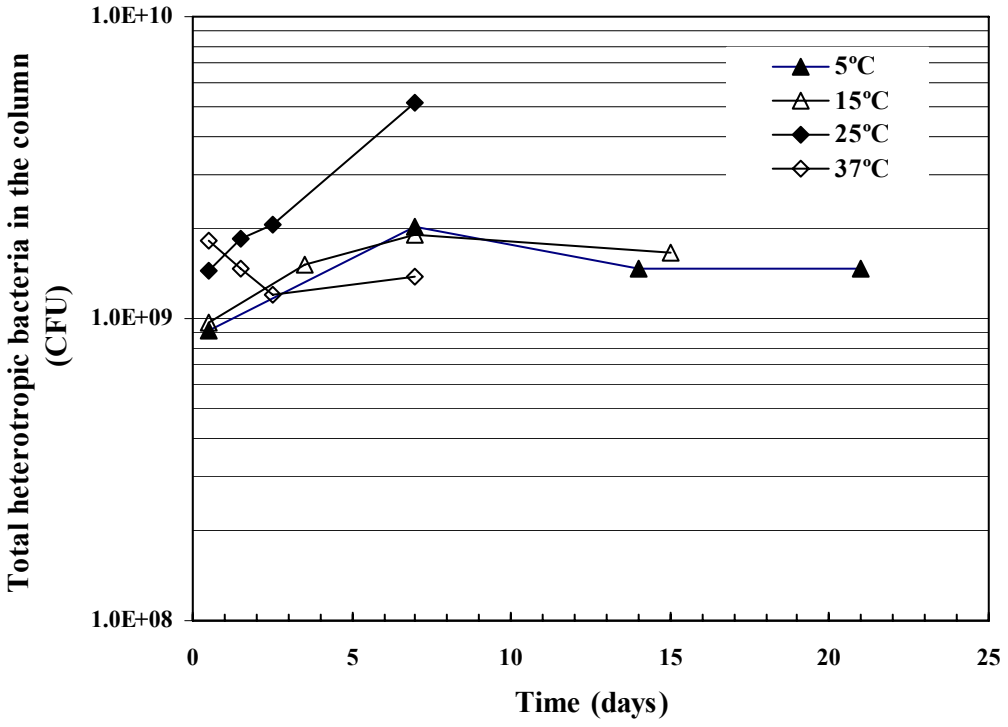


Figure 5.13 Total heterotrophic bacteria attached to CBM in the columns under different temperatures after 6-hr runoff application.

On the other hand, total number of heterotrophic bacteria associated with the CBM was higher at 37ºC than at the other temperatures after bacterial loading and gravity drainage overnight. However, the subsequent heterotrophic bacteria number decreased over time, falling to lower levels than at the other temperatures. Thus, similar to strain B6914 cells, excessive temperature (such as 37ºC or higher) is also unfavorable for the survival of the indigenous heterotrophic bacteria in CBM, which may be due to higher metabolism and lack of substrates. This suggests that the greater decay rate of strain B6914 at 37 ºC compared to 25ºC is not simply due to increased protozoan predation and competition for nutrients with heterotrophic bacteria; faster metabolism and die-off of strain B6914 cells may be a major contributor to the high decay rate for strain B6914 at 37ºC.

5.3.3 Native microorganism in the field media

A sample point (5 m away from the entrance of runoff) in cell CP was selected to observe the microbial community component at different temperatures. The data show that the concentrations of protozoa in the field media at the most depth were one-order of magnitude higher than those in laboratory CBM under the similar temperature (Figure 5.14). Consistently, it was reported that the number of bacterial-feeding protozoa in the rhizosphere may increase up to 30-fold compared to bulk soil (Griffiths, 1990). One possible explanation is that plant materials, an important component of traditional bioretention facilities, provide more favorable environments for the proliferation and metabolism of protozoa. Plants may stimulate the bacterial activity in the rhizosphere through secreting carbon sources into soil as exudates, and

thus increase the protozoan populations as bacteria are the primary food source of most soil protozoa (Lynch and Whipps, 1990; Timonen *et al.*, 2004).

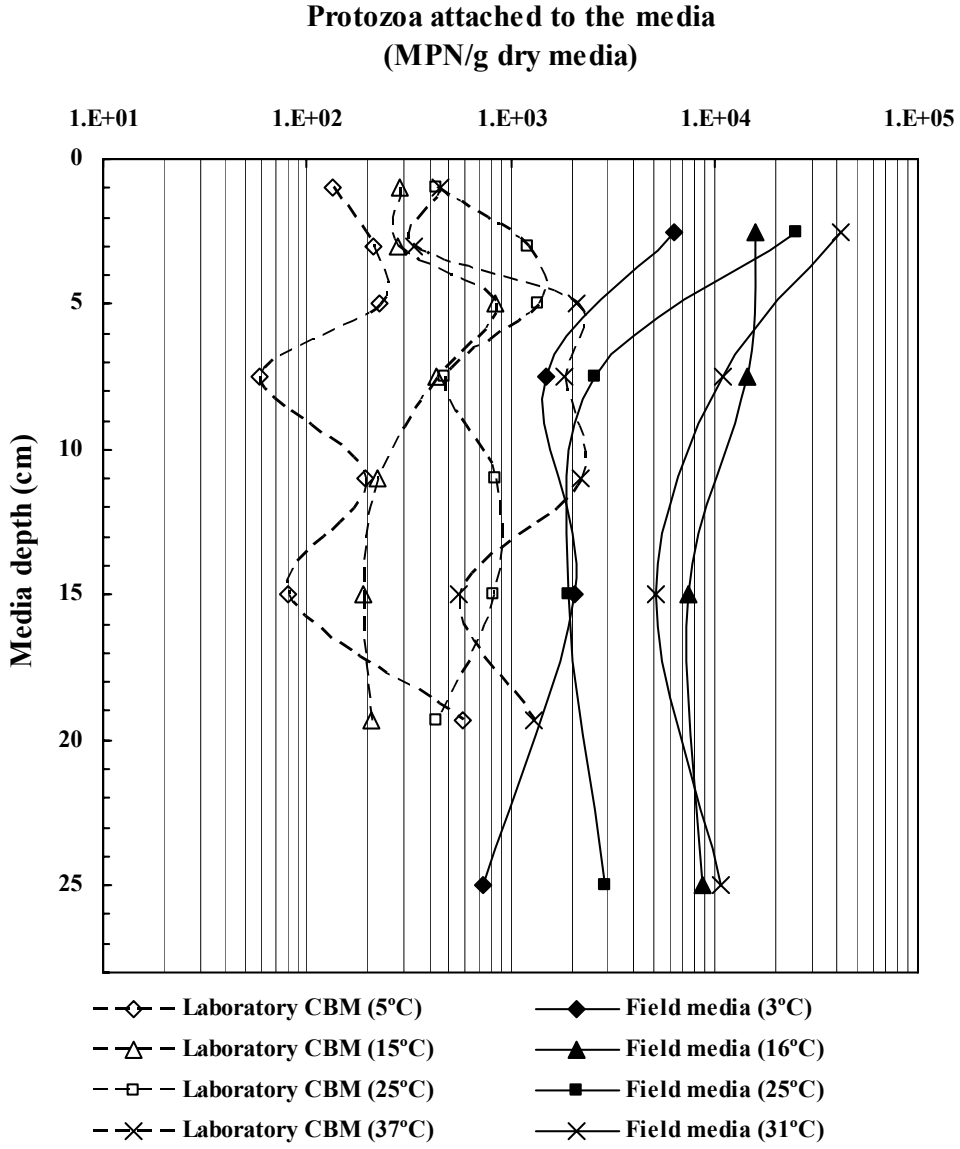


Figure 5.14 Longitudinal distribution of protozoa in the laboratory CBM and field media after stormwater runoff application and overnight drainage at different temperature.

As described in Section 5.3.2.3, the number of protozoa and heterotrophic bacteria in the laboratory CBM generally increased with increasing temperature, especially after 6-hr rainfall application and overnight drainage. However, the levels of protozoa in the field media only increase with increasing the daily temperature at the top surface (0-5 cm). At other depths of field media, the concentrations of protozoa at 5ºC were lower than at higher temperatures, but the concentrations of protozoa at 25ºC were lower than at 16 and 31ºC. The variation of daily temperature, moisture content of soil media, storm precipitation, bacterial loading from storm runoff, and density of shade over bioretention facilities may result in the difference between the laboratory and field data. More sampling data in the field studies are needed to reveal the mechanism on the effects of temperature on protozoa in the field.

The concentration heterotrophic bacteria attached to the field media at the top surface were much higher than at the lower soil layers, because the rhizosphere of plants is favorable for the growth and proliferation of microorganisms (Figure 5.15). The concentrations of protozoa in the field media were much higher than in the laboratory CBM. However, it is interesting that the concentrations of heterotrophic bacteria in the field media had no significant difference from that in CBM, expect at the top surface. One possible explanation is that less heterotrophic bacteria which are attached to field media particles and within small pore spaces are protested from the larger protozoa, and more free heterotrophic bacteria serves as the food sources of protozoa in the field media.

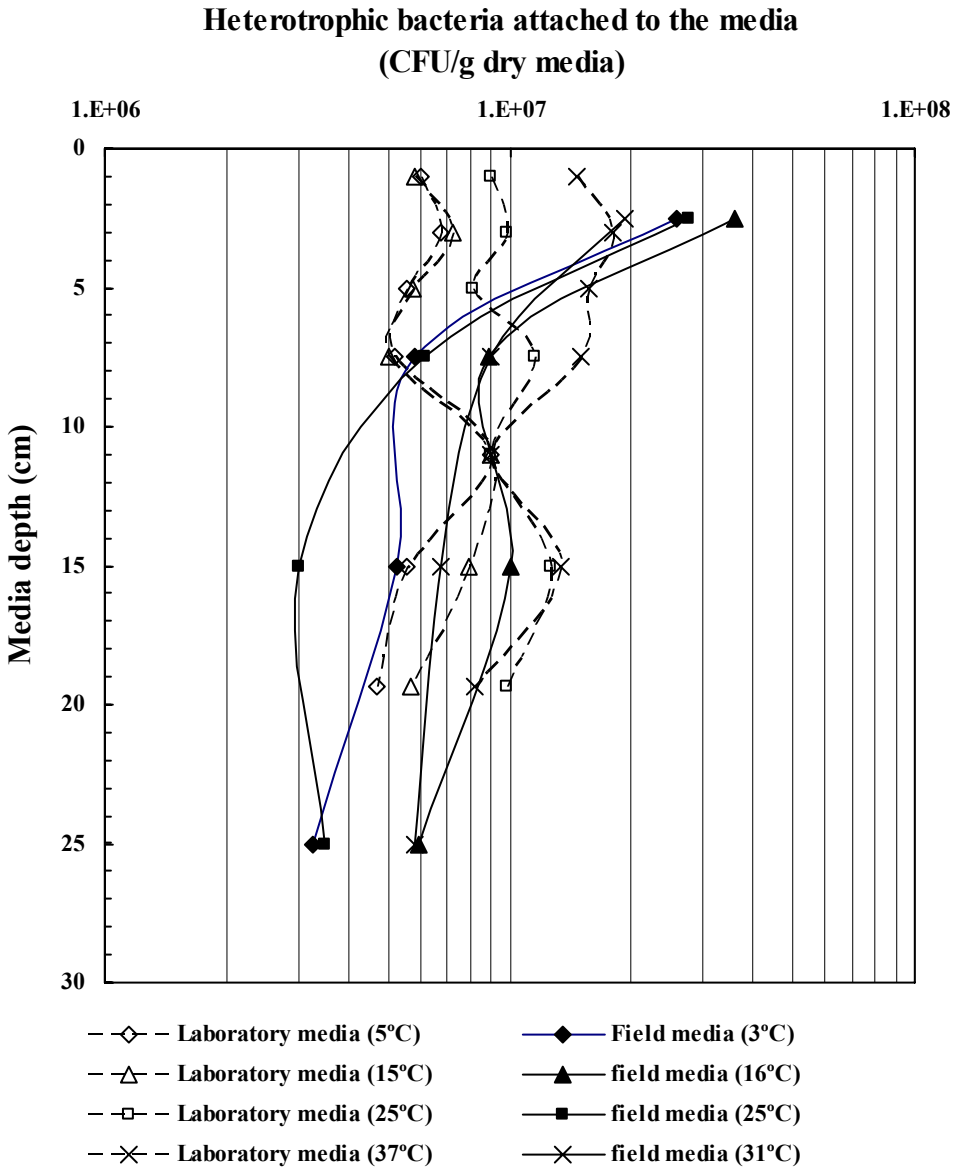


Figure 5.15 Longitudinal distribution of heterotrophic bacteria in the laboratory CBM and field media after stormwater runoff application and overnight drainage at different temperature.

5.4 Summary and conclusions

This study investigated the performance of two bioretention facilities for removal of indicator bacteria during different seasons. The 2-yr field data show that

the concentration of indicator bacteria in the input flow generally increased with increasing daily temperature. The bioretention facilities not only exhibit good hydrologic performance (such as runoff volume reduction), but reduce the concentration of indicator bacteria in the outflow in most storm events and significantly increase the probability of meeting specific water quality criteria. However, there is no clear trend in the bacterial removal efficiency with temperature.

The capture and destruction of pathogenic bacteria was further investigated at different temperatures (5, 15, 25, and 37ºC) using laboratory column studies. Temperature did not significantly influence the transport and capture of strain B6914. However, survival of trapped strain B6914 in CBM was temperature sensitive. The numbers of strain B6914 cells captured in the CBM reduced exponentially over time at each temperature, and the decay coefficients increased with increasing temperature. One possible explanation for this trend is that high temperatures increased protozoa levels. Also, heterotrophic bacteria associated with CBM grew faster as the temperature increased from 5ºC to 25ºC. The result is faster die-off of trapped strain B6914 via predation and competition for limited nutrients. However, though the total numbers of protozoa and heterotrophic bacteria in the columns at 37ºC were the highest, the growth rates of protozoa and heterotrophic bacteria were lower than those at other temperatures. Thus, faster metabolism and higher decay rates of strain B6914 cells due to the lack of substrates, may be a major cause for the rapid decline of trapped B6914 cells at 37ºC.

Chapter 6: Conclusions and recommendations

6.1 Conclusions

Bioretention performance with respect to bacterial removal was systematically evaluated in this research using laboratory column and field studies. Columns packed with CBM were used to evaluate the transport and destruction of *E. coli* O157:H7 strain B6914 from simulated urban stormwater runoff under different environmental conditions. Also, the long-term (18 months) sustainability of bacterial removal in CBM-packed columns was investigated. Additionally, field studies were completed to examine the removal of indicator bacteria (fecal coliform and *E. coli*) at two existed bioretention facilities during thirteen rainfall events. Finally, the potential of an engineered media (iron oxide-coated sand (IOCS)) for enhancing bacterial removal was evaluated using column studies of the transport and destruction of strain B6914 from simulated stormwater runoff.

CBM is a mixture of sand, sandy loam soil and mulch (leaf compost). The results of column studies indicated that CBM could achieve 80% or higher removal for strain B6914 under the test conditions, suggesting that strain B6914 can be effectively managed by CBM. However, with increased media storage time (at room temperature), the removal efficiency of CBM for strain B6914 decreased, which may be attributed to changes in the surface characteristics and/or biological diversity of the media components over time.

The basic mechanisms of bacterial transport and capture are presented in Figure 6.1. Because the dimension of strain B6914 cell is much smaller than 5 % of the

mean diameter of CBM particles, physical straining probably had a relatively small impact on the bacterial capture. Thus, bacterial adsorption may have been the main factor controlling the bacterial capture. Electrostatic interactions between strain B6914 and CBM may have been a more important mechanism compared to hydrophobic interactions, because strain B6914 is a relatively hydrophilic organisms with a weak negative charge. Consistent with this conclusion, a higher ionic strength of the simulated runoff resulted in the enhancement of adhesion of strain B6914 in CBM due to the reduction of repulsive forces and compression of the electrical double layer.

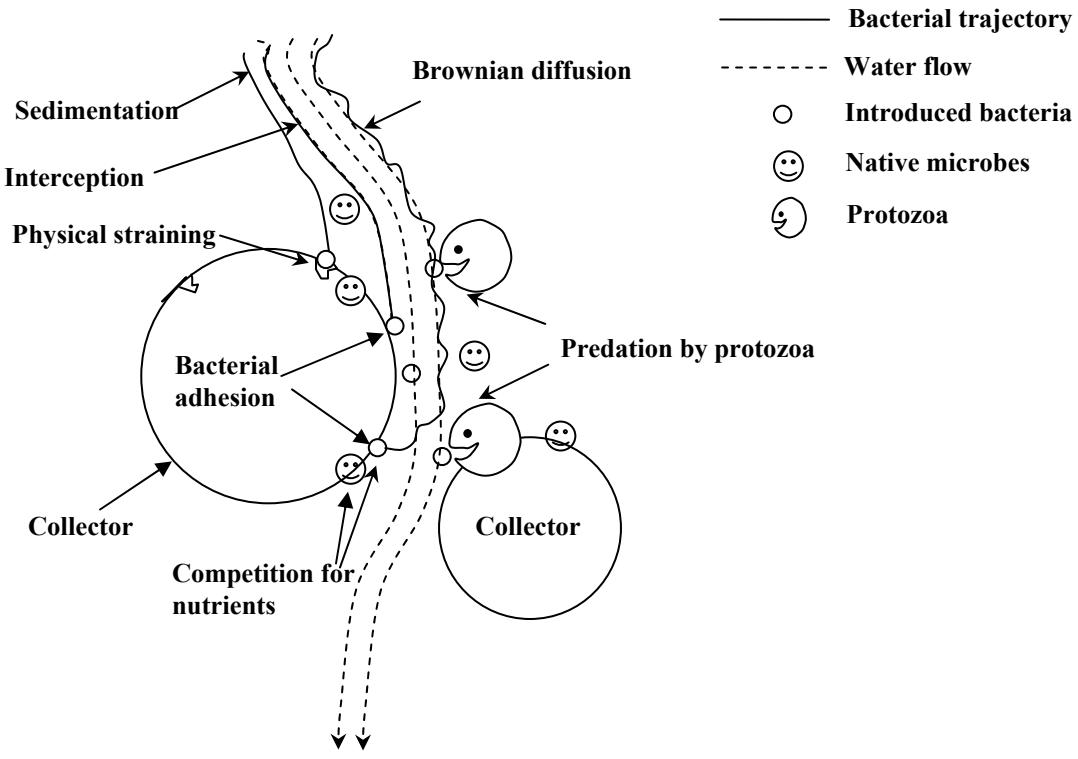


Figure 6.1 Diagram of basic bacterial transport, capture and destruction mechanisms in porous media.

IOCS significantly enhanced the capture of strain B6914 compared to in CBM, because IOCS possesses a more positive surface charge and greater specific surface area, which increase the electrostatic attraction and bacterial adhesion. However, the die-off rate of trapped cells was much faster in CBM (0.90 d^{-1}) compared to that in the IOCS (0.04 d^{-1}) under the experimental conditions. More than 99.98% of B6914 cells attached to CBM died off within one week. In comparison, only approximately 52% of trapped B6914 cells in the IOCS died off after gravity drainage for one week and about 22% cells still survived after four weeks, which may represent a potential threat for receiving waters. A major factor contributing to these different results was probably the lack of microbial predation and competition in the IOCS, as studied. Indeed, predation and competition from the native microorganisms (Figure 6.1) in CBM were verified to play a dominant role in the rapid destruction of trapped strain B6914 through comparing the bacterial survival in parallel non-sterile media, γ-irradiated sterile media and γ-irradiated sterile media coated with cultured native microorganisms. In particular, protozoan grazing appeared to play an important role, with the die-off of trapped B6914 increasing with increasing concentrations of protozoa in the media.

The long-term sustainability study demonstrated that the bacterial removal efficiency in CBM improved over time, with removal efficiencies of greater than 97% after 6 months of periodic simulated rainfall application. Additionally, the trapped B6914 cells died off rapidly between runoff application events. Therefore, there is significant potential for the bacterial removal process in CBM to be sustainable. Mechanistic studies indicate that the improvement of the bacterial capture and

destruction in CBM following the long-term runoff application was due to changes in the physical and biological characteristics of the CBM. For example, porosity was observed to decrease, and hydrodynamic dispersion increased over time; both trends are favorable for improving the physical straining of cells and for bacterial adhesion. Indigenous protozoa in the CBM grew logistically, which may play an important role in the enhancement of bacterial capture and the quick decline in the numbers of trapped strain B6914. Although competition with indigenous heterotrophic bacteria apparently had little effect on bacterial destruction, the heterotrophic bacteria may have contributed to the sustainability of the bioretention system by serving as food sources for grazing protozoa.

Another important component of this research was the field study examining the performance of two bioretention facilities for removal of indicator bacteria from actual runoff events during different seasons. The two years of field data showed that the concentration of indicator bacteria in the input flow generally increased with increasing daily temperature. In addition, the bioretention facilities not only exhibited good hydrologic performance (e.g., runoff volume reduction), but also reduced the concentration of indicator bacteria in the outflow during most storm events and significantly increased the probability of meeting specific water quality criteria. However, there was no clear trend in the bacterial removal efficiency with temperature.

Laboratory column studies at different temperatures (5, 15, 25, and 37ºC) also showed that temperature did not significantly influence the transport and capture of strain B6914. However, survival of trapped strain B6914 in CBM was temperature

sensitive. The numbers of trapped strain B6914 cells in the CBM decreased exponentially over time at each temperature, and the decay coefficients increased exponentially with increasing temperature. One possible explanation for this trend is that predatory protozoa and competitive heterotrophic bacteria associated with the CBM grew faster as the temperature increased from 5ºC to 25ºC, which resulted in faster die-off of trapped strain B6914 via predation and competition for limited nutrients (Figure 6.1). Faster metabolism and higher decay rates of strain B6914 cells due to the lack of substrate, may have been a major cause for the fastest decline of trapped B6914 cells at high temperatures, especially at 37ºC.

In summary, the results of the column study show that the removal efficiency for strain B6914 in CBM improved over time, with an initial mean removal efficiency of 70%, while the field data from the monitored bioretention facilities demonstrate a 60% or higher mean concentration-based removal for fecal coliform at 8 out of 13 storm events. Consistent with these results, Rusciano and Obropta (2007) reported a mean reduction coefficient of 91.5% for fecal coliform in bioretention columns, and Hunt *et al.* (2008) observed a 69% mean concentration-based removal for fecal coliform in a bioretention facility. Therefore, based on these studies, bioretention facilities has a greater bacterial removal capacity compared to open channel vegetated systems and sand infiltration systems, which have been reported to achieve an average of -25% and 37% bacterial removal, respectively (USEPA, 1999), and similar bacterial removal efficiency as other several BMPs, such as infiltration wet ponds and constructed wetland systems, which are reported to have achieved an average of 79% and 78% bacterial removal, respectively (USEPA, 1999). Thus, the bioretention

system is one of the BMPs that are able to effectively remove bacteria from stormwater runoff.

6.2 Recommendations

Based on the results of laboratory bioretention column studies, the removal efficiency for strain B6914 in CBM columns decreased with increasing storage time of the components of CBM. Therefore, it is recommended that the reduction of storage time of sandy soil and mulch (leaf compost) be minimized before the preparation and utilization of CBM (the planting soil in Figure 1.2) in a bioretention facility. This should allow the system to achieve better bacterial removal during the initial start-up period of the bioretention facility. For long-term operation, the laboratory sustainability studies indicated that the bacterial removal efficiencies in the columns improved over time, with little variability after 6 months of simulated runoff application. Therefore, it is expected that the monitoring data from 6-month or longer aged bioretention cells will demonstrate superior ability of bioretention facilities to treat pathogenic bacteria from stormwater runoff.

As described above, for the relative hydrophilic, less negatively charged strain B6914 cells, bacterial adsorption (especially electrostatic interactions) may have been the main mechanism controlling bacterial transport and capture in CBM. Thus, it is expected that CBM should also exhibit good removal efficiency for other less negatively charged pathogenic bacteria in stormwater runoff. In addition, bacteria with similar surface charges, but a more hydrophobic surface should tend to more readily partition from the aqueous phase and accumulate at the solid phase compared

to hydrophilic bacteria. However, for the column experiments in this study, only one pathogenic bacterium was introduced into the columns. If other pathogenic or non-pathogenic bacteria were introduced together with the strain B6914 cells, the removal efficiency for strain B6914 may decrease due to the competition for adsorption sites.

The mechanistic studies on the transport and destruction of strain B6914 demonstrated that predation by indigenous protozoa played an important role in the die-off of introduced pathogenic bacteria. Therefore, maintaining or elevating the concentration of protozoa and bacterial predators in bioretention media has potential to enhance the destruction of trapped bacteria and make the bioretention system sustainable. Plants can stimulate the bacterial activity in the rhizosphere through secreting carbon sources into soil as exudates, thus increasing the protozoan populations (Lynch and Whipps, 1990; Timonen *et al.*, 2004). Therefore, plants (such as shrubs) in bioretenion facilities may increase the number of bacteria and in turn protozoa, thus enhancing the capture and destruction of trapped pathogenic bacteria. Hunt *et al.* (2008) reported that a relatively densely vegetated bioretention cell achieved 69% and 71% mean concentration-based removals of fecal coliform and *E. coli*. This is consistent with our field data from the two bioretention cells covered with shrubs. Thus, the use of vegetation is recommended in the design of bioretention facilities for bacterial treatment. However, some pathogenic protozoa such as *Giardia* and *Cryptosporidium* have been found in stormwater runoff (USEPA, 2002). The enrichment of these harmful protozoa in the planting soil may become a concern if bioretention facility is open to the public.

In addition, it is likely the protozoa are playing other important roles in the bioretention soil microbial structure in addition to predation of pathogenic bacteria, which could merit further investigation. For example, protozoa may result in positive and negative effects on the indigenous bacterial populations in soil. Selective grazing can cause changes in bacterial community structure, effectively eliminating some types of bacteria while allowing others to flourish (Tso and Taghon, 2006). For example, the pollutant-degrading microorganisms in the soil are expected to be mainly bacteria. If the pollutant-degrading bacteria are eaten by the protozoa in the soil and the number of these bacteria decreases, the degradation capacity of the system for these pollutants may noticeably deteriorate. On the other hand, protozoa may also aid in the cycling of inorganic nutrients in the soil, especially P and N, and may also excrete growth factors required by auxotrophic bacteria, which would facilitate the growth of bacteria through the microbial loop factors (Alexander, 1990; Mattison and Harayama, 2001). Grazing by protozoa (such as ciliates) has been reported to stimulate the growth of some pollutant-degrading bacteria and concomitant hydrocarbon degradation. For example, grazing by the ciliate *Colpidium colpoda* was shown to enhance the microbial degradation of crude oil (Rogerson and Berger, 1983). Therefore, in order to effectively reduce the concentration of various pollutants found in urban stormwater runoff, the effect of protozoa on the native pollutant-degrading bacteria also must be considered in the design of bioretention facility.

The two years of field data indicated higher loadings of microbial pollutants at higher air temperatures. Bioretention systems reduced the concentrations of indicator

bacteria in the outflow in most storm events and increased the probability of meeting specific water quality criteria. However, there was no clear trend in the bacterial removal efficiency with temperature based on the field study. Laboratory column studies also exhibited no significant difference for strain B6914 removal efficiency at 5, 15, 25, and 37ºC. Thus, bioretention discharge may still be a potential threat for receiving waters after some summer storm events. However, faster die-off of trapped bacteria in CBM is expected at higher temperatures when higher bacterial loadings are likely, which should make the bioretention system sustainable in summer.

IOCS column experiments exhibited a 99.4% removal for strain B6914 under the tested conditions, which was greater than observed in the CBM-packed columns. However, the bacteria trapped on IOCS survived much longer due to the lack of native microorganisms. Therefore, future work is needed to develop a reengineered design (e.g., adding an underlying layer with an optimal fraction of IOCS and CBM in the vertical profile of bioretention) for optimizing the capture and destruction of pathogenic bacteria. This may increase the probability that the bioretention discharge will meet specific water criteria, especially during the summer storm events when high microbial pollutants loadings are expected. However, capture of other pollutants and hydrological performance also must be considered in the design.

In addition, other environmental factors need to be evaluated for optimizing bacterial attenuation in bioretention facilities in future work. For example, as described above, vegetation may increase the activity and growth of indigenous bacteria and protozoa, thus enhancing the destruction of trapped pathogenic bacteria. However, dense vegetation will reduce direct sunlight in bioretention cells. Sunlight

is expected to cause quicker bacterial die-off. Therefore, it is of important research interest to investigate the optimal density of vegetation in bioretention facilities for destruction of pathogenic bacteria. Additionally, the data for bacterial survival in non-sterile media and γ-irradiated sterile media demonstrate that the increased available nutrients as a result of γ-irradiation enhance bacterial survival, and even resulted in an increase in bacterial numbers. Thus, it is recommended that the effect of nutrient availability in bioretention media on the survival of introduced pathogenic bacteria should be further investigated.

Finally, the results of this study suggest the possibility of bioretention application beyond treatment of urban stormwater runoff. In this study, the concentration of *E. coli* O157:H7 strain B6914 in the synthetic runoff influent was set at 1×10^6 cells/mL to allow the enumeration of the bacteria in the effluent using the heterotrophic plate count method. This concentration of bacteria is higher than typical concentrations found in urban stormwater runoff, and more closely approximate bacterial levels in agricultural stormwater runoff. The results of column studies indicate that CBM can achieve 80% or higher removal for strain B6914 under this condition. Thus, the data suggest that bioretention media has the ability to effectively reduce the microbial pollutants from agricultural runoff.

Appendices

Appendix-1 The transport of strain B6914 in columns packed with different aged media.

Storage time of media (months)	Flow rate (mL/h)	B6914 $C_{influent}$ ($X10^6$ CFU/mL)	B6914 $C_{effluent}/C_{influent}$ at time t									Removal efficiency of B6914 (%)
			0	0.5 h	1 h	1.5 h	2 h	3 h	4 h	5 h	6 h	
5-month (03/2006)	39.0	0.7 ± 0.13	0	0.010	0.07	0.16	0.15	0.18	0.15	0.29	0.29	82.9
	40.8	1.6 ± 0.30	0	0.007	0.03	0.09	0.09	0.08	0.08	0.14	0.15	91.2
	39.1	0.8 ± 0.21	0	0.003	0.02	0.06	0.06	0.11	0.09	0.12	0.14	91.8
8-month (06/2006)	39.8	1.31 + 0.12	0		0.08		0.20	0.24	0.20	0.18	0.20	83.2
	41.0	1.01 ± 0.07	0		0.06		0.21	0.26	0.21	0.23	0.31	81.3
	40.3	1.31 + 0.12	0		0.03		0.13	0.21	0.19	0.17	0.21	86.2
	39.8	1.01 ± 0.07	0		0.02		0.08	0.08	0.09	0.10	0.11	92.8
	39.1	1.01 ± 0.07	0		0.08		0.23	0.20	0.24	0.31	0.32	79.7
10-month (08/2006)	41.7	1.22 ± 0.18	0	0.012	0.06	0.10	0.15	0.14	0.15	0.10	0.10	88.7
	41.2	0.96 ± 0.13	0	0.005	0.16	0.21	0.19	0.22	0.19	0.20	0.22	81.8
	40.1	1.57 ± 0.14	0	0.044	0.19	0.22	0.22	0.26	0.33	0.27	0.29	75.8
	40.0	1.38 ± 0.17	0	0.013	0.05	0.11	0.18	0.20	0.25	0.22	0.16	83.2
14-month (02/2007)	40.2	0.82 ± 0.17	0		0.17		0.31	0.29	0.23	0.23	0.16	78.4
16-month (04/2007)	39.2	0.68 ± 0.13	0		0.35		0.38	0.34	0.34	0.29	0.29	69.3
19-month (06/2007)	39.0	1.11 ± 0.12	0		0.40		0.45	0.67	0.63	0.50	0.45	52.1
	39.8	1.0 ± 0.07	0		0.36		0.37	0.52	0.46	0.63	0.42	57.7
26-month (01/2008)	40.2	1.19 ± 0.12	0	0.076	0.23	0.34	0.37	0.39	0.59	0.66	0.57	56.3
	40.2	1.19 ± 0.12	0	0.052	0.30	0.42	0.54	0.46	0.63	0.63	0.60	50.9
	40.6	1.12 ± 0.19	0	0.104	0.26	0.42	0.43	0.39	0.64	0.56	0.44	56.1
	40.4	1.12 ± 0.19	0	0.152	0.31	0.41	0.47	0.37	0.55	0.65	0.53	54.1

Appendix-2 The transport of strain B6914 in CBM (made by 1-yr media), sand and IOCS.

Media	Flow rate mL/h)	B6914 $C_{influent}$ ($x10^6$ CFU/mL)	B6914 $C_{effluent}/C_{influent}$ at time t								Removal efficiency of B6914 (%)
			0	0.5 h	1 h	1.5 h	2 h	3 h	4 h	5 h	6 h
CMB	41.7	1.22 ± 0.16	0	0.012	0.064	0.101	0.145	0.142	0.147	0.103	0.100	88.7
	41.2	0.96 ± 0.13	0	0.005	0.162	0.210	0.185	0.220	0.189	0.200	0.220	81.8
	40.0	1.57 ± 0.12	0	0.044	0.194	0.220	0.216	0.265	0.330	0.266	0.294	75.8
	40.1	1.38 ± 0.17	0	0.013	0.052	0.114	0.177	0.196	0.247	0.217	0.164	83.2
Fine IOCS	40.7	1.22 ± 0.16	0	0	0.0189	0.0216	0.0269	0.046	0.053	0.0644	0.070	95.9
	41.5	0.96 ± 0.13	0	0	0.0011	0.0013	0.0014	0.002	0.004	0.0032	0.004	99.8
	40.0	1.57 ± 0.12	0	0	1.3E-06	0.0E+00	2.4E-05	2.2E-05	2.1E-05	1.6E-04	3.6E-04	100.0
	40.8	1.38 ± 0.17	0	0	0.0E+00	2.8E-06	3.0E-06	1.2E-05	8.9E-05	9.3E-05	7.2E-05	100.0
Fine sand	40.2	1.22 ± 0.16	0	0.100	0.397	0.396	0.452	0.500	0.507	0.556	0.436	55.4
Coarse sand	39.2	0.68 ± 0.13	0	0.337	0.424	0.453	0.467	0.420	0.495	0.580	0.564	52.5
Coarse IOCS	39.0	0.68 ± 0.13	0	0.081	0.109	0.116	0.120	0.223	0.179	0.138	0.214	84.7

Appendix-3 The survival of trapped strain B6914 in CBM and fine IOCS.

Media	Drainage time (days)	Media depth (cm)	Moisture (%)	Strain B6914		
				log CFU/g dry media	CFU/g dry media ($x10^5$)	Total CFU in each depth ($x10^6$)
CBM (made by 1-yr media)	0.5	0-2	19.68	5.709	5.117	7.275
		2-4	18.55	5.594	3.931	5.729
		4-6	16.20	5.619	4.157	5.489
		6-9	21.99	5.413	2.588	4.996
		9-13	23.50	5.315	2.068	5.545
		13-17	30.04	4.987	0.970	2.410
		17-21	29.80	4.907	0.807	2.432
	1.5	0-2	20.80	5.284	1.924	2.582
		2-4	19.00	4.620	0.417	0.668
		4-6	16.12	4.215	0.164	0.220
		6-9	21.96	3.986	0.097	0.196
		9-13	30.71	4.439	0.275	0.719
		13-17	29.28	4.652	0.449	1.229
		17-21	28.26	4.525	0.335	1.010
	2.5	0-2	14.12	4.852	0.711	0.895
		2-4	17.02	4.034	0.108	0.159
		4-6	17.14	3.854	0.071	0.103
		6-9	19.36	3.843	0.070	0.133
		9-13	24.46	3.571	0.037	0.092
		13-17	23.26	3.721	0.053	0.138
		17-21	29.40	3.916	0.082	0.248
	7	0-2	11.18	3.624	0.042	0.048
		2-4	13.70	2.609	0.004	0.006
		4-6	15.85	2.480	0.003	0.004
		6-9	19.31	2.468	0.002	0.006
		9-13	21.19	2.468	0.003	0.007
		13-17	28.14	2.086	0.001	0.003
		17-21	29.39	2.918	0.008	0.025
Fine IOCS	0.5	0-2	4.82	5.938	8.665	14.635
		2-4	6.23	6.093	12.383	18.601
		4-6	9.62	6.270	18.639	26.782
		6-9	16.12	5.912	8.165	18.002
		9-13	24.46	5.710	5.130	14.581
		13-17	23.85	5.319	2.086	5.927
		17-21	24.03	5.494	3.116	9.388
	7	0-2	3.79	6.014	10.321	15.661
		2-4	8.56	5.944	8.787	15.313
		4-6	11.50	6.208	16.131	23.240
		6-9	19.82	5.998	9.956	21.182
		9-13	28.46	5.702	5.031	15.025
		13-17	25.05	5.291	1.954	5.980

		17-21	24.61	4.562	0.365	1.098
	14	0-2	3.03	5.691	4.913	7.163
		2-4	4.16	6.556	35.952	59.615
		4-6	5.27	6.478	30.074	44.848
		6-9	7.52	6.437	27.375	61.533
		9-13	16.38	5.848	7.042	20.878
		13-17	22.31	4.373	0.236	0.705
		17-21	21.80	3.498	0.032	0.095
	28	0-2	20.09	5.693	4.936	5.566
		2-4	23.58	6.272	18.720	35.102
		4-6	25.58	6.471	29.591	45.747
		6-9	26.46	6.457	28.620	62.704
		9-13	28.70	6.050	11.227	34.037
		13-17	28.39	4.498	0.314	0.963
		17-21	25.83	3.061	0.012	0.035

Appendix-4 The effect of native microorganisms on bacterial transport.

Media	Flow rate (mL/h)	B6914 $C_{influent}$ ($X10^6$ CFU/mL)	B6914 $C_{effluent}/C_{influent}$ at time t								Removal efficiency of B6914 (%)
			0	0.5 h	1 h	1.5 h	2 h	3 h	4 h	5 h	6 h	
CMB made by 1-yr media	41.7	1.22 ± 0.16	0	0.012	0.064	0.101	0.145	0.142	0.147	0.103	0.100	88.7
	41.2	0.96 ± 0.13	0	0.005	0.162	0.210	0.185	0.220	0.189	0.200	0.220	81.8
	40.0	1.57 ± 0.12	0	0.044	0.194	0.220	0.216	0.265	0.330	0.266	0.294	75.8
	40.1	1.38 ± 0.17	0	0.013	0.052	0.114	0.177	0.196	0.247	0.217	0.164	83.2
CMB made by 2-yr media	40.2	1.19 ± 0.12	0	0.076	0.226	0.344	0.372	0.392	0.586	0.660	0.575	56.3
	40.2	1.19 ± 0.12	0	0.052	0.303	0.424	0.537	0.460	0.626	0.633	0.598	50.9
	40.6	1.12 ± 0.19	0	0.104	0.263	0.417	0.431	0.392	0.640	0.556	0.444	56.1
	40.4	1.12 ± 0.19	0	0.152	0.312	0.413	0.475	0.371	0.551	0.653	0.528	54.1
γ-sterile CBM	40.7	1.22 ± 0.16	0	0.020	0.219	0.249	0.315	0.481	0.522	0.520	0.471	61.4
	41.5	0.96 ± 0.13	0	0.044	0.292	0.467	0.509	0.592	0.490	0.503	0.560	53.7
	40.0	1.57 ± 0.12	0	0.119	0.252	0.335	0.317	0.452	0.712	0.772	0.790	50.0
	40.8	1.38 ± 0.17	0	0.083	0.267	0.442	0.450	0.595	0.710	0.564	0.557	50.1
γ-sterile CBM coated with native microorganisms	40.7	1.58 ± 0.36	0	0.083	0.501		0.275	0.718	0.448	0.553	0.636	56.6
	40.2	1.58 ± 0.36	0	0.083	0.277		0.228	0.447	0.569	0.495	0.580	63.2
	40.8	1.58 ± 0.36	0	0.083	0.360		0.268	0.409	0.588	0.353	0.540	64.8
	39.5	1.58 ± 0.36	0	0.100	0.351		0.353	0.524	0.637	0.407	0.435	60.6
γ-sterile CBM coated with native microorganisms containing reduced protozoa	40.3	1.07 ± 0.11	0.000	0.156	0.600		0.717	0.665	0.735	0.514	0.641	42.0
	40.2	1.07 ± 0.11	0.000	0.146	0.428		0.594	0.641	0.735	0.430	0.718	47.4
	40.5	1.07 ± 0.11	0.000	0.227	0.502		0.552	0.731	0.496	0.456	0.532	50.1
	41.6	1.07 ± 0.11	0.000	0.152	0.647		0.782	0.819	0.780	0.603	0.622	35.7

Appendix-5 The effect of native microorganisms on bacterial survival.

Media	Drainage time (days)	Media depth (cm)	Moisture (%)	Strain B6914			Heterotrophic bacteria			Protozoa	
				log CFU/g dry media	CFU/g dry media ($x10^5$)	Total CFU in each depth ($x10^6$)	log CFU/g dry media	CFU/g dry media ($x10^7$)	Total CFU in each depth ($x10^7$)	MPN/g dry media	Total MPN in each depth
CMB (made by 2-yr media)	0.5	0-2	19.68	5.709	5.117	7.275	6.953	0.90	12.75	432.8	6152.4
		2-4	18.55	5.594	3.931	5.729	6.990	0.98	14.26	1213.9	17691.3
		4-6	16.20	5.619	4.157	5.489	6.907	0.81	10.66	1354.1	17882.1
		6-9	21.99	5.413	2.588	4.996	7.061	1.15	22.19	469.4	9060.8
		9-13	23.50	5.315	2.068	5.545	7.187	1.54	41.27	837.2	22455.9
		13-17	30.04	4.987	0.970	2.410	7.100	1.26	31.25	815.8	20261.8
		17-21	29.80	4.907	0.807	2.432	6.992	0.98	29.61	437.1	13168.7
	1.5	0-2	20.80	5.284	1.924	2.582	7.306	2.02	21.28	3268.5	43858.3
		2-4	19.00	4.620	0.417	0.668	7.213	1.63	26.16	552.2	8848.5
		4-6	16.12	4.215	0.164	0.220	7.217	1.65	22.10	2426.5	32513.1
		6-9	21.96	3.986	0.097	0.196	7.143	1.39	28.24	1368.8	27802.0
		9-13	30.71	4.439	0.275	0.719	7.054	1.13	29.65	582.2	15235.7
		13-17	29.28	4.652	0.449	1.229	6.947	0.89	24.22	318.8	8724.0
		17-21	28.26	4.525	0.335	1.010	7.097	1.25	37.67	1242.8	37442.8
	2.5	0-2	14.12	4.852	0.711	0.895	7.002	1.00	12.64	1369.3	17236
		2-4	17.02	4.034	0.108	0.159	7.274	1.88	27.70	1173.4	17292
		4-6	17.14	3.854	0.071	0.103	7.094	1.24	17.96	2352.3	34016
		6-9	19.36	3.843	0.070	0.133	7.269	1.86	35.58	2393.6	45796
		9-13	24.46	3.571	0.037	0.092	7.162	1.45	35.98	2360.7	58494
		13-17	23.26	3.721	0.053	0.138	7.037	1.09	28.45	2414.7	63135
		17-21	29.40	3.916	0.082	0.248	7.297	1.98	59.71	2263.5	68194
	7	0-2	11.18	3.624	0.042	0.048	7.732	5.39	60.92	2951.3	36038
		2-4	13.70	2.609	0.004	0.006	7.623	4.19	62.29	3726.4	58161
		4-6	15.85	2.480	0.003	0.004	7.557	3.60	51.09	1773.8	24135
		6-9	19.31	2.468	0.002	0.006	7.606	4.03	85.23	8372.7	170744

		9-13	21.19	2.468	0.003	0.007	7.703	5.04	124.49	2254.2	57510
		13-17	28.14	2.086	0.001	0.003	7.632	4.29	110.21	2086.2	53015
		17-21	29.39	2.918	0.008	0.025	7.627	4.24	127.73	812.4	24477
γ-sterile CBM	0.5	0-2	4.82	5.938	8.665	14.635					
		2-4	6.23	6.093	12.383	18.601					
		4-6	9.62	6.270	18.639	26.782					
		6-9	16.12	5.912	8.165	18.002					
		9-13	24.46	5.710	5.130	14.581					
		13-17	23.85	5.319	2.086	5.927					
		17-21	24.03	5.494	3.116	9.388					
	1.5	0-2	3.79	6.014	10.321	15.661					
		2-4	8.56	5.944	8.787	15.313					
		4-6	11.50	6.208	16.131	23.240					
		6-9	19.82	5.998	9.956	21.182					
		9-13	28.46	5.702	5.031	15.025					
		13-17	25.05	5.291	1.954	5.980					
		17-21	24.61	4.562	0.365	1.098					
	2.5	0-2	3.03	5.691	4.913	7.163					
		2-4	4.16	6.556	35.952	59.615					
		4-6	5.27	6.478	30.074	44.848					
		6-9	7.52	6.437	27.375	61.533					
		9-13	16.38	5.848	7.042	20.878					
		13-17	22.31	4.373	0.236	0.705					
		17-21	21.80	3.498	0.032	0.095					
	7	0-2	20.09	5.693	4.936	5.566					
		2-4	23.58	6.272	18.720	35.102					
		4-6	25.58	6.471	29.591	45.747					
		6-9	26.46	6.457	28.620	62.704					
		9-13	28.70	6.050	11.227	34.037					
		13-17	28.39	4.498	0.314	0.963					

		17-21	25.83	3.061	0.012	0.035					
γ-sterile CBM coated with native microbes	0.5	0-2	10.43	6.254	17.950	23.486	6.953	0.897	12.75	432.8	6152
		2-4	10.53	6.399	25.039	34.991	6.990	0.978	14.26	1213.9	17691
		4-6	11.87	6.134	13.613	17.468	6.907	0.807	10.66	1354.1	17882
		6-9	12.60	6.313	20.554	37.581	7.061	1.150	22.19	469.4	9061
		9-13	19.29	5.293	1.961	4.984	7.187	1.539	41.27	837.2	22456
		13-17	23.98	5.162	1.452	3.550	7.100	1.258	31.25	815.8	20262
		17-21	23.18	4.964	0.921	2.774	6.992	0.983	29.61	437.1	13169
	1.5	0-2	8.47	5.191	1.553	1.887	7.306	2.023	21.28	3268.5	43858
		2-4	9.85	5.487	3.070	4.459	7.213	1.633	26.16	552.2	8849
		4-6	11.13	5.318	2.079	2.859	7.217	1.650	22.10	2426.5	32513
		6-9	11.48	5.257	1.807	3.185	7.143	1.391	28.24	1368.8	27802
		9-13	13.85	5.062	1.153	3.064	7.054	1.133	29.65	582.2	15236
		13-17	18.61	4.365	0.232	0.574	6.947	0.885	24.22	318.8	8724
		17-21	21.86	4.215	0.164	0.494	7.097	1.250	37.67	1242.8	37443
	2.5	0-2	8.14	5.440	2.752	3.274	7.002	1.004	12.64	1369.3	17236
		2-4	9.96	5.092	1.235	1.802	7.274	1.879	27.70	1173.4	17292
		4-6	9.86	5.004	1.009	1.330	7.094	1.242	17.96	2352.3	34016
		6-9	10.97	5.015	1.036	1.997	7.269	1.860	35.58	2393.6	45796
		9-13	13.22	4.715	0.519	1.272	7.162	1.452	35.98	2360.7	58494
		13-17	16.61	4.578	0.378	1.004	7.037	1.088	28.45	2414.7	63135
		17-21	21.45	3.959	0.091	0.274	7.297	1.982	59.71	2263.5	68194
	7	0-2	7.10	4.237	0.173	0.211	7.732	5.392	60.92	2951.3	36038
		2-4	8.68	4.092	0.123	0.193	7.623	4.195	62.29	3726.4	58161
		4-6	9.76	4.040	0.110	0.149	7.557	3.605	51.09	1773.8	24135
		6-9	10.56	3.861	0.073	0.148	7.606	4.032	85.23	8372.7	170744
		9-13	11.43	3.486	0.031	0.078	7.703	5.042	124.49	2254.2	57510
		13-17	14.73	2.829	0.007	0.017	7.632	4.290	110.21	2086.2	53015
		17-21	13.27	2.991	0.010	0.030	7.627	4.239	127.73	812.4	24477

γ-sterile CBM coated with native microbes containing with reduced protozoa	0.5	0-2	12.33	5.713	5.164	6.457	7.749	5.609	70.13	165.0	2064
		2-4	18.31	5.428	2.679	4.048	7.937	8.641	130.59	297.9	4502
		4-6	17.88	5.208	1.613	2.151	7.713	5.165	68.88	186.0	2480
		6-9	23.06	5.442	2.764	5.569	7.655	4.515	90.97	255.1	5139
		9-13	21.75	5.395	2.484	6.698	7.539	3.457	93.22	145.0	3908
		13-17	20.35	5.383	2.417	7.150	7.620	4.173	123.45	397.0	11745
		17-21	19.87	5.608	4.053	12.211	7.946	8.832	266.08	234.2	7055
	1.5	0-2	14.35	4.480	0.302	0.428	7.747	5.589	79.30	269.7	3827
		2-4	17.42	4.628	0.425	0.637	7.910	8.127	121.98	131.4	1973
		4-6	20.63	4.651	0.448	0.592	7.697	4.972	65.75	300.0	3967
		6-9	22.47	4.938	0.867	1.716	7.748	5.603	110.95	260.6	5161
		9-13	25.31	5.114	1.299	3.337	7.820	6.610	169.84	319.0	8197
		13-17	19.23	5.266	1.844	4.898	7.709	5.122	136.06	211.2	5612
		17-21	18.32	5.160	1.444	4.351	7.937	8.654	260.73	288.0	8678
	2.5	0-2	9.43	4.717	0.521	0.699	7.843	6.964	93.37	1352.5	18134
		2-4	12.57	4.561	0.364	0.579	7.748	5.600	89.20	364.8	5810
		4-6	15.16	4.264	0.184	0.250	7.668	4.655	63.53	578.1	7888
		6-9	17.39	4.164	0.146	0.288	7.723	5.286	104.41	303.8	6000
		9-13	17.78	4.755	0.569	1.585	7.640	4.364	121.71	230.6	6432
		13-17	17.96	4.830	0.676	1.824	8.067	11.655	314.48	116.5	3143
		17-21	16.00	4.383	0.242	0.728	7.761	5.772	173.91	395.4	11912
	7	0-2	12.98	3.270	0.019	0.025	7.524	3.344	41.81	1414.0	17682
		2-4	16.41	2.675	0.005	0.007	7.546	3.518	53.17	308.6	4664
		4-6	18.48	2.879	0.008	0.012	7.483	3.038	40.52	2794.2	37260
		6-9	17.98	3.925	0.084	0.169	7.636	4.322	87.07	1691.4	34076
		9-13	17.24	3.536	0.034	0.092	7.709	5.122	138.11	257.1	6933
		13-17	19.43	3.547	0.035	0.091	7.717	5.217	154.31	215.2	6364
		17-21	19.75	3.977	0.095	0.286	7.703	5.047	152.05	142.8	4303

Appendix-6 Bacterial transport in CBM during long-term experiments.

Column	Data of bacteria loading	Flow rate (L/h)	B6914 $C_{influent}$ ($x10^6$ CFU/mL)	Heterotrophic bacteria in the wash water ($x10^5$CFU/mL)	B6914 $C_{effluent}/C_{influent}$ at time t							Removal efficiency of B6914 (%)
					0	1 h	2 h	3 h	4 h	5 h	6 h	
2-month	4/4/2007	39	0.7 ± 0.13	4.9	0	0.35	0.38	0.14	0.34	0.29	0.29	70.2
	4/17/2007	40.8	1.6 ± 0.30	2.1	0	0.35	0.48	0.47	0.48	0.34	0.29	59.9
	5/2/2007	39.1	0.8 ± 0.21	2.4	0	0.44	0.36	0.51	0.37	0.38	0.49	57.4
	5/15/2007	41.8	1.1 ± 0.22	1.1	0	0.39	0.43	0.49	0.76	0.43	0.43	51.1
	6/4/2007	39.4	0.6 ± 0.08	1.0	0	0.29	0.32	0.32	0.60	0.48	0.36	60.5
	6/14/2007	39.6	1.0 ± 0.14	0.4	0	0.03	0.06	0.06	0.08	0.09	0.08	93.4
5-month	6/26/2007	38.7	0.5 ± 0.04	2.0	0	0.40	0.45	0.55	0.55	0.50	0.45	51.7
	7/6/2007	41	1.1 ± 0.2	2.6	0	0.36	0.37	0.42	0.46	0.55	0.42	57.1
	7/25/2007	39	0.6 ± 0.07	4.6	0	0.22	0.34	0.40	0.35	0.30	0.38	66.8
	8/7/2007	40.4	1.7 ± 0.25	6.9	0	0.15	0.31	0.28	0.29	0.35	0.27	72.5
	8/21/2007	38	0.5 ± 0.09	1.6	0	0.20	0.27	0.30	0.24	0.22	0.19	76.3
	9/4/2007	40.2	0.8 ± 0.25	1.0	0	0.17	0.32	0.29	0.36	0.21	0.28	72.8
	9/18/2007	38.8	1.1 ± 0.12	3.7	0	0.07	0.20	0.19	0.28	0.21	0.25	80.0
	10/3/2007	40.2	1.4 ± 0.34	3.5	0	0.12	0.19	0.17	0.16	0.12	0.18	84.3
	10/16/2007	42	1.2 ± 0.25	3.9	0	0.09	0.12	0.13	0.08	0.14	0.12	88.7
	10/31/2007	40.8	0.6 ± 0.07	5.6	0	0.14	0.15	0.25	0.20	0.08	0.16	83.7
	11/12/2007	40.8	1.3 ± 0.34	1.9	0	0.03	0.04	0.06	0.04	0.06	0.07	95.0
9-month	2/8/2007	37.6	0.6 ± 0.14	8.6	0	0.38	0.25	0.44	0.38	0.43	0.34	63.1
	2/21/2007	40.2	0.8 ± 0.17	3.4	0	0.17	0.31	0.29	0.23	0.23	0.16	77.0
	3/6/2007	39.9	0.9 ± 0.08	1.0	0	0.64	0.73	0.57	0.27	0.26	0.27	54.1
	3/21/2007	41.3	0.8 ± 0.09	1.9	0	0.64	0.73	0.57	0.27	0.26	0.27	54.1
	4/4/2007	40.7	0.7 ± 0.13	3.3	0	0.27	0.15	0.07	0.15	0.16	0.21	83.2
	4/17/2007	40.6	1.6 ± 0.30	0.3	0	0.06	0.09	0.09	0.12	0.10	0.12	90.4
	5/2/2007	40.1	0.8 ± 0.21	2.7	0	0.10	0.10	0.10	0.07	0.05	0.08	91.7
	5/15/2007	39.1	1.1 ± 0.22	0.9	0	0.04	0.07	0.06	0.07	0.05	0.06	94.0

	6/4/2007	40.2	0.6 ± 0.08	0.7	0	0.04	0.02	0.06	0.05	0.08	0.04	95.0
	6/14/2007	38.9	1.0 ± 0.14	1.3	0	0.02	0.03	0.03	0.03	0.04	0.03	97.0
	6/26/2007	40.3	0.5 ± 0.04	0.9	0	0.04	0.07	0.05	0.09	0.16	0.08	91.8
	7/6/2007	40.8	1.1 ± 0.2	3.9	0	0.06	0.02	0.03	0.03	0.04	0.03	96.5
	7/25/2007	39.9	0.6 ± 0.07	0.7	0	0.01	0.03	0.04	0.04	0.04	0.04	96.6
	8/7/2007	38.4	1.7 ± 0.25	0.6	0	0.01	0.00	0.02	0.01	0.01	0.02	98.9
	8/21/2007	39.5	0.5 ± 0.09	0.5	0	0.04	0.05	0.05	0.05	0.05	0.06	95.2
	9/4/2007	39.2	0.8 ± 0.25	0.7	0	0.07	0.08	0.09	0.11	0.10	0.09	91.0
	9/18/2007	43.6	1.1 ± 0.12	1.0	0	0.02	0.03	0.02	0.02	0.01	0.02	98.1
	10/3/2007	40.1	1.4 ± 0.34	4.2	0	0.04	0.05	0.05	0.06	0.05	0.05	95.4
	10/16/2007	38.3	1.2 ± 0.25	3.3	0	0.01	0.01	0.01	0.01	0.01	0.01	99.2
	10/31/2007	40.4	0.6 ± 0.07	2.2	0	0.02	0.06	0.05	0.06	0.08	0.06	94.7
13-month	6/1/2006	43	1.3 ± 0.10		0	0.03	0.12	0.14	0.09	0.12	0.13	89.5
	6/14/2006	39.8	1.0 ± 0.07		0	0.02	0.08	0.08	0.09	0.10	0.11	91.9
	6/28/2006	39.8	1.4 ± 0.13		0	0.01	0.05	0.05	0.07	0.08	0.07	94.3
	7/12/2006	40.6	0.8 ± 0.23		0	0.05	0.05	0.07	0.10	0.08	0.09	92.6
	7/27/2006	40.8	0.9 ± 0.12		0	0.00	0.01	0.03	0.04	0.03	0.04	97.6
	8/9/2006	39.3	0.8 ± 0.07		0	0.00	0.03	0.05	0.06	0.05	0.06	95.6
	8/23/2006	40.5	1.5 ± 0.19		0	0.00	0.03	0.04	0.05	0.04	0.05	96.5
	9/6/2006	40.5	0.6 ± 0.10		0	0.01	0.07	0.08	0.08	0.07	0.08	93.5
	9/19/2006	36.8	0.9 ± 0.06		0	0.00	0.02	0.03	0.03	0.03	0.04	97.4
	10/3/2006	41	1.6 ± 0.12		0	0.002	0.01	0.02	0.02	0.03	0.03	98.2
	10/17/2006	37.8	0.7 ± 0.03		0	0.04	0.05	0.05	0.06	0.05	0.05	95.4
	10/31/2006	39	0.6 ± 0.15		0	0.00	0.01	0.05	0.04	0.05	0.05	96.6
	11/14/2006	39.1	1.1 ± 0.22		0	0.004	0.01	0.01	0.02	0.02	0.04	98.2
	11/28/2006	40.7	1.4 ± 0.22		0	0.002	0.00	0.00	0.01	0.02	0.03	98.9
	12/12/2006	44.5	1.5 ± 0.10		0	0.004	0.01	0.01	0.02	0.02	0.04	98.1
	1/3/2007	41	1.0 ± 0.11		0	0.006	0.01	0.02	0.02	0.02	0.03	98.2
	1/9/2007	39.9	1.0 ± 0.09		0	0.001	0.00	0.01	0.01	0.02	0.02	99.0
	1/24/2007	39.2	1.6 ± 0.10		0	0.008	0.01	0.01	0.02	0.02	0.03	98.2
	2/8/2007	38.4	0.6 ± 0.14	1.5	0	0.001	0.01	0.00	0.00	0.07	0.03	98.0

	2/21/2007	39.8	0.8 ± 0.17	3.2	0	0.000	0.00	0.00	0.00	0.01	0.00	99.7
	3/6/2007	37.6	0.9 ± 0.08	3.6	0	0.005	0.01	0.02	0.02	0.02	0.01	98.6
	3/21/2007	38.4	0.8 ± 0.09	4.0	0	0.036	0.04	0.08	0.08	0.10	0.09	93.1
	4/4/2007	38.6	0.7 ± 0.13	2.1	0	0.000	0.001	0.000	0.004	0.003	0.017	99.6
	4/17/2007	38.7	1.6 ± 0.30	2.1	0	0.005	0.024	0.024	0.032	0.029	0.031	97.6
	5/2/2007	37.9	0.8 ± 0.21	2.0	0	0.003	0.002	0.003	0.003	0.004	0.008	99.6
	5/15/2007	36.4	1.1 ± 0.22	2.8	0	0.000	0.000	0.000	0.001	0.003	0.000	99.9
	6/4/2007	39	0.6 ± 0.08	1.4	0	0.001	0.001	0.001	0.001	0.001	0.003	99.9
	6/14/2007	39	1.0 ± 0.14	0.6	0	0.000	0.000	0.000	0.000	0.000	0.000	100.0
	6/26/2007	38	0.5 ± 0.04	0.5	0	0.008	0.015	0.007	0.015	0.026	0.021	98.5
	7/6/2007	40.3	1.1 ± 0.2	4.0	0	0.00	0.00	0.00	0.00	0.00	0.01	99.8
18-month	6/1/2006	40.2	1.3 ± 0.10		0	0.20	0.27	0.30	0.24	0.22	0.19	76.3
	6/14/2006	39.1	1.0 ± 0.07		0	0.08	0.23	0.20	0.24	0.31	0.32	77.0
	6/28/2006	40	1.4 ± 0.13		0	0.07	0.15	0.19	0.16	0.21	0.15	84.4
	7/12/2006	39.5	0.8 ± 0.23		0	0.03	0.09	0.11	0.17	0.10	0.10	90.0
	7/27/2006	39.7	0.9 ± 0.12		0	0.02	0.06	0.06	0.08	0.07	0.04	94.6
	8/9/2006	38.3	0.8 ± 0.07		0	0.02	0.06	0.10	0.09	0.09	0.11	92.1
	8/23/2006	38.9	1.5 ± 0.19		0	0.03	0.14	0.16	0.12	0.12	0.15	87.9
	9/6/2006	37.9	0.6 ± 0.10		0	0.04	0.12	0.13	0.14	0.16	0.17	87.2
	9/19/2006	38.8	0.9 ± 0.06		0	0.04	0.05	0.05	0.06	0.05	0.05	95.4
	10/3/2006	39.5	1.6 ± 0.12		0	0.00	0.01	0.05	0.04	0.05	0.05	96.6
	10/17/2006	38.7	0.7 ± 0.03		0	0.02	0.08	0.08	0.09	0.10	0.11	91.9
	10/31/2006	41.6	0.6 ± 0.15		0	0.02	0.09	0.01	0.10	0.12	0.13	92.0
	11/14/2006	43.6	1.1 ± 0.22		0	0.02	0.06	0.05	0.06	0.08	0.13	93.4
	11/28/2006	39.8	1.4 ± 0.22		0	0.00	0.01	0.01	0.07	0.01	0.02	98.2
	12/12/2006	40.9	1.5 ± 0.10		0	0.02	0.06	0.05	0.06	0.08	0.15	93.0
	1/3/2007	39.8	1.0 ± 0.11		0	0.010	0.009	0.013	0.002	0.003	0.003	99.3
	1/9/2007	40.2	1.0 ± 0.09		0	0.003	0.009	0.013	0.111	0.025	0.034	96.8
	1/24/2007	41.5	1.6 ± 0.10		0	0.002	0.003	0.006	0.077	0.011	0.016	98.1
	2/8/2007	38	0.6 ± 0.14	0.7	0	0.001	0.001	0.003	0.001	0.002	0.002	99.8
	2/21/2007	39.3	0.8 ± 0.17	2.2	0	0.000	0.000	0.001	0.000	0.001	0.000	100.0

	3/6/2007	39.4	0.9 ± 0.08	1.4	0	0.006	0.006	0.011	0.002	0.003	0.003	99.5
	3/21/2007	38.5	0.8 ± 0.09	1.7	0	0.009	0.012	0.011	0.009	0.018	0.020	98.7
	4/4/2007	39.4	0.7 ± 0.13	1.2	0	0.005	0.008	0.003	0.004	0.003	0.005	99.5
	4/17/2007	40.7	1.6 ± 0.30	0.5	0	0.002	0.003	0.005	0.006	0.003	0.004	99.6
	5/2/2007	35.5	0.8 ± 0.21	1.8	0	0.000	0.000	0.000	0.001	0.001	0.002	99.9
	5/15/2007	39.5	1.1 ± 0.22	0.1	0	0.000	0.000	0.000	0.001	0.001	0.000	100.0
	6/4/2007	39	0.6 ± 0.08	0.4	0	0.000	0.000	0.000	0.000	0.000	0.000	100.0
	6/14/2007	36.8	1.0 ± 0.14	0.3	0	0.000	0.000	0.000	0.000	0.000	0.000	100.0
	6/26/2007	40.3	0.5 ± 0.04	0.5	0	0.009	0.007	0.001	0.009	0.013	0.010	99.2
	7/6/2007	40.7	1.1 ± 0.2	0.2	0	0.000	0.000	0.000	0.000	0.000	0.000	100.0
	7/25/2007	39	0.6 ± 0.07	0.2	0	0.000	0.000	0.000	0.000	0.001	0.001	100.0
	8/7/2007	40.4	1.7 ± 0.25	0.2	0	0.000	0.000	0.000	0.000	0.000	0.000	100.0
	8/21/2007	40.5	0.5 ± 0.09	0.3	0	0.001	0.002	0.003	0.003	0.003	0.003	99.8
	9/4/2007	41	0.8 ± 0.25	0.3	0	0.000	0.000	0.000	0.000	0.000	0.005	99.9
	9/18/2007	38.8	1.1 ± 0.12	0.5	0	0.000	0.000	0.000	0.000	0.000	0.003	100.0
	10/3/2007	40.3	1.4 ± 0.34	0.1	0	0.000	0.000	0.000	0.000	0.000	0.006	99.9
	10/16/2007	41	1.2 ± 0.25	1.4	0	0.000	0.000	0.000	0.000	0.000	0.007	99.9
	10/31/2007	37.2	0.6 ± 0.07	2.9	0	0.000	0.001	0.002	0.001	0.001	0.001	99.9
	11/12/2007	40.3	1.3 ± 0.34	0.5	0	0.000	0.001	0.001	0.001	0.002	0.002	99.9
	11/26/2007	40.1	1.0 ± 0.38	0.4	0	0.000	0.000	0.000	0.000	0.001	0.001	100.0

Appendix-7 The bromide breakthrough and best-fit parameters in columns packed with different aged CBM.

Column		Bromide $C_{effluent}/C_{influent}$ at time t												flow rate (mL/h)	Best-fit parameters		
		0	0.25	0.5	0.75	1	1.25	1.5	2	3	4	5	6		ν (cm/h)	ε	D (cm^2/h)
2-month	Experimental data	0	0.000	0.000	0.051	0.569	0.912	0.996	1.022	1.000	1.002	1.000	1.000				
	model-fit	0	0.000	0.000	0.0636	0.561	0.927	0.994	1.000	1.000	1.000	1.000	1.000	40.7	21.95	0.378	7.1
5-month	Experimental data	0	0.002	0.052	0.336	0.725	0.900	0.996	1.000	1.000	1.000	1.000	1.000				
	model-fit	0	0.000	0.216	0.619	0.950	0.995	1.029	1.000	1.000	1.000	1.000	1.000	40.7	23.04	0.36	13.88
9-month	Experimental data	0	0.000	0.000	0.264	0.743	0.904	0.953	1.000	1.000	1.000	1.002	1.000				
	model-fit	0	0.000	0.014	0.285	0.713	0.925	0.985	1.000	1.000	1.000	1.000	1.000	40.7	24.25	0.342	17.38
13-month	Experimental data	0	0.000	0.044	0.235	0.597	0.771	0.900	1.003	1.010	1.000	1.000	1.000				
	model-fit	0	0.000	0.022	0.242	0.583	0.819	0.932	1.000	1.000	1.000	1.000	1.000	40.3	22.06	0.372	24.8
18-month	Experimental data	0	0.000	0.050	0.350	0.741	0.851	0.960	1.003	1.000	1.000	1.002	1.000				
	model-fit	0	0.000	0.052	0.363	0.704	0.890	0.964	0.997	1.000	1.000	1.000	1.000	40.1	24.52	0.333	28.75

Appendix-8 The survival of trapped strain B6914 and other microbial composition over time in columns packed with different aged CBM.

Column	Media depth (cm)	Moisture (%)	Strain B6914			Heterotrophic bacteria			Protozoa	
			log CFU/g dry media	CFU/g dry media ($x10^5$)	Total CFU in each depth ($x10^6$)	log CFU/g dry media	CFU/g dry media ($x10^7$)	Total CFU in each depth ($x10^7$)	MPN/g dry media	Total MPN in each depth
2-month	0-2	8.96	6.189	15.451	22.122	7.065	1.162	16.634	3358.9	48093
	2-4	16.47	5.702	5.038	6.775	7.008	1.018	13.687	1150.2	15466
	4-6	18.83	5.685	4.841	7.554	7.108	1.284	20.033	1343.0	20958
	6-9	20.08	5.438	2.741	5.360	7.076	1.191	23.292	1068.7	20896
	9-13	18.94	5.342	2.199	6.582	7.021	1.051	31.452	1038.7	31093
	13-17	24.94	5.000	0.999	2.576	6.962	0.916	23.613	139.7	3600
	17-21	25.48	4.426	0.267	0.804	6.948	0.887	26.736	248.1	7475
5-month	0-2	10.83	5.957	9.061	15.034	7.096	1.246	20.680	2262.9	32696
	2-4	11.05	5.769	5.872	8.070	6.940	0.871	11.967	3007.4	44418
	4-6	15.00	5.777	5.982	8.725	6.981	0.958	13.969	2991.9	42791
	6-9	14.78	5.534	3.424	6.773	6.905	0.804	15.908	1083.3	21387
	9-13	18.36	5.527	3.368	9.006	7.012	1.028	27.481	1897.8	53949
	13-17	20.82	5.453	2.839	7.323	6.990	0.976	25.178	2352.6	65606
	17-21	25.84	4.639	0.436	1.312	7.030	1.071	32.276	2923.2	88071
9-month	0-2	11.84	5.748	5.596	8.086	6.990	0.977	14.120	2942.1	42509
	2-4	21.66	5.942	8.749	12.922	6.912	0.817	12.074	3007.4	44418
	4-6	18.00	5.482	3.032	4.336	7.097	1.250	17.880	2451.3	35059
	6-9	20.68	5.169	1.474	2.911	6.966	0.925	18.265	1856.5	36651
	9-13	23.59	5.018	1.042	2.962	6.858	0.721	20.501	1873.0	53244
	13-17	26.66	4.637	0.433	1.209	6.853	0.714	19.900	2505.9	69883
	17-21	27.31	3.657	0.045	0.137	6.791	0.619	18.638	3661.0	110298
13-month	0-2	20.09	5.436	2.273	3.109	7.032	1.077	14.730	704.0	10080

	2-4	23.58	6.020	8.736	11.682	7.173	1.489	19.913	2807.6	37754
	4-6	25.58	5.887	6.421	8.992	7.029	1.070	14.986	2202.0	34363
	6-9	26.46	5.895	6.548	14.018	6.873	0.746	15.977	2888.3	56476
	9-13	28.70	5.559	3.017	7.264	6.804	0.900	21.669	3984.7	119279
	13-17	28.39	4.708	0.426	1.090	7.005	1.011	25.891	2916.6	75172
	17-21	25.83	3.876	0.063	0.189	6.916	0.824	24.824	14206.8	428021
18-month	0-2	18.70	5.297	1.983	2.761	6.824	0.667	9.287	588.2	8190
	2-4	22.32	5.463	2.902	4.404	6.862	0.727	11.039	1148.1	17422
	4-6	24.17	6.002	10.041	12.713	6.700	0.502	6.351	2711.8	34333
	6-9	27.18	5.591	3.898	7.598	6.659	0.456	8.883	2982.5	58127
	9-13	28.26	5.738	5.469	14.720	6.790	0.617	16.615	1968.7	52991
	13-17	24.89	5.249	1.775	4.832	6.818	0.658	17.920	3804.9	103577
	17-21	24.92	4.159	0.144	0.434	6.871	0.743	22.400	3811.6	114836

Appendix-9 The effect of temperature on bacterial transport in CBM (made by 2-yr aged media).

T (ºC)	Flow rate (mL/h)	B6914 $C_{influent}$ ($X10^6$ CFU/mL)	B6914 $C_{effluent}/C_{influent}$ at time t									Removal efficiency of B6914 (%)
			0	0.5 h	1 h	1.5 h	2 h	3 h	4 h	5 h	6 h	
5	41.7	1.22 ± 0.16	0	0.103	0.278	0.342	0.386	0.561	0.625	0.561	0.671	52.8
	41.2	0.96 ± 0.13	0	0.105	0.337	0.453	0.572	0.696	0.751	0.524	0.600	45.2
	40.0	1.57 ± 0.12	0	0.076	0.276	0.623	0.456	0.434	0.777	0.773	0.668	45.7
	40.1	1.38 ± 0.17	0	0.085	0.467	0.557	0.763	0.517	0.568	0.573	0.728	44.4
15	40.2	1.19 ± 0.12	0	0.100	0.200	0.321	0.532	0.479	0.520	0.535	0.648	55.0
	40.2	1.19 ± 0.12	0	0.054	0.157	0.302	0.509	0.450	0.541	0.492	0.651	57.1
	40.6	1.12 ± 0.19	0	0.150	0.221	0.312	0.324	0.333	0.632	0.537	0.640	58.5
	40.4	1.12 ± 0.19	0	0.216	0.282	0.371	0.333	0.335	0.541	0.576	0.592	58.1
25	40.2	1.19 ± 0.12	0	0.076	0.226	0.344	0.372	0.392	0.586	0.660	0.575	56.3
	40.2	1.19 ± 0.12	0	0.052	0.303	0.424	0.537	0.460	0.626	0.633	0.598	50.9
	40.6	1.12 ± 0.19	0	0.104	0.263	0.417	0.431	0.392	0.640	0.556	0.444	56.1
	40.4	1.12 ± 0.19	0	0.152	0.312	0.413	0.475	0.371	0.551	0.653	0.528	54.1
37	40.7	1.58 ± 0.36	0	0.219	0.457	0.420	0.395	0.638	0.575	0.508	0.668	50.0
	40.2	1.58 ± 0.36	0	0.016	0.029	0.350	0.281	0.463	0.388	0.354	0.353	69.0
	40.8	1.58 ± 0.36	0	0.027	0.402	0.337	0.308	0.395	0.438	0.406	0.433	64.2
	39.5	1.58 ± 0.36	0	0.002	0.365	0.325	0.284	0.452	0.327	0.313	0.333	68.5

Appendix-10 The effect of temperature on the survival of trapped strain B6914 and microbial composition in CBM (made by 2-yr aged media).

T (ºC)	Drainage time (days)	Media depth (cm)	Moisture (%)	Strain B6914			Heterotrophic bacteria			Protozoa	
				log CFU/g dry media	CFU/g dry media ($x10^5$)	Total CFU in each depth ($x10^6$)	log CFU/g dry media	CFU/g dry media ($x10^7$)	Total CFU in each depth ($x10^7$)	MPN/g dry media	Total MPN in each depth
5	0.5	0-2	17.62	5.709	6.381	24.023	6.781	0.604	8.476	134.1	1881
		2-4	17.58	5.594	6.387	24.378	6.829	0.674	9.499	210.3	2963
		4-6	18.61	5.619	6.218	16.521	6.746	0.557	7.757	224.9	3130
		6-9	22.58	5.413	6.096	12.479	6.716	0.520	10.639	58.3	1191
		9-13	23.33	5.315	5.981	9.568	6.860	0.900	25.611	192.6	5481
		13-17	22.35	4.987	5.942	8.755	6.746	0.557	15.532	80.0	2233
		17-21	21.62	4.907	5.928	8.470	6.670	0.467	14.080	578.1	17418
	7	0-2	15.71	5.963	9.185	12.008	6.982	0.960	12.546	218.6	2857
		2-4	17.67	6.124	13.312	20.263	7.090	1.231	18.739	363.7	5536
		4-6	18.23	6.148	14.049	20.912	6.959	0.909	13.531	133.6	1989
		6-9	21.89	6.035	10.830	22.979	7.293	1.963	41.642	183.1	3885
		9-13	24.99	5.714	5.173	14.656	6.950	0.900	25.496	300.8	8522
		13-17	25.98	5.691	4.905	13.886	7.270	1.862	52.696	484.1	13705
		17-21	25.24	5.861	7.259	21.870	7.089	1.228	37.011	383.1	11541
	14	0-2	12.61	4.947	0.886	1.393	7.037	1.089	17.125	642.1	10094
		2-4	13.94	5.149	1.409	1.936	6.926	0.844	11.603	441.3	6066
		4-6	14.28	5.151	1.417	1.861	6.759	0.574	7.535	534.2	7018
		6-9	17.59	5.214	1.637	3.391	6.830	0.677	14.018	436.9	9051
		9-13	19.70	4.976	0.945	2.686	6.675	0.900	25.572	832.6	23658
		13-17	23.03	4.970	0.933	2.740	7.001	1.003	29.463	362.2	10638
		17-21	23.35	5.034	1.083	3.262	7.146	1.399	42.136	3272.7	98599
	21	0-2	14.05	4.959	0.910	1.128	7.152	1.419	17.585	1338.4	16588
		2-4	21.34	5.093	1.240	2.020	7.068	1.170	19.061	1324.2	21577

		4-6	19.54	4.889	0.775	1.072	7.096	1.248	17.256	1768.7	24458
		6-9	22.71	4.773	0.593	1.284	7.082	1.209	26.174	2116.6	45829
		9-13	25.20	4.816	0.655	1.726	7.072	0.900	23.704	2934.9	77297
		13-17	28.44	5.056	1.137	3.418	6.822	0.663	19.937	567.0	17043
		17-21	29.39	5.030	1.072	3.230	6.857	0.719	21.653	471.3	14199
15		0-2	16.74	6.121	13.200	17.111	6.768	0.586	7.591	288.4	3738
		2-4	17.93	6.536	34.372	55.106	6.860	0.724	11.608	281.8	4518
		4-6	16.88	6.401	25.149	31.081	6.759	0.574	7.089	842.0	10406
	0.5	6-9	22.68	6.424	26.543	42.984	6.700	0.501	8.117	429.2	6950
		9-13	20.51	5.845	6.997	15.532	6.695	0.900	19.978	221.5	4916
		13-17	24.75	5.671	4.691	15.025	6.897	0.789	25.274	190.1	6090
		17-21	24.12	5.739	5.481	16.513	6.755	0.568	17.124	206.8	6232
		0-2	10.58	5.489	3.085	4.275	6.562	0.365	5.053	530.6	7354
		2-4	18.97	6.045	11.094	19.034	7.067	1.168	20.035	283.2	4859
		4-6	13.34	5.834	6.826	8.952	6.856	0.718	9.423	888.2	11648
	3.5	6-9	22.40	6.003	10.076	20.216	7.026	1.061	21.280	374.2	7508
		9-13	22.48	5.812	6.482	18.190	6.834	0.900	25.255	1367.1	38362
		13-17	22.79	5.952	8.948	24.424	6.974	0.942	25.709	295.2	8057
		17-21	20.51	6.135	13.632	41.072	7.157	1.435	43.234	366.1	11030
		0-2	11.14	5.098	1.253	2.042	7.239	1.735	28.275	1957.0	24540
		2-4	11.54	5.237	1.724	2.601	7.233	1.711	25.805	627.7	7284
		4-6	10.59	5.029	1.068	1.555	7.204	1.601	23.294	213.8	51215
	7	6-9	16.04	5.038	1.091	2.158	7.141	1.385	27.387	4566.2	19791
		9-13	18.71	5.162	1.451	4.152	7.218	0.900	25.742	2928.8	64439
		13-17	21.69	6.031	10.733	30.596	7.082	1.206	34.393	1294.6	28387
		17-21	22.79	6.037	10.898	32.834	6.939	0.869	26.167	1834.0	42503
	14	0-2	7.82	5.257	1.807	2.505	7.035	1.084	15.030	1319.4	18294
		2-4	7.35	4.842	0.696	1.109	7.049	1.120	17.849	437.6	6976
		4-6	11.75	4.746	0.557	0.727	7.134	1.360	17.781	3907.0	51063
		6-9	12.35	4.399	0.250	0.502	7.137	1.372	27.501	4145.3	83089

		9-13	13.89	4.836	0.686	2.008	6.617	0.900	26.343	998.8	29234
		13-17	18.87	5.201	1.589	4.614	7.020	1.047	30.415	1175.5	34140
		17-21	20.74	5.144	1.392	4.194	7.007	1.016	30.610	1742.4	52494
25		0-2	19.68	5.709	5.117	7.275	6.953	0.90	12.75	432.8	6152
		2-4	18.55	5.594	3.931	5.729	6.990	0.98	14.26	1213.9	17691
		4-6	16.20	5.619	4.157	5.489	6.907	0.81	10.66	1354.1	17882
	0.5	6-9	21.99	5.413	2.588	4.996	7.061	1.15	22.19	469.4	9061
		9-13	23.50	5.315	2.068	5.545	7.187	1.54	41.27	837.2	22456
		13-17	30.04	4.987	0.970	2.410	7.100	1.26	31.25	815.8	20262
		17-21	29.80	4.907	0.807	2.432	6.992	0.98	29.61	437.1	13169
		0-2	20.80	5.284	1.924	2.582	7.306	2.02	21.28	3268.5	43858
		2-4	19.00	4.620	0.417	0.668	7.213	1.63	26.16	552.2	8849
		4-6	16.12	4.215	0.164	0.220	7.217	1.65	22.10	2426.5	32513
	1.5	6-9	21.96	3.986	0.097	0.196	7.143	1.39	28.24	1368.8	27802
		9-13	30.71	4.439	0.275	0.719	7.054	1.13	29.65	582.2	15236
		13-17	29.28	4.652	0.449	1.229	6.947	0.89	24.22	318.8	8724
		17-21	28.26	4.525	0.335	1.010	7.097	1.25	37.67	1242.8	37443
		0-2	14.12	4.852	0.711	0.895	7.002	1.00	12.64	1369.3	17236
		2-4	17.02	4.034	0.108	0.159	7.274	1.88	27.70	1173.4	17292
		4-6	17.14	3.854	0.071	0.103	7.094	1.24	17.96	2352.3	34016
	2.5	6-9	19.36	3.843	0.070	0.133	7.269	1.86	35.58	2393.6	45796
		9-13	24.46	3.571	0.037	0.092	7.162	1.45	35.98	2360.7	58494
		13-17	23.26	3.721	0.053	0.138	7.037	1.09	28.45	2414.7	63135
		17-21	29.40	3.916	0.082	0.248	7.297	1.98	59.71	2263.5	68194
	7	0-2	11.18	3.624	0.042	0.048	7.732	5.39	60.92	2951.3	36038
		2-4	13.70	2.609	0.004	0.006	7.623	4.19	62.29	3726.4	58161
		4-6	15.85	2.480	0.003	0.004	7.557	3.60	51.09	1773.8	24135
		6-9	19.31	2.468	0.002	0.006	7.606	4.03	85.23	8372.7	170744
		9-13	21.19	2.468	0.003	0.007	7.703	5.04	124.49	2254.2	57510
		13-17	28.14	2.086	0.001	0.003	7.632	4.29	110.21	2086.2	53015

		17-21	29.39	2.918	0.008	0.025	7.627	4.24	127.73	812.4	24477
37	0.5	0-2	13.86	5.051	1.126	1.449	1.501	1.460	18.788	457.3	5886
		2-4	14.46	4.807	0.641	0.979	7.029	1.798	27.473	339.6	5187
		4-6	14.34	4.700	0.501	0.766	6.953	1.564	23.920	2114.2	32327
		6-9	18.54	4.834	0.682	1.338	6.804	1.501	29.474	1837.6	36077
		9-13	18.57	4.438	0.274	0.696	6.617	0.900	22.851	2189.4	55589
		13-17	19.57	4.152	0.142	0.361	6.647	1.336	34.040	555.7	14161
		17-21	28.73	4.455	0.285	0.860	6.372	0.819	24.666	1305.3	39327
	1.5	0-2	16.68	4.146	0.140	0.174	7.319	2.082	25.860	850.6	10563
		2-4	17.31	3.730	0.054	0.088	7.165	1.461	23.783	1322.8	21538
		4-6	20.85	3.495	0.031	0.042	7.176	1.501	20.170	1387.3	18639
		6-9	18.82	3.464	0.029	0.058	7.107	1.278	25.421	1150.4	22879
		9-13	20.54	2.920	0.008	0.023	7.076	0.900	24.463	1107.2	30096
		13-17	26.11	3.006	0.010	0.028	6.700	0.501	13.889	560.4	15545
		17-21	25.37	2.749	0.006	0.017	6.652	0.449	13.530	1412.1	42544
	2.5	0-2	8.32	3.303	0.020	0.025	7.053	1.131	14.077	2130.7	26524
		2-4	14.37	3.051	0.011	0.019	7.010	1.024	17.146	2153.1	36052
		4-6	10.80	2.351	0.002	0.003	7.027	1.065	15.019	2081.4	29342
		6-9	12.32	2.626	0.004	0.008	7.020	1.046	20.544	1196.1	23490
		9-13	16.66	2.225	0.002	0.004	6.894	0.900	23.631	2754.6	72327
		13-17	21.09	2.357	0.002	0.006	6.772	0.592	16.350	2112.8	58358
		17-21	23.89	2.097	0.001	0.004	6.653	0.450	13.560	587.7	17707
	7	0-2	7.82	5.257	1.807	2.505	7.041	1.099	14.983	806.4	10997
		2-4	7.35	4.842	0.696	1.109	7.012	1.028	17.568	2663.5	45529
		4-6	11.75	4.746	0.557	0.727	7.044	1.107	14.010	2209.8	27960
		6-9	12.35	4.399	0.250	0.502	6.939	0.869	15.474	1798.0	32021
		9-13	13.89	4.836	0.686	2.008	6.916	0.900	25.769	1120.7	32087
		13-17	18.87	5.201	1.589	4.614	6.927	0.845	21.958	2374.2	61665
		17-21	20.74	5.144	1.392	4.194	6.979	0.952	28.681	577.5	17398

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