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Transport of Potential Microbial Source Tracking Markers in Sandy Material

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TRANSPORT OF POTENTIAL MICROBIAL SOURCE TRACKING MARKERS

IN SANDY MATERIALS

by

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ABSTRACT

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Groundwater, a primary source of drinking water for nearly half the people in the United States, can be contaminated by pathogenic bacteria from fecal materials causing outbreaks of waterborne illness. Therefore, early identification of the presence of fecal contamination in groundwater can help prevent such outbreaks, and determining whether bacteria originate from human or animal feces can narrow down the location of potential pollution sources, allowing timely remediation and reduced potential for future outbreaks.

Pathogens are found in relatively low concentration in feces leading to difficulties in their detection in groundwater samples. In addition, a wide variety of pathogenic bacteria and viruses may exist in feces making it costly to analyze groundwater directly for all potential pathogens. As a result, groundwater samples are routinely analyzed for non-pathogenic fecal indicator bacteria (FIB), which are used as a proxy for the potential contamination by fecal

pathogens. An ideal FIB would be abundant in the source material, easy and inexpensive to analyze, mobile in the subsurface so that it does not lag behind the pathogens, and host-specific to help identify the contaminant source.

Bacteria which can be identified as originating selectively from human vs nonhuman sources (animals) are especially helpful in determining the source of contamination when multiple potential sources are present. *Escherichia coli* (*E. coli*) has long been used as a FIB due to its abundance in fecal matter. However *E. coli* is found in many different hosts, which limits its use for source identification. Recent research has focused on identifying microbial source tracking (MST) bacteria which have markers that are specific to human or animal hosts, and these host-specific markers can be critical in early source identification efforts. This potential for MST is especially promising if combined with the other characteristics of an ideal FIB, such as abundance and mobility in the subsurface.

This research focuses on evaluating the subsurface mobility of two bacteria, *Enterococcus faecium* (*E. faecium*) and *Bacteriodes fragilis* (*B. fragilis*), in order to better understand their potential use as source-tracking FIB. These bacteria are both abundant in fecal matter and they have shown promise as having human-specific markers. We performed column experiments to compare their subsurface transport through sandy material. Bacteria with relatively high attachment to sand have lower mobility in groundwater and may therefore be less effective as early tracers of fecal contamination

The first part of our research compares two strains of *E. faecium*; one with and one without Enterococcal surface protein (Esp), a marker which recent research has linked to human

sources, to evaluate whether the presence of Esp affects bacterial attachment to sand. The results indicate that in water with neutral pH (~7.2) the presence of Esp is linked to increased attachment to sand, thereby reducing the mobility of the Esp positive *E. faecium*. Because indicator bacteria should have relatively high mobility, this increased attachment potentially decreases the usefulness of Esp for MST. The results are consistent with calculations using the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory of colloidal attachment, which predicts that attachment in bacteria with Esp should be greater than in those without Esp due to the presence of a higher energy barrier for the bacteria without Esp.

The second part of this research compares the transport of the common aerobic fecal indicator bacteria *E. coli*, which has had limited success in source tracking, to the much more abundant anaerobic *B. fragilis*, which has shown promise as a potential MST bacteria. The results indicate that in water with neutral pH and low total ionic strength conditions, both *E. coli* and *B. fragilis* have similar attachment to sand, but at high ionic strength, such as may be found in areas near the source of contamination, the *B. fragilis* has lower attachment (and thus potentially higher mobility) than *E. coli*. The XDLVO calculations indicate a secondary energy minimum exists at higher ionic strength for both bacteria. This secondary minimum, which is absent at low ionic strength, occurs at a distance of 1 to 20 nm from the sand surface and appears to be the result of compression of the electrostatic double layer. The depth of this energy minimum is greater for *E. coli* than for *B. fragilis*, leading to greater attachment in the *E. coli* than the *B. fragilis*.

Dedication

To my wonderful family;

my children Ian, Patrick, and Megyn have been waiting a long time to call me Dr. Mom,

and my wonderful husband Steve has been my rock throughout this long, long process …and coming from a geologist, that means a lot!

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A	Hamaker constant (J)
a_b	Radius of bacterial cells (nm)
AB	Lewis Acid-Base interaction
B. fragilis	*Bacteriodes fragilis*
C	Effluent concentration of bacterial cell suspension (cells/mL)
Co	Influent concentration of bacterial cell suspension (cell/mL)
CPS	Capsular polysaccharide complex
DLVO	classical Derjaguin-Landau-Verwey-Overbeek theory
e	Electron charge
E. faecium	*Enterococcus faecium*
E. coli	*Escherichia coli*
EDL	Electrostatic Double Layer interaction
Esp	Enterococcal surface protein
FIB	fecal indicator bacteria
$\Delta G\,\frac{AB}{h_0}$	Hydrophobicity interaction free energies per unit area (mJ/m^2)
ΔG_{iwi}	Free energy of interaction between two bacterial cells in water (mJ/m^2)

h	Separation distance between bacteria and sand grain (nm)
h_0	Minimum equilibrium distance between the cell and sand surface (nm)
i	Subscript representing known surface tension parameter for a substance
I	Ionic strength
k	Boltzmann's constant
k_d	First-order deposition (attachment) rate coefficient (min^{-1})
L	Length of the column representing the packed bed (cm)
LPS	Lipopolysaccharides
LW	Lifshitz/van der Waals interaction
MST	microbial source tracking
PCR	Polymerized chain reaction
PV	Pore volume for columns used in transport experiments
t	Time (min)
T	Absolute temperature (K)
TS	Tryptic Soy
v	Specific discharge (cm/min) = discharge (cm^3/min)/area (cm^2)
XDLVO	extended Derjaguin-Landau-Verwey-Overbeek theory

ZPS	zwitterionic polysaccharides (in *Bacteriodes* capsule)
γ_i^L	Interfacial tension parameter for probe liquid (*i*)
α	Attachment efficiency of bacteria-sand system
γ^+	Electron accepting interfacial tension parameter
γ^-	Electron donating interfacial tension parameter
γ^{LW}	LW interfacial tension parameter (mJ/m^2)
ε	Porosity of the sand
ε_0	Dielectric permittivity of vacuum
ε_w	Dielectric constant of water
η	single-collector contact efficiency (frequency bacterial cells strike sand)
θ	Contact angle (deg)
κ^{-1}	Debye length (nm)
λ	Characteristic wavelength (nm)
λ_w	Characteristic decay length for AB interactions in water (nm)
π	Pi (= 3.1415927…)
ρ	Soil bulk density (kg/m^3)
$\boldsymbol{\phi}^{AB}$	Acid-Base interaction energy (J)

$\boldsymbol{\phi}^{EDL}$	Electrostatic Double Layer interaction energy (J)
$\boldsymbol{\phi}^{LW}$	Lifshitz-van der Waals interaction energy (J)
$\boldsymbol{\phi}^{Total}$	Total interaction energy (J)
ψ_b	Surface potential of bacterial cells (V)
ψ_s	Surface potential of sand (V)

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CHAPTER 1. Introduction

Groundwater is the primary source of drinking water for more than forty percent of the U.S. population (Kenny, Barber et al. 2009), and approximately 72% of the groundwater used for drinking water purposes is not disinfected (Yates 1994, Hutson, Barber et al. 2004, Kenny, Barber et al. 2009). Shallow groundwater is susceptible to bacterial contamination from sources at and near the ground surface. In particular, microbial contamination often occurs when bacteria and viruses originating in fecal matter infiltrate into the groundwater system. It has been estimated that such pathogens in groundwater have caused over half of the outbreaks of waterborne illness in the United States (Herwaldt, Craun et al. 1992, Craun, Brunkard et al. 2010, Craun 2012).

1.1 Fecal Indicator Bacteria and Microbial Source Tracking

Detection of the actual pathogenic bacteria and viruses in groundwater can be problematic, because so many different types of bacteria are present in fecal matter that analyzing all possible pathogens is cost prohibitive. In addition, pathogens are orders of magnitude less abundant than non-pathogenic bacteria. According to the US Environmental Protection Agency (2012), the presence in groundwater of bacteria which are abundant in the gastro-intestinal tracts of most warm-blooded animals provides an indication that the groundwater is likely contaminated by fecal materials. Under the Groundwater Rule (USEPA 2007) public groundwater systems are tested for microbial contamination by analyzing for the presence of total coliforms, and if detected, further tested for *E. coli*, enterococci, or somatic coliphage.

These FIB can be used to provide a warning of the potential presence of human pathogens in groundwater. However these standard microbial analysis do little to determine the source of any FIB detected in groundwater samples.

Effective indicator bacteria should have a number of characteristics: they should be abundant in the source material, and be easy, quick and inexpensive to detect. They should also be mobile enough in the subsurface to travel from the contamination source to a point of human contact (such as a well or a surface water body) as quickly as the pathogens. And ideally they would be amenable to microbial source tracking (MST), so that they could be used to determine if the source of contamination has human or animal origins, in order to help track down the source of contamination to hasten the ability to remediate.

FIB in groundwater can originate from animal sources, such as concentrated animal feeding operations (CAFOs), manure spread on fields, dog parks, or areas of concentrated wildlife such as geese or gulls. They can also originate from human sources, such as leaky sewer lines or faulty septic systems. Human-derived waste contains more known human-specific pathogenic organisms than animal waste does (Scott 2002). Therefore identifying whether contamination is from a human or animal source can assist in the identification, management and mitigation of contamination sources. Unfortunately, many of the commonly used FIB, such as *E. coli*, are abundant in both humans and other animals and are therefore not useful in distinguishing the source of contamination.

More recently, research has focused on identifying host-specific markers which may be found in certain FIB (Ahmed, Goonetilleke et al. 2009, Ahmed, Sawant et al. 2009). The use of these

markers in tracing the origins of contaminated water samples is a current area of research (Scott 2002, Ahmed, Stewart et al. 2007, Ahmed, Goonetilleke et al. 2009, Wicki, Karabulut et al. 2011, Johanson, Feriancikova et al. 2012, Feriancikova, Bardy et al. 2013, Tian 2013). MST methods adhere to the premise that some enteric microorganisms demonstrate specific characteristics related to the host organism (specificity) and/or they have adapted differently to different host gut conditions, thereby enabling identification of the host species. Various methods have been employed to detect these markers from contaminated water samples, and to evaluate their ability to distinguish contamination from known sources (Bower, Scopel et al. 2005, Dick, Bernhard et al. 2005, Scott, Jenkins et al. 2005, Sauer, VandeWalle et al. 2011).

1.2 Motivation

Recent studies focused on the ability to use indicator bacteria to distinguish microbial sources indicates good potential for using *B. fragilis* for tracking human source markers (Bower, Scopel et al. 2005, Layton, McKay et al. 2006, Gawler, Beecher et al. 2007, Ahmad, Tourlousse et al. 2009, Mieszkin, Furet et al. 2009, Sauer, VandeWalle et al. 2011). Similarly, a protein specific to the *Enterococcus* bacteria, known as Enterococcal surface protein (Esp), has been found to be predominantly from human sources (Scott, Jenkins et al. 2005, Whitman, Przybyla-Kelly et al. 2007, Ahmed, Goonetilleke et al. 2009, Scott, Harwood et al. 2009), which makes Esp a potential candidate for MST. The mobility of these promising MST bacteria within the aquifer system is relatively unstudied, and has important implications for their effectiveness in the early detection of microbial contamination (Bolster, Walker et al. 2006). Bacteria which adhere to aquifer materials make poor indicators. Pathogens that are more mobile than the indicator

bacteria can be transported more readily in the groundwater system, potentially impacting drinking water supplies in advance of the indicators. Alternatively, indicator species with higher mobility can be transported more rapidly through the groundwater providing opportunities for earlier detection at source water locations, preventing potential contamination related outbreaks. Therefore understanding the mobility of potential MST bacteria in the subsurface has important implications in evaluating its potential effectiveness as an indicator bacteria.

E. coli are very abundant aerobic, Gram-negative bacteria found in the gut of warm blooded animals. The transport of *E. coli* within saturated porous media has been extensively studied (Walker, Redman et al. 2004, Foppen, van Herwerden et al. 2007, Bolster, Haznedaroglu et al. 2009, Kim and Walker 2009, Bolster, Cook et al. 2010, Foppen, Lutterodt et al. 2010), however these studies have had limited success in MST methods, and the usefulness of *E. coli* as a fecal indicator has been called into question due to its ability to replicate in the shallow subsurface, such as beach sands (Scott 2002, Kon, Weir et al. 2007). Relatively few studies have examined the transport of anaerobic species such as *Bacteriodes*, or the Gram-positive *Enterococcus* species (Schinner, Letzner et al. 2010). And although Esp may provide a human marker for MST, recent studies of hospital-derived bacteria indicate that Esp may also be involved in biofilm formation (Toledo-Arana, Valle et al. 2001, Eaton and Gasson 2002, Tendolkar, Baghdayan et al. 2004, Heikens, Bonten et al. 2007) suggesting that Esp may increase bacterial adherence to abiotic surfaces. Therefore Esp may also negatively affect the mobility of *Enterococcus* in the groundwater system. Understanding more about the mobility of *Bacteriodes* and *Enterococcus* in the subsurface, therefore, can provide valuable insights into their potential usefulness as FIB.

1.3 Objectives and Approach

The objective of this dissertation work is to evaluate the transport properties of several bacteria which may have strong potential as MST indicator bacteria. The selected bacteria include *B. fragilis* and *E. faecium*. Our research also included *E. coli* for comparison due to its prevalent use as a FIB. In our research, we performed laboratory-scale column experiments to examine the transport of *B. fragilis* and *E. faecium* within saturated sand packs, and evaluate how their mobility could impact their usefulness as FIB. The extended Derjaguin-Landau –Verwey-Overbeek (XDLVO) theory was used to evaluate bacterial attachment results. Additional information about each of the selected bacteria is provided in Chapter 2.

CHAPTER 2. Overview of Selected MST Fecal Indicator Bacteria

The bacteria selected for investigation in this study include *E. faecium*, because of its potential human-specific marker Esp, and *B. fragilis*, which has human-specific markers. These bacteria were specifically selected for their potential as indicator bacteria due to their abundance in the human intestinal tract, and due to recent research indicating their potential for MST. In addition, *E. coli* was included in the investigation due to its prevalent use as a groundwater FIB.

2.1 *Enterococcus faecium* and Esp

Enterococcus is a Gram-positive, facultatively anaerobic, non-motile spherical or ovoid bacterium that can occur in pairs or chains. It has pili and lacks obvious capsules (Hardy-Diagnostics 1996-2016, Mandlik, Gaspar et al. 2009). Gram-positive bacteria have a cell envelope consisting of a cell wall, which is composed of a relatively thick (15-18 nm) peptidoglycan layer sandwiched between the interior plasma membrane and the exterior lipopolysaccharide (LPS) capsule (Figure 2.1). Teichoic acids, which are unique to the Gram-positive cell wall, run perpendicular to the peptidoglycan sheets. *Enterococcus* species are generally from 0.6 to 2.5 nm diameter (Hardy-Diagnostics 1996-2016, Kokkinosa, Fasseas et al. 1998), are very common gut bacteria in humans and other mammals, and are also among the most prevalent fecal bacterial species found in the environment (Kühn, Iversen et al. 2003). This abundance is a strong factor in favor of their use as a FIB. Although generally commensal, some *Enterococcus* strains are opportunistic pathogens. Two species in particular, *E. faecalis* and *E. faecium*, are the most often detected in hospital-diagnosed infections. These nosocomial Enterococcal infections also generally detected Esp, which indicates that Esp is present in

human sources. Although *E. faecalis* has been the more commonly studied in terms of human infections, *E. faecium* is increasingly being recognized as a human pathogen (Huycke, Sahm et al. 1998, Eaton and Gasson 2002, Sadowy and Luczkiewicz 2014), and in recent years the relative proportion of *E. faecium* to *E. faecalis* is increasing in infections in the US and Europe (Sadowy and Luczkiewicz 2014). In addition, a particular strain of this protein found in *E. faecium* has been noted as a human specific marker (Scott, Harwood et al. 2009). Therefore in this study we focus on the transport properties of two strains of *E. faecium*; one with Esp and one without Esp.

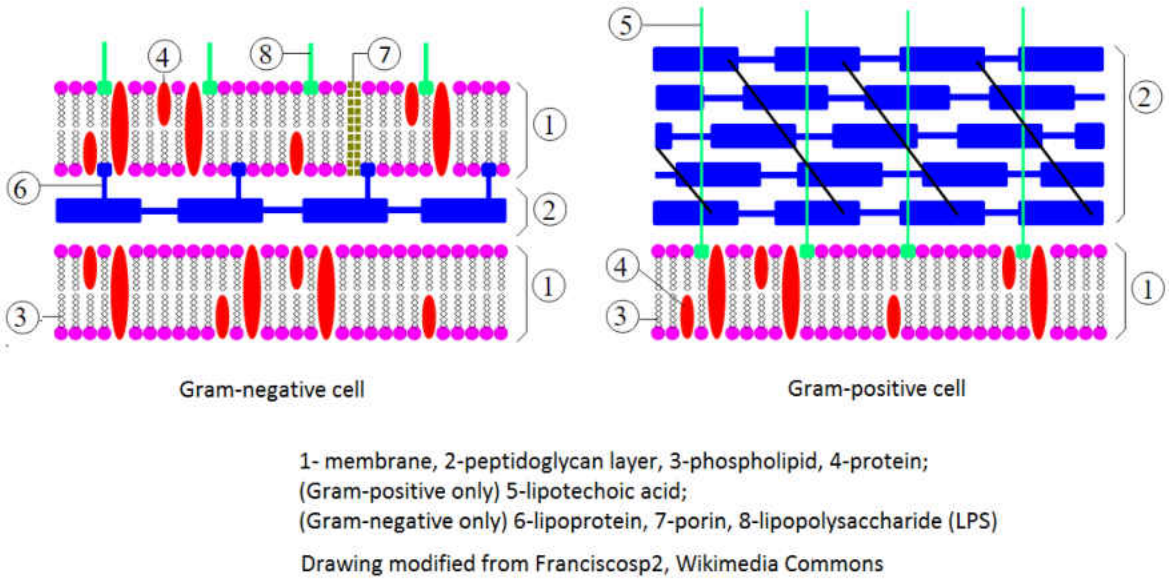

Figure 2.1. General structure of the cell envelope for Gram-positive and Gram-negative bacteria. The top of each drawing represents the outer surface of the cell, the bottom is the interior cell wall. An outer capsule may also be present on some bacteria.

Esp is a large surface protein without significant similarity to other known proteins. As is clear by its name, Esp is found on the outer surface of the Enterococcal cell envelope, where it

attaches to the outer peptidoglycan layer (Hendrickx, Willems et al. 2009). It contains a hydrophobic region that spans the cell membrane. The presence of Esp increases the hydrophobicity of the bacterial cell (Toledo-Arana, Valle et al. 2001). Recent studies have indicated that Esp is a virulence factor and Esp-expressing strains of *E. faecium* are becoming more common in hospital infections involving *E. faecium* (Eaton and Gasson 2002, Scott 2002, Hendrickx, Willems et al. 2009, Heikens, Singh et al. 2011, Sadowy and Luczkiewicz 2014), and importantly, Esp is generally found on *E. faecium* of human origins (Scott 2002, Scott, Jenkins et al. 2005, Van Wamel, P. et al. 2007, Ahmed, Goonetilleke et al. 2009, Hendrickx, Willems et al. 2009, Scott, Harwood et al. 2009) making this a candidate for MST.

We therefore focused particularly on the effects of Esp on the transport of *E. faecium* because: (1) Esp was reported to be highly associated with the ability of *Enterococcus* to form biofilms through facilitating cell attachment to various engineered materials such as polystyrene, polyvinyl chloride and polypropylene (Toledo-Arana, Valle et al. 2001, Tendolkar, Baghdayan et al. 2004, Heikens, Bonten et al. 2007, Hendrickx, Willems et al. 2009) and (2) recent results of polymerase chain reaction (PCR) assays suggested that *E. faecium* specific Esp gene (esp_{fm}) was more prevalent in sewage and septic system samples than livestock, wild animal, and bird samples and it could potentially be used as a molecular marker to identify human sources of fecal pollution (Scott, Jenkins et al. 2005, Brownell, Harwood et al. 2007, Ahmed, Goonetilleke et al. 2009, Hendrickx, Willems et al. 2009, Scott, Harwood et al. 2009, Masago, Pope et al. 2011). Some recent research cautions that Esp may not be consistent spatially or temporally as a human marker, but still suggest Esp is one potentially valuable line of evidence for source tracking (Whitman, Przybyla-Kelly et al. 2007, Byappanahalli, Przybyla-Kelly et al. 2008, Kim, Lee

et al. 2010, Johnston, Byappanahalli et al. 2013). Investigations into the transport of *E. faecium* with and without Esp within saturated quartz sands could therefore provide valuable information about the mobility of *E. faecium* from human and nonhuman sources. The findings also have useful implications for the effectiveness of this emerging tool as an MST index of human fecal pollution for the groundwater system.

2.2 *Bacteriodes fragilis*

B. fragilis is a rod-shaped obligate anaerobe that is Gram-negative, non-spore-forming, a found in abundance in the intestinal tract of humans and other animals. Fecal anaerobes are several orders of magnitude more prevalent in gastrointestinal tracts than fecal coliforms. *Bacteroides* is the most prevalent fecal anaerobe in the colon, and outnumbers E. coli by orders of magnitude (Todar 2008-2012). Although avoided in the past because of the difficulty of cultivation, anaerobic bacteria have been long suggested as alternative indicators for fecal contamination (Fiksdal, Maki et al. 1985), and qPCR-based analysis now allows detection in groundwater samples without the need for cultivation.

Gram-negative bacteria have a cell envelope that consists of a relatively thin (~10 nm) peptidoglycan layer sandwiched between an inner plasma membrane that is composed of a phospholipid bilayer, and an outer membrane. The outer membrane is also comprised of a bilayer structure similar to the inner membrane; however while the inner layer of this bilayer is comprised mainly of phospholipids similar to the inner plasma membrane, the outer layer contains some phospholipids, but also has abundant lipopolysaccharides (LPS) and proteins, along with porins (Figure 2.1). The outer LPS molecules, also known as endotoxins, are

amphiphilic (polar and nonpolar). Their makeup consists of a nonpolar lipid A "head" that is buried in the membrane, and a polar polysaccharide "tail" that extends into the aqueous environment. The tail is a core polysaccharide and an o-specific (o=outer) polysaccharide. LPS increase the negative charge of a cell membrane. Where lipid A connects to the polysaccharide tail an excess negative charge builds up, causing a magnesium ion to chelate between adjacent LPS molecules (Cooper and Hausman 2000, Todar 2008-2012).

The structure of *B. fragilis* includes a polysaccharide capsule and/or LPS side chains anchored in the outer membrane that form a visible fringe under transmission electron microscopy (Oyston and Handley 1991, Pumbwe, Skilbeck et al. 2006). This LPS fringe, also known as endotoxin in other Gram-negative bacteria (such as *E. coli*), is orders of magnitude less toxic in *B. fragilis*, and thus is generally not referred to as 'endotoxin' (Pumbwe, Skilbeck et al. 2006). Previous studies also noted the presence of peritrichous fimbrae (or pili) and a polysaccharide capsule (Oyston and Handley 1990, Oyston and Handley 1991, Wexler 2007). The Capsular Polysaccharide Complex (CPS) of *B. fragilis* is interesting in that it includes zwitterionic capsular polysaccharides (ZPS) whereas most carbohydrates (saccharides) are either neutral or negatively charged at physiologic pHs (Cobb and Kasper 2005). For example, PS A and PS B, each with repeating positively charged amino groups and negatively charged carboxyl or phosphate groups, were the ZPS first identified on *B. fragilis* (Tzianabos, Kasper et al. 1995). *Bacteriodes* also has the ability to modulate its surface polysaccharides (Wexler 2007). Either the polysaccharide capsule, the LPS outer layer, or the fimbrae may be responsible for adhesion (Wexler 2007). Previous research also indicates *B. fragilis* has low hydrophobicity (Oyston and Handley 1990).

Bacteriodes are promising as targets for MST assays not only because of their abundance, but because of apparent differences in strains as these bacteria have co-evolved with their hosts (Ballesté, Bonjoch et al. 2010, Johnston, Byappanahalli et al. 2013). As such, host-specific nucleotides in *B. fragilis* can be used in qPCR analysis to identify human sources (Ballesté, Bonjoch et al. 2010, Lee and Lee 2010, Alsalah, Al-Jassim et al. 2015). In addition, although some aerobic bacteria such as *E*. *coli* have been shown to persist and even grow outside the original hosts, *B. fragilis* is an obligatory aerobic bacteria so there is little concern over regrowth in the environment (Johnston, Byappanahalli et al. 2013). Experiments performed in aerobic surface water environments showed that detectable *B. fragilis* can survive for more than 6 days, and that the detection of *Bacteroides* spp. can be more sensitive than the enumeration of *E. coli*. More importantly, host-specific *Bacteriodes* markers such as the human-specific *Bacteroides* 16S rRNA gene can be used to help determine the source of fecal pollution (Fiksdal, Maki et al. 1985, Bernhard and Field 2000, Bower, Scopel et al. 2005, Layton, McKay et al. 2006, Gawler, Beecher et al. 2007, Ahmad, Tourlousse et al. 2009, Mieszkin, Furet et al. 2009, Alsalah, Al-Jassim et al. 2015). *Bacteriodes* spp. thus represents a promising groundwater fecal contamination indicator that may be useful for MST. Understanding variations in transport and attachment of these common fecal bacteria have important implications for the use of *Bacteriodes* spp. as MST indicator bacteria.

2.3 *Escherichia coli*

E. coli is a facultatively anaerobic, Gram-negative, rod-shaped, motile bacteria which has been extensively studied and is commonly used for FIB purposes. Although it comprises a small

proportion of the gut bacteria, it is the predominant facultative anaerobic organism in the human intestine (Todar 2008-2012). Its usefulness for tracking human sources, however, is not well established, with issues including lack of human specificity and potential to grow outside of the original host (Scott 2002, Ivanetich, Hsu et al. 2006, Beversdorf, Bornstein-Forst Sm Fau - McLellan et al. 2007, Kon, Weir et al. 2007).

The outer surface of *E*. *coli* is structured similar to other Gram-negative bacteria such as *B. fragilis*, as described in section 2.2, and shown on Figure 2.1. It generally has fimbrae (pili) as well as flagella. However, the strain used in this research, E. coli K-12 cells, lack O-antigen (the repeating polysaccharide component of LPS) (Walczak, Wang et al. 2012).

CHAPTER 3. The Extended Derjaguin, Landau, Verwey and Overbeek (XDLVO) Theory

3.1 Introduction

Groundwater flow has the potential to transport colloidal particles such as bacteria through a porous media. As the bacteria are transported, attachment to the porous media is a major mechanism which can immobilize the bacterium (Wu and Cheng 2016). Attractive and repulsive forces between and among the colloids and the surrounding media influence the likelihood of particle adhesion to the substrate. The DLVO theory of colloid stability was developed by Boris Derjaguin, Lev Landau, Evert Verwey and Jan Theodoor Overbeek in the 1940s to quantitatively address the behavior of colloids in an aqueous medium (Derjaguin and Landau 1941, Verwey, Overbeek et al. 1948).

Colloids are tiny particulate matter, generally in the range of 1 to 1000 nm, with an electrostatic surface charge (Fetter 1999). They tend to remain dispersed in liquid rather than settling out. Colloidal behavior has been extensively studied to evaluate whether these particles will move with the bulk solution, or be permanently or temporarily attached to the porous media through which they pass. Most bacterial cells have diameters ranging from 1 to 10 µm (Cooper and Hausman 2000), and bacteria are negatively charged in most natural aqueous environments due to their low point of zero charge (Wu and Cheng 2016). These similar charges repel one another, and the surface frictional forces in these bacterial particles are therefore greater than the gravitational force. Thus, bacteria in an aqueous solution will behave like colloids, remaining dispersed in the solution rather than settling out, and consequently their interaction energies can be modeled using the XDLVO method.

XDLVO theory is an extension of the classical DLVO theory which describes the interaction energies between particles immersed in an aqueous solution. The DLVO theory is based on the Derjaguin approximation, which relates the forces acting between two colloidal particles to the free energy between two plates. The total interaction energy between particles immersed in an aqueous solution can be described as the sum of the attractive and repulsive forces between the particles. Originally developed to describe energy interactions between identical colloidal particles, the DLVO theory has been adapted to predict forces between particles of different sizes and between different interfaces, such as a particle and a plane. When the particles in question have a very large size disparity, such as a bacteria and a sand grain, the particle/planar case serves as an appropriate approximation.

The classical DLVO theory models the free energy per unit area by adding the contributions of two physicochemical forces: the Lifschitz -van der Waals interaction (LW) force and the electric double layer (EDL) force. The strength of these interaction forces vary based on the separation distance between the particles. The DLVO theory can be extended when necessary to take other factors into account, such as hydrophobicity, which can be of particular importance in bacterial systems (Van Oss, Chaudhury et al. 1988). The modification is called the extended DLVO, or XDLVO theory, which also assumes that the total force acting on the particle is the sum of all acting forces. Each of the forces is described below.

3.2 Forces acting on the particles

3.2.1 Electrostatic Double Layer

At the interface between the negatively charged bacterium and sand particle that are immersed in an aqueous solution, a layer of positively charged dissolved ions is strongly attracted to the negatively charged particle. These counter-ions function as a screening layer between the negatively charged particle surface and the bulk solution. The resulting structure, which extends from the particle surface into the aqueous solution, is known as the electrostatic double layer (EDL). It consists of a thin inner layer of strongly adsorbed counter- ions at the particle's surface, known as the Stern layer, and an outer layer of counter- ions that becomes increasingly more diffuse with distance. The net charge in the double layer balances the net surface charge of the particle.

As shielding from the counter-ions increases with distance from the particle, at some distance the diffuse layer is no longer attracted strongly enough to the particle to overcome shear forces from the movement of the bulk fluid flowing past, and it is therefore not attached to the particle but is free to move with the fluid. This slippage distance, which is typically on the order of a few nanometers in a dilute solution, is where the zeta potential is measured, and this distance from the particle is known as the Debye length (κ^{-1}). It is reciprocally proportional to the square root of the solution concentration. Therefore the thickness of the double layer decreases with increasing solution concentration.

Because both the sand and bacterial cells have negatively charged surfaces (Mills, Herman et al. 1994, Wu and Cheng 2016), when a bacteria approaches a sand grain and their double layers

overlap it leads to a repulsive force between the particles, which is the EDL force in the XDLVO theory. This EDL repulsion decreases the potential for bacterial adhesion to the sand surface.

3.2.2 Lifschitz/van der Waals forces

The Lifschitz/van der Waals force (LW) is generally an attractive force that occurs when the bacteria are very close to the substrate; generally within about 20 nm (Tabor and Winterton 1968) and drops off rapidly with distance. LW forces occur as a result of dipole attractions which can occur between permanent or instantaneously induced dipoles, as described by Keesom force, Debye force, and London dispersion force.

The LW force between macroscopic objects such as bacteria and sand is proportional to the size of the bacteria and the magnitude of the Hamaker constant, which generally has a magnitude of 10^{-19} to 10^{-21} J. The value of the Hamaker constant is calculated from the interfacial tension parameters for sand, water, and the bacteria. These parameters can be characterized for a given bacteria by using contact angle measurements as described later. A larger bacterial radius or a larger Hamaker constant leads to an increased LW attraction.

3.2.3 Hydrophobic Interactions

In bacterial solutions, hydrophobicity is a major factor influencing particle adhesion. Hydrophobic interactions generally arise from electron-accepting and electron-donating Lewis acid–base interaction (AB force). The ability to form hydrogen bonds with water makes bacteria more hydrophilic and more likely to remain in the aqueous solution whereas lack of hydrogen

bonding capability leads to hydrophobic interactions. Hydrogen bond attractions are generally much stronger than dispersion forces, and as a result the hydrophobic force can be stronger than EDL or LW forces by an order of magnitude or more.

3.3 XDLVO Theory Overview

3.3.1 Energy barrier and primary energy minimum.

The classical DLVO theory models the free energy per unit area by adding the contributions of two physicochemical forces; the Lifschitz van der Waals interaction (LW) which is generally an attractive force, and the electrostatic double layer (EDL), which is repulsive in situations like a sand-bacteria system where the sand and bacteria both are negatively charged. The XDLVO theory also includes the hydrophobic (AB) force. The strength of these forces vary based on the separation distance between the particles, and using the XDLVO theory, each of these energies can be calculated at any given separation distance *h* between the sand and the bacterium, as illustrated in Figure 3.1, which represents the classical DLVO model. The repulsive EDL forces can act over longer distances than the attractive LW forces, thus setting up a net energy barrier to a colloid approaching a sand grain, and decreasing the chances of attachment. However at very close approach distances the strength of the LW forces are much greater than the EDL forces, overcoming the repulsive forces and creating a strong primary energy minimum, such that attachment occurring within the primary minimum is irreversible. The effect of a hydrophilic AB force is to increase the height of the energy barrier, whereas a hydrophobic AB force can decrease the energy barrier.

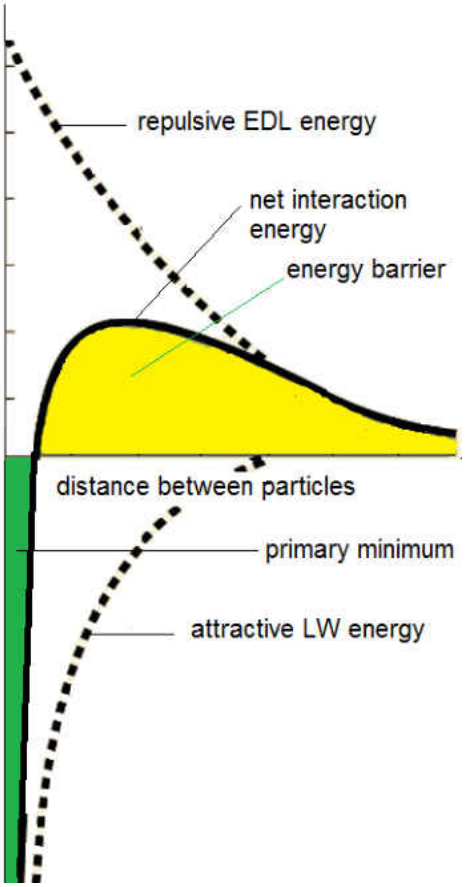

Figure 3.1 Diagram of interaction energy from the classical DLVO theory. Dashed lines represent the individual interaction energy values, and the solid line represents the net energy, which highlights the energy barrier and primary minimum.

3.3.2 Secondary energy minimum.

In general, the DLVO theory predicts the interaction forces are dominated by EDL repulsion at low ionic concentration such that an energy barrier deterring attachment exists at an approach distance that is based on where the EDLs begin to overlap. However at higher ionic concentrations the thickness of the EDL of the bacteria and sand are compressed such that the EDL overlap occurs when the interacting particles are at closer range, where the LW attractive forces are stronger. This can lead to a secondary net energy minimum farther from the sand

surface, with a magnitude less than the primary minimum. However this secondary minimum can be deep enough to attach the bacteria to the sand. This attachment may be reversible under appropriate conditions (e.g., when the ionic strength is lowered).

3.4 Calculating XDLVO Interaction Energy

The overall forces between the bacteria and the sand grain are described using the combined interaction energies of these components:

$$\Phi^{Total} = \Phi^{\mathrm{LW}} + \Phi^{\mathrm{EDL}} + \Phi^{\mathrm{AB}} \tag{3.1}$$

Where Φ^{LW} represents the LW interaction (Lifschitz-van der Waals dispersion forces), Φ^{EDL} represents the EDL interaction caused by the overlap of the double layers, i.e. the layer of positively charged ions from the electrolyte solution attracted closely to the negatively charged sand or bacteria surface, and ΦAB represents the AB interaction caused by hydrophobic or hydrophilic attractions between the bacterium and the sand and water. For the cell-sand geometry, the system interaction energies are represented as sphere-plate geometry (bacterium as the sphere and sand grain as a plate).

The values required to calculate interaction energies were determined through measuring cell size a_b, measuring zeta potential as a substitute for surface potential (Walker, Redman, Elimelich, 2004), and measuring contact angles to determine interfacial tension parameters for each type of bacteria. Details of such measurements and the corresponding calculations are included in chapter 4, where the cell properties of the *Enterococcus* and *Bacteroides* cells are examined.

CHAPTER 4. Materials and Methods

The column transport experiments and physicochemical measurements performed on the selected bacteria followed similar procedures. Common materials and methods are discussed below. Chapters 5 and 6 provide more specific details about each separate experiment.

4.1 General Procedure.

To compare the mobility of these bacteria in the subsurface, multiple column transport experiments were conducted under various ionic strength conditions. Column transport experiments are designed to provide information about the interactions between the bacteria in aqueous solution and the porous sand through which it flows. A solution with a known concentration of bacteria is injected into a sand-packed column, and the concentration of bacteria is measured after the solution exits the column. Bacteria can either travel with the solution, or attach to the sand surface. Each experiment was performed using bacteria suspended in an electrolyte solution prepared with a specific total ionic strength. The pH of the solution was tested both before and after the experiment to verify stable pH conditions during the experiment. Each column transport experiment was run in duplicate.

Additional measurements were required to determine the values of specific variables needed for the XDLVO calculations. The zeta potential and bacterial cell size measurements were determined for bacterial suspensions under each of the ionic strength conditions used in the experiments in order to calculate thermodynamic properties associated with Lifschitz - van der Waals forces and electrostatic double layer forces. Contact angle measurements were performed to evaluate the Lewis acid-base (AB) hydrophobic/hydrophilic tendencies of the

various bacteria by determining the interfacial tension parameters of the bacteria used in the experiments. These measurements are described in more detail below.

4.2 Preparation of Electrolyte and Bacterial Solutions.

The electrolyte solutions were prepared in a similar manner for each test condition. Four to five different ionic strengths, ranging from 1 mM to 50 mM, were selected to cover ranges for natural and contaminated groundwater. Solutions were prepared using sterile deionized water with 0.2 mM sodium bicarbonate ($NaHCO_3$), then adjusted to the desired total ionic strength using NaCl. For example, a buffered solution with a 5 mM total ionic strength had 0.2 mM $NaHCO_3$ and 4.8 mM NaCl. Buffered solutions had a pH of approximately 7.2, which is typical of groundwater in southeast Wisconsin (Masarik, Janke et al. 2006). The concentrations for the solutions used in our experiments are provided in subsequent chapters.

Bacterial solutions were prepared by introducing cultured bacteria into a prepared electrolyte solution of specific ionic strength and diluting the bacterial concentration to 4×10^7 cells/mL, which correlates to an absorbance of 0.3 at 220 nm. The dilution was made by first zeroing the spectrophotometer (Shimadzu UV-1700) using the electrolyte solution in a flow through cell, and then diluting the bacteria + electrolyte solution to an absorbance of 0.3. The bacterial solutions prepared in this manner were used for transport experiments, for zeta potential measurements and for cell size measurements.

The bacteria used to create the test solutions were grown in appropriate conditions (aerobic for *E. facium* and *E. coli*, and anaerobic for *B. fragilis*). The *E. coli* K-12 and *E. faceum* were cultured from preserved cells stored at -80°C, which was streaked on to Tryptic Soy (TS) agar plates and

incubated at 37°C overnight. One colony from the plate was transferred by sterile transfer loop into a sterile culture tube containing 15 ml sterile TS broth. This starter culture was incubated under agitation for six hours at 37° C. Then 0.5 ml of the starter culture was transferred into 250 ml sterile TS broth, and incubated under agitation at 37° C for an additional 18 hours (late exponential growth phase). They were then harvested through centrifugation (4000 g, 10 min, 4°C). The harvested bacterial pellets were rinsed four times using the appropriate electrolyte solution to remove the growth media (Payan, Ebdon et al. 2005, Feriancikova, Bardy et al. 2013)

The rinsed bacterial cell pellets were used to prepare cell suspensions for the column transport experiments, and to collect physicochemical measurements. Using the background electrolyte solutions to dilute the cell concentration, each suspension was adjusted to approximately 4×10^7 cells/ml by measuring the absorbance at a wavelength of 220 nm using a spectrophotometer. Column transport experiments were initiated three hours after harvesting.

4.3 Column Transport Experiments.

Quartz sand with a size range of 0.211-0.297 mm were used to represent aquifer media in the column transport experiments. Clean quartz sands have been frequently utilized to investigate specific conditions related to microbial transport within the subsurface system and this size fraction was selected because it represents a major portion of natural porous media (Redman, Walker et al. 2004, Walczak, Wang et al. 2012, Yang, Kim et al. 2012). Sand was cleaned by boiling in concentrated nitric acid for 24 hours to remove metal hydroxides, then soaking in diluted NaOH solution for 24 hours to remove natural clay particles, and boiling again using nitric acid to remove metal residues (Xu, Liao et al. 2008). Following each cleaning step, the

sand was thoroughly rinsed with deionized water. The clean sand was dried and then stored in high density polyethylene containers until used. The porosity of the sand was 0.369, as measured using the bulk density method (Weight 2008).

Column experiments were performed in vertically-oriented duplicate glass chromatography columns measuring 2.5 cm diameter and 15 cm length. A diagram of the equipment setup is shown in Figure 4.1. The clean quartz sand was wet-packed into the columns using the background electrolyte solution. Columns were equilibrated by flushing with more than 30 pore volumes (PV) of the bacteria-free electrolyte solution using peristaltic pumps. The flow rate was adjusted to a specific discharge of 0.31 cm/min prior to injection of the bacterial cell suspension. The bacterial cell suspension was injected into the top of the column for 60 minutes (~3.5 PV). Solution pH and concentration were measured prior to and following testing to identify changes in testing conditions. The results of these tests indicated that both pH and cell concentration were stable throughout the experiments.

The column effluent was connected to flow-through quartz cells in a spectrophotometer, and bacterial cell concentration in the effluent was monitored by measuring the effluent absorbance at a wavelength of 220 nm. Following injection the column was flushed with bacteria-free electrolyte solution until the effluent returned to background absorbance values (~1-2 PV).

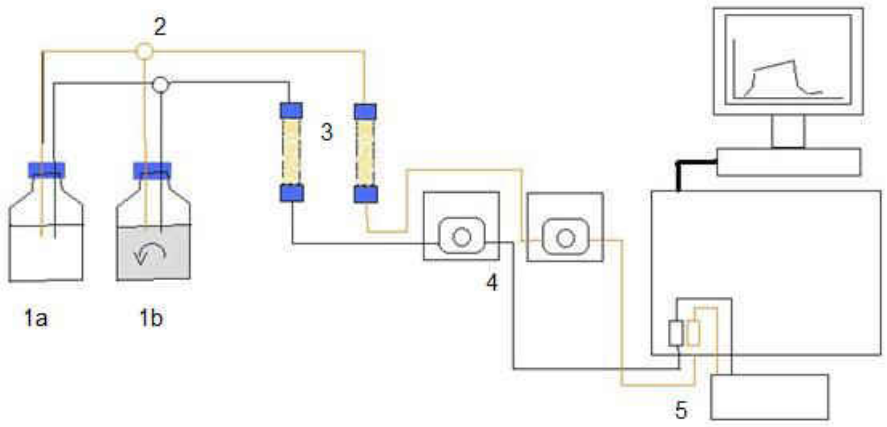

Figure 4.1. Diagram of column transport experiment setup. Solution flows from 1a (without bacteria) or 1b (with bacteria) which can be switched with a valve (2) to flow through the packed columns (3). Flow rate is controlled by the peristaltic pumps (4), and the effluent concentration is monitored at the flow-through cells in the spectrophotometer (5) connected to the computer.

4.4 Deposition Rate Kinetics.

The clean-bed deposition rate coefficients (k_d) which measure the rates at which bacterial cells were being removed from the aqueous phase under pristine conditions (i.e., the sand surfaces are free of bacterial cells) were estimated from the early cell breakthrough concentrations in the effluent (Kretzschmar, Barmettler et al. 1997, Walker, Redman et al. 2005, Castro and Tufenkji 2007)

$$k_d = -\frac{v}{\varepsilon L}\ln\left(\frac{C}{C_0}\right) \tag{4.1}$$

where ε is porosity, v is the specific discharge, L is the column length and C/C_0 is the normalized breakthrough concentration relevant to clean-bed conditions. The latter value was obtained from the average bacterial breakthrough concentrations between 1.8-2.0 PV, which is

consistent with many previous studies (Walker, Redman et al. 2005, Castro and Tufenkji 2007). Hydrodynamic dispersion of colloid-sized particles (such as the bacterial cells) has been previously shown to be negligible for relatively uniform sands such as were used in this experiment (Xu, Gao et al. 2006, Feriancikova, Bardy et al. 2013).

According to colloid-filtration theory (Yao, Habibian et al. 1971, Rajagopalan and Tien 1976, Tufenkji and Elimelech 2004), k_d is a function of the frequency at which the suspended cells strike the surface of the sands (the single-collector contact efficiency η) and the probability that a cell that strikes the surface of the sand will get attached (attachment efficiency α):

$$k_d = \frac{3(1-\varepsilon)v}{2d_g}\alpha\eta \tag{4.2}$$

where d_g is sand-grain diameter. The value of η depends on parameters such as fluid velocity and viscosity as well as cell size and density and can be estimated from empirical correlation equations (Yao, Habibian et al. 1971, Tufenkji and Elimelech 2004). The value of α is strongly dependent on the energy interactions between the bacterial cells and the surface of the quartz sands.

4.5 Cell Characterization.

The equivalent bacteria radius a_b was determined by capturing images of the cells in each electrolyte solution using a Nikon Eclipse 50i microscope equipped with a Photometric Coolsnap ES digital camera and Metamorph software. The length and width of at least 30 bacterial cells suspended in each electrolyte solution was determined using ImageJ software,

and the equivalent radii of the cells were calculated as $\left(\sqrt{\frac{L_c \times W_c}{\pi}}\right)$ where L_c and W_c represent the length and width of the cell, respectively.

Zeta potentials for bacterial solutions were measured using cell suspensions prepared with the background electrolyte solution similar to the column transport experiments. For the quartz sand, colloid size particles were prepared by pulverizing the sand, which was then suspended in the background electrolyte solutions. The zeta potential of the bacterial cells and the sand particles suspended in each solution was measured a minimum of five times using a ZetaPALS analyzer (Brookhaven Instruments Corporation).

4.6 Contact Angle Measurement.

The interfacial tension values for bacterial cells (γ^{LW}, γ^{+}, and γ^{-}) were determined by contact angle measurement using a Rame-Hart goniometer. A bacterial lawn was produced by vacuum filtering the bacterial solution onto a microfilter. A drop of probe liquid was applied to the lawn, and the contact angle (θ) between the liquid drop and the bacteria was measured (Figure 4.2).

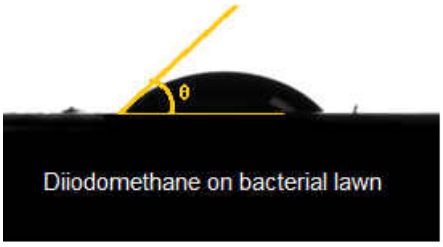

Figure 4.2. Contact angle measurement. Image shows a drop of diiodomethane on a lawn of bacteria, illustrating the contact angle measurement.

Three different probe liquids with known surface tension parameters (Table 4.1) were used, and contact angles between the liquid drop and the bacterial lawn were measured (Morrow, Stratton et al. 2005). The interfacial tension parameters for the bacteria (γ^{LW}, γ^{+} and γ^{-}) could then be calculated using a system of three equations, one for each probe liquid, to find the three unknown parameters:

$$\gamma_i^L(1+\cos\theta) = 2\sqrt{\gamma_i^{LW}\gamma^{LW}} + 2\sqrt{\gamma_i^{+}\gamma^{-}} + 2\sqrt{\gamma_i^{-}\gamma^{+}} \quad (4.3)$$

where the subscript *i* represents the probe liquid used. The three probe liquids used in this study include water, glycerol, and diiodomethane. The interfacial values of the probe liquids, which have been previously defined, are in Table 4.1.

Table 4.1. Interfacial surface tension parameters (mJ/m^2) for the sand-liquid interface for three liquids

liquid used for contact angle measurement	γ^L	γ^{LW}	γ^+	γ^-	γ^{AB}
water	72.8	21.8	25.5	25.5	51.0
glycerol	64.0	34.0	3.92	57.4	30.0
diiodomethane	50.8	50.8	0	0	0

4.7 XDLVO Interactions Between Bacterial Cells and Sand Surfaces

During the transport experiments, the bacterial cells either passed through the packed column along with the electrolyte solution, or became attached to the sand in the column. The relative bacterial concentration in the effluent compared to the influent solution (C/C_0) was used to monitor bacterial attachment as a known volume of water (pore volumes, PV) passed through

the column. The resulting relative bacterial concentrations at given pore volumes were used to calculate the XDLVO interaction energies Φ^{Total}, Φ^{LW}, Φ^{EDL}, and Φ^{AB}.

CHAPTER 5. Influence of Enterococcal Surface Protein (Esp) on the Transport of *Enterococcus faecium* within Saturated Quartz Sands

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5.1 Introduction

In the United States, approximately 40% of the population depends on groundwater as the primary source of drinking water (Kenny, Barber et al. 2009). Surveillance data collected by the Centers for Disease Control and Prevention (CDC) showed that untreated and inadequately treated groundwater was responsible for 52% of the waterborne disease outbreaks in the United States between 1971 and 2006 (Craun, Brunkard et al. 2010). Pathogens within the groundwater system thus represent a serious threat to public health (Yates 1994, Howard, Bartram et al. 2006). The fast and reliable detection of groundwater microbial contamination and the identification of the contamination sources are of critical importance to the protection of public health.

Fecal materials are the most common causes of groundwater microbial contamination. To identify groundwater fecal contamination, US EPA selected *E*. *coli*, enterococci, and coliphage as fecal indicator microorganisms because they are present in higher concentrations than pathogens in fecal materials; their detection is generally simple, reliable, and inexpensive; their presence often implies the presence of pathogens; and illness can result from the consumption of groundwater with fecal contamination in the absence of identified pathogens (USEPA 2007).

The transport behavior of the indicator microorganisms within the aquifer system has important implications for their effectiveness in the detection of groundwater microbial contamination (Bolster, Walker et al. 2006). As the transport of the Gram-negative *E. coli* within saturated porous media has been extensively studied (Walker, Redman et al. 2004, Foppen, van Herwerden et al. 2007, Torkzaban, Tazehkand et al. 2008, Bolster, Haznedaroglu et al. 2009, Kim and Walker 2009, Bolster, Cook et al. 2010, Foppen, Lutterodt et al. 2010), relatively few studies have examined the transport of the Gram-positive *Enterococcus* species (Schinner, Letzner et al. 2010). In this research, we performed laboratory-scale column experiments to examine the transport of *Enterococcus faecium*, which is increasingly being recognized as a human pathogen (Huycke, Sahm et al. 1998, Eaton and Gasson 2002), within saturated sand packs. Particularly, we focused on the effects of enterococcal surface protein (Esp) on the transport of *E. faecium* because: (1) Esp was reported to be highly associated with the ability of *Enterococcus* to form biofilms through facilitating cell attachment at various engineered materials such as polystyrene, polyvinyl chloride and polypropylene (Toledo-Arana, Valle et al. 2001, Tendolkar, Baghdayan et al. 2004); and (2) recent results of polymerase chain reaction (PCR) assays suggested that *E. faecium* specific Esp gene (esp_{fm}) was more prevalent in sewage and septic system samples than livestock, wild animal, and bird samples and it could potentially be used as a molecular marker to identify human sources of fecal pollution (Scott, Jenkins et al. 2005, Whitman, Przybyla-Kelly et al. 2007, Ahmed, Goonetilleke et al. 2009, Scott, Harwood et al. 2009). Investigations into the transport of *E. faecium* with and without Esp within saturated quartz sands could provide valuable information about the mobility of *E. faecium* from human

and nonhuman sources. The findings will also have important implications for the effectiveness of this emerging tool as an index of human fecal pollution for the groundwater system.

5.2 Materials and Methods

5.2.1 *E. faecium* Strains and the Preparation of Cell Suspensions

The Esp -negative *E. faecium* mutant (E1162Δesp) used in this research was created from the wild type strain (E1162) using the insertion-deletion approach (Heikens, Bonten et al. 2007). The wild-type strain E1162 was originally isolated from the blood of a patient (Heikens, Bonten et al. 2007). Preserved cells stored in 20% glycerol at –80 °C were streaked onto Tryptic soy (TS) agar (Difco Laboratories) plates, which were incubated at 37 °C overnight. One single colony from each plate (E1162 or E1162Δesp) was transferred by a sterile transfer loop into culture tubes that contained 15 mL sterile TS broth (Difco Laboratories). Following six hour incubation at 37 °C with 90 rpm shaking, 0.5 mL of the starter culture was added to 250 mL sterile TS broth stored in an Erlenmeyer flask (Fisher Scientific), which was incubated at 37 °C with 90 rpm shaking for 18 hours. The bacterial cells were harvested through centrifugation (4000 g, 10 min, 4 °C) and the pellets were rinsed four times using appropriate electrolyte solutions to remove the growth media. All of the electrolyte solutions were buffered with 0.2 mM NaHCO3 (Fisher Scientific) and total ionic strength was adjusted using NaCl (Fisher Scientific) to 1, 2.5, 5, 20, and 50 mM, respectively. The ionic strengths were selected to cover the range observed for natural groundwater (Mills, Herman et al. 1994, Gotkowitz 2000, Kim and Walker 2009). The pH of the solutions was ~7.2. The rinsed cell pellets were diluted to prepare cell suspensions (4 × 10^7cells/mL) that were subsequently used for the column transport experiments.

5.2.2 Column Transport Experiments

The column transport experiments were performed using duplicate glass chromatography columns measuring 2.5 cm in diameter and 15 cm in length (Kontes). The vertically oriented columns were wet-packed with clean quartz sands (size range: 0.211–0.297 mm) (US Silica). The sand-cleaning steps involved boiling the sands in concentrated nitric acid for 24 hours to remove metal hydroxides, soaking the sands in diluted NaOH solution for 24 hours to remove natural clay particles, and boiling the sands again using nitric acid to remove metal residues (Xu, Liao et al. 2008). The porosity of the sands was measured using the bulk density method and equaled 0.37 (Weight 2008). The packed saturated sand columns were equilibrated by pumping more than 30 pore volumes (PV) of the buffered NaCl solutions (total ionic strength of 1, 2.5, 5, 20, and 50 mM, respectively) using peristaltic pumps (MasterFlex). The specific discharge was maintained at 0.31 cm/min.

Following the equilibration step, the transport experiments were initiated by injecting the *E. faecium* cell suspensions prepared using similar buffered NaCl solutions to the top of the columns and concentrations of the bacterial cells in the effluent were determined through measuring the absorbance at a wavelength of 220 nm using a Shimadzu UV-1700 spectrophotometer. After 60 minutes of injection (3.5 PV), the columns were flushed with bacteria-free buffered NaCl solution until the absorbance of effluent returned to the background values.

For the relatively uniform sands used in this research, it was previously shown that the effect of hydrodynamic dispersion on the transport of colloid-sized particles was negligible (Xu, Gao et

al. 2006). The clean-bed deposition rate coefficients (k_d) of the bacterial cells within the saturated sand packs could be estimated from the early cell breakthrough concentrations in the effluent (Kretzschmar, Barmettler et al. 1997, Walker, Redman et al. 2005):

$$k_{d=-\frac{v}{\varepsilon L}\ln\left(\frac{C}{C_o}\right)} \tag{5.1}$$

where ε is porosity, υ is the specific discharge, L is the column length, and C/C_0 is the normalized breakthrough concentration relevant to clean-bed conditions, which was obtained from the average bacterial breakthrough concentrations between 1.8 and 2.0 PV (Walker, Redman et al. 2005, Castro and Tufenkji 2007).

5.2.3 Energy Interactions between Bacterial Cells and Sand Surfaces

The transport of *E. faecium* cells within the saturated sands packs was determined by the energy interactions between the bacterial cells and the surface of quartz sands. According to the extended Derjaguin–Landau–Verwey–Overbeek (XDLVO) theory, the energy interactions include the Lifshitz–van der Waals (LW) interaction, the electrostatic double layer (EDL) interaction as well as the Lewis acid–base (AB) interaction:

$$\Phi^{Total} = \Phi^{\mathrm{LW}} + \Phi^{\mathrm{EDL}} + \Phi^{\mathrm{AB}} \tag{5.2}$$

The LW, EDL, and AB interaction energies (Φ^{LW},Φ^{EDL} and Φ^{AB}) for the cell-sand (sphere-plate geometry) system can be calculated using the following equations (Elimelech 1994, Ong, Razatos et al. 1999, Redman, Walker et al. 2004, Morrow, Stratton et al. 2005, Bayoudh, Othmane et al. 2006, Bayoudh 2009, Farahat 2009, Huang, Bhattacharjee et al. 2010):

$$\Phi^{\mathrm{LW}} = -\frac{Aa_b}{6h} \tag{5.3}$$

$$\Phi^{\mathrm{EDL}} = \pi\varepsilon_0\varepsilon_w a_b \left\{2\psi_b\psi_s \ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + (\psi_b^2 + \psi_s^2)\ln[1-\exp(-2\kappa h)]\right\} \tag{5.4}$$

$$\Phi^{\mathrm{AB}} = 2\pi a_b \lambda_w \Delta G_{h_0}^{\mathrm{AB}} exp\left(\frac{h_0 - h}{\lambda_w}\right) \tag{5.5}$$

where *A* represents the Hamaker constant; a_b is the radius of the bacterial cells; *h* is the separation distance between the bacterium and sand surface; ε_0 is the dielectric permittivity of vacuum, ε_w is the dielectric constant of water; κ is the inverse of Debye length; ψ_b and ψ_s are the surface potentials of the bacterial cells and sand, respectively; λ_w (= 0.6 nm) is the characteristic decay length of AB interactions in water; h_0 represents the minimum equilibrium distance between the cell and sand surface and equals to 0.157 nm; and $\Delta G_{h0}^{\mathrm{AB}}$ represents the hydrophobicity interaction free energies per unit area corresponding to h_0.

The values of *A*, $\Delta G_{h0}^{\mathrm{AB}}$, a_b, κ, ψ_b, and ψ_s were required for the interaction energy calculations. The Hamaker constant can be calculated from the LW interfacial tension parameters of bacteria (γ_b^{LW}), water (γ_w^{LW}) and sand (γ_w^{LW}) (van Oss 1993):

$$A = 24\pi h_0^2 \left(\sqrt{\gamma_b^{\mathrm{LW}}} - \sqrt{\gamma_w^{\mathrm{LW}}}\right)\left(\sqrt{\gamma_s^{\mathrm{LW}}} - \sqrt{\gamma_w^{\mathrm{LW}}}\right) \tag{5.6}$$

The value of $\Delta G_{h0}^{\mathrm{AB}}$ can be obtained from the electron-accepting (γ^+) and electron-donating (γ^-) interfacial tension parameters (van Oss 1993):

$$\Delta G_{h_0}^{AB} = 2\left[\sqrt{\gamma_w^+}\left(\sqrt{\gamma_b^-} + \sqrt{\gamma_s^-} - \sqrt{\gamma_w^-}\right) + \sqrt{\gamma_w^-}\left(\sqrt{\gamma_b^+} + \sqrt{\gamma_s^+} - \sqrt{\gamma_w^+}\right) - \sqrt{\gamma_b^-\gamma_s^+} - \sqrt{\gamma_b^+\gamma_s^-}\right] \tag{5.7}$$

where the subscripts of *b*, *w*, and *s* represent bacteria, water, and sand, respectively.

For equations 5.6 and 5.7, the values of γ_w^{LW}, γ_w^+, and γ_w^- (for water) are 21.8, 25.5, and 25.5 mJ m^{-2}, respectively (van Oss 1993). Previously reported values of γ_s^{LW} (39.2 mJ m^{-2}), γ_s^+ (1.4 mJ m^{-2}), and γ_s^- (47.8 mJ m^{-2}) (for quartz sand) (Morrow, Stratton et al. 2005) were used in this research. For the wild-type (E1162) and Esp mutant (E1162 Δesp) *E. faecium* cells, the values of γ_b^{LW}, γ_b^+, and γ_b^- were determined through measuring the contact angles (θ) using three different probe liquids (water, glycerol, and diiodomethane) with known surface tension parameters (van Oss 1993, Morrow, Stratton et al. 2005):

$$\gamma_i^{\mathrm{L}}(1+\cos\theta) = 2\sqrt{\gamma_i^{\mathrm{LW}}\gamma^{\mathrm{LW}}} + 2\sqrt{\gamma_i^+\gamma^-} + 2\sqrt{\gamma_i^-\gamma^+} \tag{5.8}$$

where the subscript i represents water (γ^L = 72.8, γ^{LW} = 21.8 and $\gamma^+ = \gamma^-$ = 25.5 mJ m–2), glycerol (γ^L = 64.0, γ^{LW} = 34.0, γ^+ = 3.92 and γ^- = 57.4 mJ m–2) or diiodomethane (γ^L = 50.8, γ^{LW} = 50.8 and $\gamma^+ = \gamma^-$ = 0 mJ m–2) (van Oss 1993). The contact angles were acquired with a Rame-Hart goniometer using bacterial lawns produced by filtering cells onto porous membrane(Ong, Razatos et al. 1999).

Using the LW (γ^{LW}), electron-accepting (γ^+) and electron-donating (γ^-) interfacial tension parameters, values of ΔG_{iwi}, which express the free energy of interaction between two cells (i) (E1162 and E1162Δesp) in water (w), can be calculated as a measure of cell hydrophobicity (Ong, Razatos et al. 1999, Morrow, Stratton et al. 2005):

$$\Delta G_{iwi} = 2\left(\sqrt{\gamma_b^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 - 4(\gamma_b^+ - \gamma_w^+)(\gamma_b^- - \gamma_w^-) \tag{5.9}$$

The Debye length was calculated as follows:

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_0 \varepsilon_w kT}{Ie^2}} \tag{5.10}$$

where ε_0 and ε_w were previously defined, *k* is Boltzmann's constant, *T* is absolute temperature, *I* is ionic strength, and *e* is the electron charge. For our experimental conditions (25 °C), the values of Debye length were 9.61 nm (1 mM), 6.08 nm (2.5 mM), 4.30 nm (5 mM), 2.15 nm (20 mM), and 1.36 nm (50 mM), respectively.

Images of the E1162 and E1162 Δesp cells suspended in the electrolyte solutions were obtained using a Nikon Eclipse 50i microscope that was equipped with a Photometric CoolSnap ES digital camera and MetaMorph software. For each electrolyte solution, the diameter of a minimum of 30 cells was determined using the ImageJ software.

Cell suspensions were prepared in a similar fashion as the column transport experiments and the quartz sands were pulverized and the colloid-sized quartz particles were suspended in the buffered NaCl solutions (Porubcan and Xu 2011). The zeta potential of the bacterial cells and colloidal quartz particles suspended in each solution was then measured for a minimum of five times using a ZetaPALS analyzer (Brookhaven Instruments Corporation). The measured zeta potential values were used in place of surface potentials for the XDLVO calculations (Walker, Redman et al. 2004).

5.3 Results and Discussion

5.3.1 Transport of *E. faecium* E1162 and E1162Δesp within Saturated Sand Packs

The breakthrough curves of the E1162 and E1162Δesp cells under various ionic strength conditions are shown in Figure 5.1. For both strains, the breakthrough concentrations decreased monotonically with the increase in ionic strength. For instance, the breakthrough concentrations of E1162Δesp decreased from ~ 99% to ~ 3% as ionic strength increased from 1 to 50 mM. It was also observed that, under the same ionic strength condition, the breakthrough concentrations of E1162 were always lower than the corresponding breakthrough concentrations of E1162Δesp. When the ionic strength was 5 mM, for instance, the breakthrough concentrations of E1162 and E1162Δesp were ~ 47% and ~ 80%, respectively.

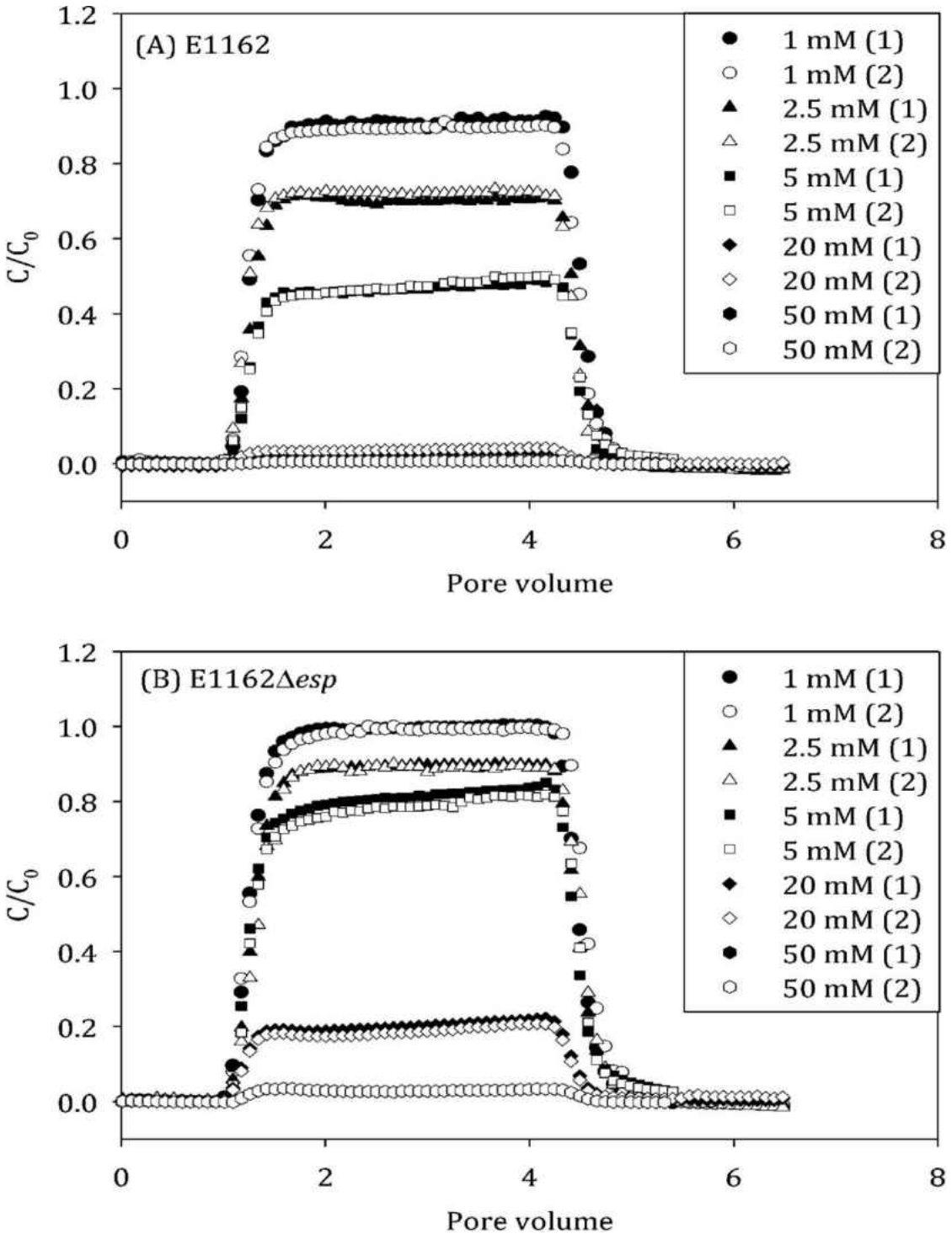

Figure 5.1. Breakthrough concentrations of (A) *E. faecium* E1162 and (B) *E. faecium* E1162Δesp under ionic strength conditions of 1 to 50 mM. The pH of the solutions was buffered at 7.2 using 0.2 mM NaHCO3. The ionic strength was adjusted using NaCl. Duplicate experiments (numbers in the parentheses) were performed for each ionic strength condition.

The clean-bed deposition rate coefficients (k_d) were calculated from the early breakthrough concentrations using eq 5.1 (Figure 5.2). The calculated values of k_d for E1162 were 50% (50 mM) to 450% (1 mM) higher than those of E1162Δesp under the ionic strength conditions tested in this research, and results of Student's *t*-tests showed that the difference was statistically significant (p = 0.05), suggesting that the Esp increased the attachment of *E. faecium* cells to the surface of the quartz sands. For both strains, the k_d values also increased with ionic strength.

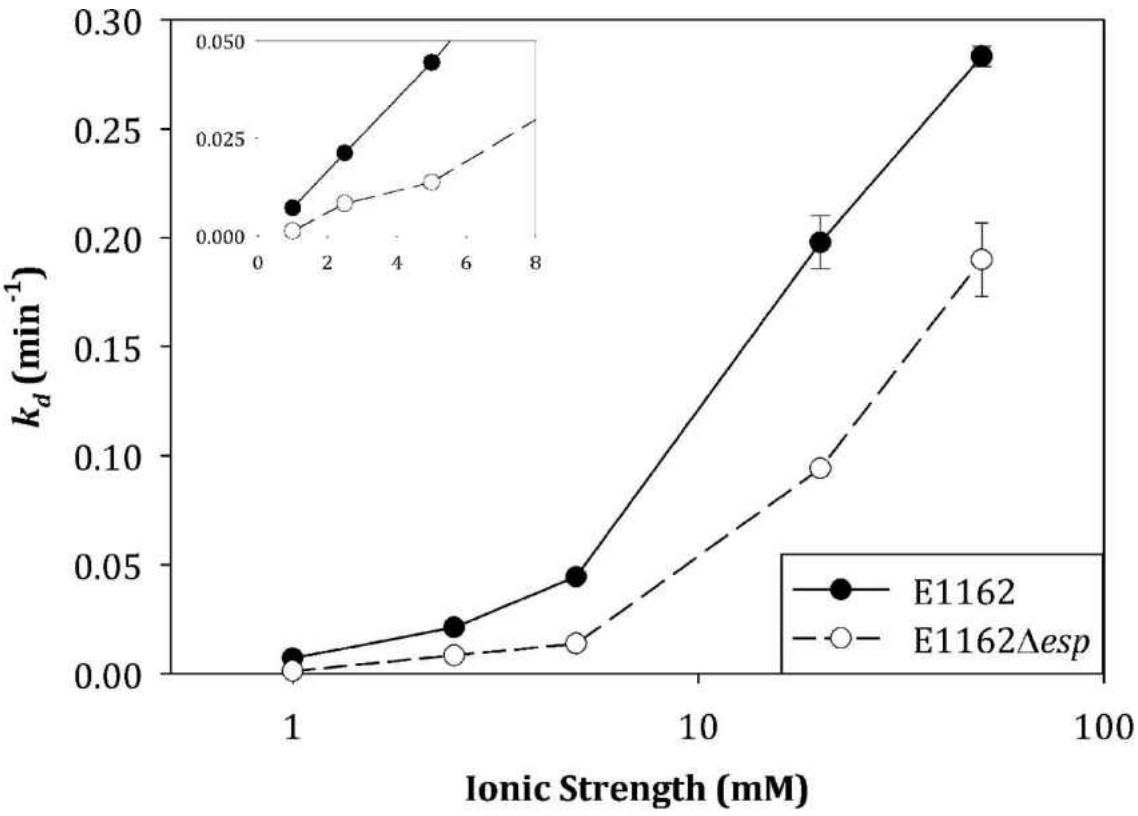

Figure 5.2. Average deposition rate coefficients (k_d) for *E. faecium* E1162 and E1162Δesp under ionic strength conditions of 1, 2.5, 5, 20, and 50 mM. The pH of the solutions was buffered at 7.2 using 0.2 mM $NaHCO_3$. The values of k_d were calculated using eq 5.1. Figure inset shows the comparison of the k_d values under low ionic strength conditions.

Previous results of batch experiments showed that Esp could facilitate the attachment of *Enterococcus* cells to various engineered materials such as polystyrene, polyvinyl chloride, and polypropylene (Toledo-Arana, Valle et al. 2001, Tendolkar, Baghdayan et al. 2004, Heikens, Bonten et al. 2007) and could facilitate the formation of biofilm (Tendolkar, Baghdayan et al. 2004). Specifically, for the *E. faecium* strains (E1162 and E1162Δesp) used in this research, it was observed that strain E1162 displayed higher attachment efficiency to polystyrene than E1162Δesp (Heikens, Bonten et al. 2007). Our results further suggested that Esp could enhance the attachment of *E. faecium* cells onto the surfaces of mineral materials such as quartz sands.

5.3.2 XDLVO Energy Interactions

Microscopic measurements (>30 cells for each ionic strength condition) showed that the E1162 and E1162Δesp cells were spherical and their sizes did not vary when suspended in the buffered NaCl solutions. On average, the diameter of the E1162 and E1162Δesp cells was 1.29(±0.12) μm and 1.30(±0.12) μm, respectively, suggesting that the removal of Esp from cell wall thus did not affect cells size. The corresponding radius values were then used for the calculation of XDLVO interaction energies.

Contact angle measurements showed that the removal of Esp had noticeable impacts on cell surface properties (Table 5.1). Results of Student's *t*-tests showed that the differences in the contact angle values of E1162 and E1162 Δesp were statistically significant ($p = 0.05$). Using equation 5.8, the LW (γ^{LW}), electron-accepting (γ^{+}) and electron-donating (γ^{-}) interfacial tension parameters for E1162 and E1162Δesp cells were then resolved (Table 5.1). The Hamaker constants (A) for the cell-water-sand system were determined as 4.2×10^{-21} J and 4.8×10^{-21} J

for E1162 and E1162Δesp, respectively. These values were generally consistent to previously reported values for the bacterium-water-sand system (Ong, Razatos et al. 1999, Cail and Hochella Jr 2005, Morrow, Stratton et al. 2005, Farahat 2009). For instance, Cail et al. (2005) suggested a Hamaker constant of 4.1×10^{-21} J for the *Enterococcus*-water–glass system. Additionally, the values of ΔG_{h0}^{AB} were determined using equation 5.7 and equaled 24.1 and 31.4 mJ/m^2 for E1162 and E1162Δesp, respectively. The AB interaction between E1162 or E1162Δesp cells and quartz sands was thus repulsive and the repulsion increased as a result of the removal of Esp.

Table 5.1. Contact Angle Measurements, Surface Tension Components, Hamaker Constant, ΔG_{h0}^{AB} and ΔG_{iwi} for E1162 and E1162Δesp[a]

properties		**E1162**	**E1162Δesp**
contact angle (deg) ($n \geq 4$)	water	21.2 (±4.1)	16.1 (±1.6)
	glycerol	24.3 (±1.6)	32.5 (±4.6)
	diiodomethane	48.2 (±2.5)	44.2 (±0.2)
surface tension components (mJ/m^2)	γ^{LW}	35.3	37.4
	γ^+	3.1	1.4
	γ^-	44.7	53.5
Hamaker constant (x10^{-21} J), A		4.2	4.8
ΔG_{h0}^{AB}(mJ/m^2)		24.1	31.4
ΔG_{iwi}(mJ/m^2)		18.3	31.0

The differences between the contact angle values of E1162 and E1162Δesp were statistically significant (p = 0.05) based on Student's t-test.

The calculated ΔG_{iwi} values for E1162 and E1162Δesp cells were both positive (Table 5.1), suggesting that the cells of both strains were hydrophilic (Morrow, Stratton et al. 2005). Additionally, because E1162Δesp had a higher ΔG_{iwi} value than E1162, E1162Δesp cells were more hydrophilic than E1162 cells (Ong, Razatos et al. 1999, Morrow, Stratton et al. 2005). This was in good agreement with previous observations that the removal of Esp increased the hydrophilicity of *Enterococcus* cells as determined through microbial adhesion to hydrocarbon (MATH) tests (Toledo-Arana, Valle et al. 2001, Tendolkar, Baghdayan et al. 2004). Furthermore, the finding that removing Esp increased the hydrophilicity and mobility of *E. faecium* cells was consistent to several recent publications which suggested that increase in cell hydrophilicity could enhance the mobility of bacterial cells within clean quartz sands (McCaulou, Bales et al. 1994, Walker, Redman et al. 2005, Bolster, Walker et al. 2006, Park, Kim et al. 2010, Walczak, Bardy et al. 2011). Walker et al., (2005) for instance, reported that the more hydrophilic exponential-phase *E. coli* D21g (16% of cells partitioned into the hydrocarbon phase) displayed slightly higher mobility than the less hydrophilic stationary-phase cells (34% of cells partitioned into the hydrocarbon phase). Walczak et al. (2011) also showed that desiccation at high relative humidity levels (>75% of relative humidity) increased the mobility of *E. coli* cells through increasing cell hydrophilicity.

Under the experimental conditions, the surfaces of the quartz sands as well as the E1162 and E1162Δesp cells were all negatively charged as revealed by the zeta potential measurements (Figure 5.3). The EDL interactions between the bacterial cells and the quartz sands were thus

repulsive. Although the measured zeta potential values of E1162Δesp cells were slightly more negative than those of the E1162 cells, the results of Student's *t* test suggested that the differences were statistically insignificant (p = 0.05). In general, there was little variation in the zeta potential values of the bacterial cells within the ionic strength range of 1–5 mM. Further increase in ionic strength to 20 mM led to significant (Student's *t* test, p = 0.05) increase in zeta potential values likely due to the compression of the electrostatic double layer (Kim and Walker 2009). Similarly, the zeta potential values of the quartz sands were stable within the range of 1–20 mM. There was significant (Student's *t* test, p = 0.05) increase in the zeta potential of sands as ionic strength increased from 20 mM to 50 mM.

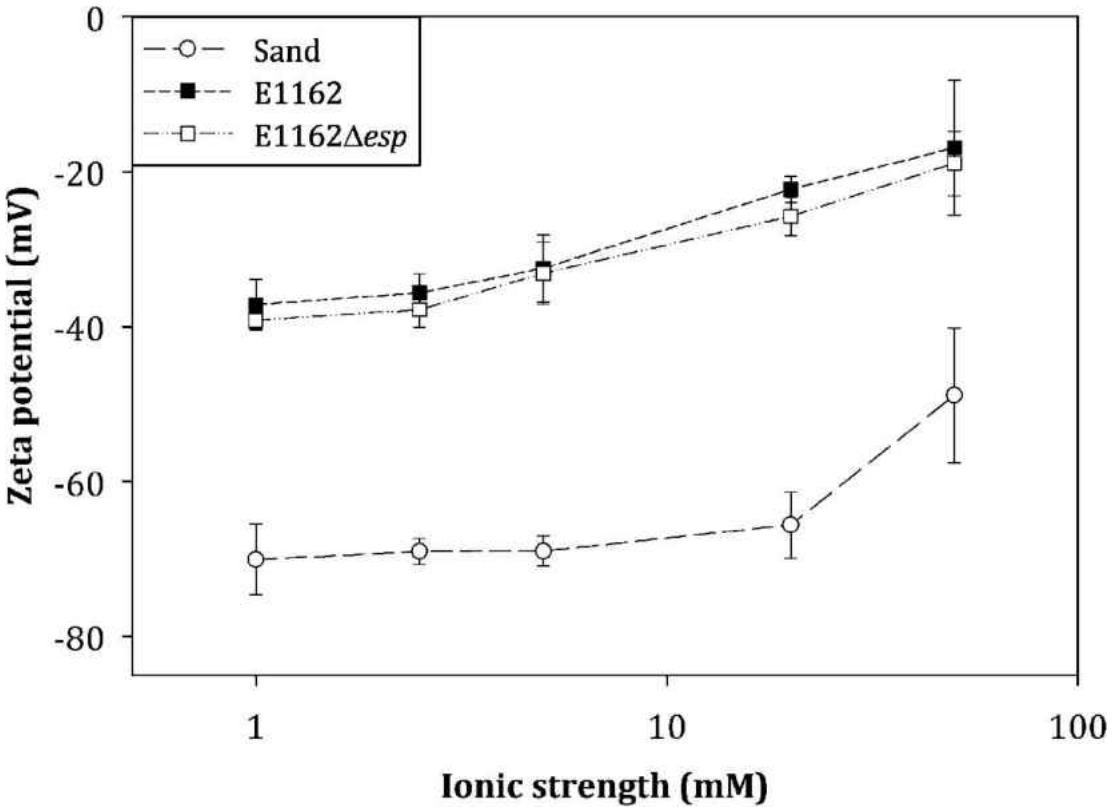

Figure 5.3. Zeta potential of the *E. faecium* E1162 and E1162Δesp cells and the silica sands suspended in 1, 2.5, 5, 20, and 50 mM buffered (pH = 7.2) NaCl solutions. Error bars represent standard deviation of 5 measurements.

The calculated XDLVO energy interaction profiles (Figure 5.4) indicated the presence of energy barrier for the deposition of E1162 and E1162Δesp cells onto the surface of quartz sands. The height of the energy barrier between E1162 cells and the quartz sands was lower than the height of the energy barrier between E1162Δesp cells and quartz sands by 135 (1 mM), 172 (2.5 mM), 46 (5 mM), 185 (20 mM), and 55 (50 mM) kT, respectively. This comparison of the magnitude of energy barriers suggested that it was more likely for the E1162 cells to attach to the surface of quartz sands. This was consistent to the observation that the clean-bed deposition rate coefficients of E1162 were higher than those of E1162Δesp. Inspection of the LW, EDL, and AB components of the interaction energy profiles showed that the stronger AB and EDL repulsive interactions between E1162Δesp cells and quartz sands were responsible for the higher energy barriers. Additionally, the magnitude of the energy barriers for both E1162 and E1162Δesp strains decreased with ionic strength (Figure 5.4). This could explain the observed increase in cell deposition rate coefficients with ionic strength (Figure 5.2). As the LW and AB interactions were independent of ionic strength, the decrease in the magnitude of the energy barriers was due to the decrease in Debye length (given similar zeta potential values, smaller Debye length generally leads to lower energy barrier) and the slight increase (i.e., becoming less negative) of the zeta potential values of both bacterial cells and the quartz sands with increasing ionic strength, which in turn decreased the magnitude of the repulsive EDL interactions between the bacterial cells and the quartz sands. The calculation of the XDLVO energy interaction profiles also indicated the presence of secondary energy minimum. Under such conditions, bacteria deposition could occur at the secondary energy minimum (Redman, Walker et al. 2004, Wang, Xu et al. 2011). In general, the magnitude of the secondary energy

minimum increased with increasing ionic strength, which was consistent with the observed trend in *E. faecium* deposition kinetics. The effects of Esp on the calculated secondary energy minimum, however, seemed to be negligible, suggesting that the secondary energy minimum was not a main cause of the observed differences in the transport behavior of E1162 and E1162Δesp.

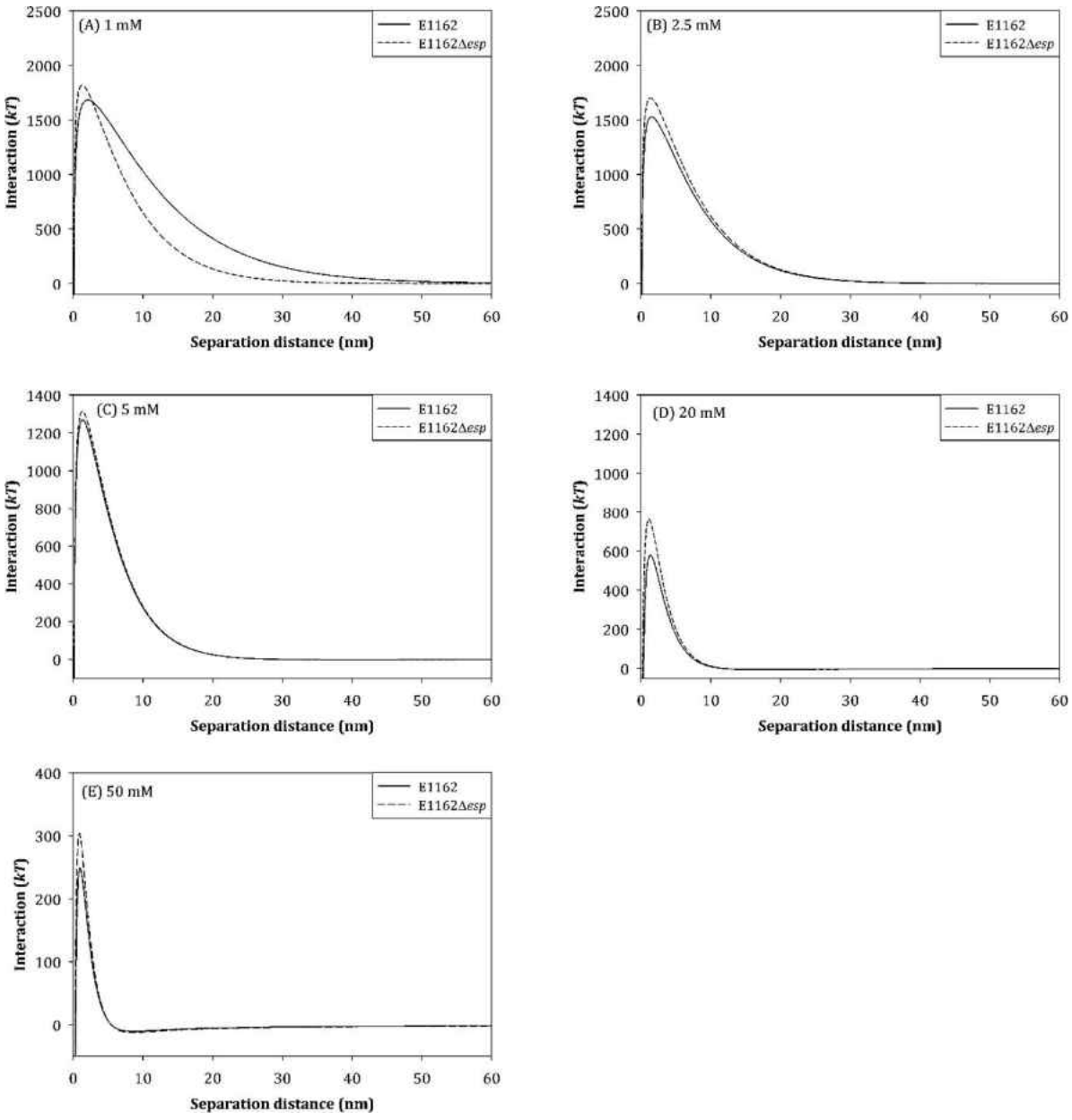

Figure 5.4. Calculated XDLVO interaction energy profiles as a function of separation distance for (A) 1 mM, (B) 2.5 mM, (C) 5 mM, (D) 20 mM and (E) 50 mM ionic strength conditions. The height of the energy barrier between E1162 cells and the quartz sands was lower than the height of the energy barrier between E1162Δesp cells and quartz sands by 135 (1 mM), 172 (2.5 mM), 46 (5 mM), 185 (20 mM) and 55 (50 mM) kT, respectively.

Through the analysis of the Esp gene structure, it was inferred that the Esp protein contains 1873 amino acids with a predicted mass of 202 kDa (Shankar, Baghdayan et al. 1999). Antibody tests showed that the Esp protein is anchored to the cell wall through the C-terminus and the

N-terminus is displayed on the cell surface (Shankar, Baghdayan et al. 1999). It was further shown that the N-terminal domain of the Esp protein is sufficient to facilitate the biofilm formation by *E. faecalis* (Tendolkar, Baghdayan et al. 2005). It is thus likely that the N-terminal domain of the Esp protein leads to alterations in cell surface properties (e.g., hydrophobicity), which in turn affects the mobility of *E. faecium* within saturated sand packs.

5.3.3 Environmental Implications

Sewage and manure represent major sources of enterococci that are released into the natural environment (Huycke, Sahm et al. 1998, Walczak, Bardy et al. 2011). Results obtained in this research suggested that the surface protein Esp could lower the mobility of *E. faecium* within saturated sand packs. Using eq 5.1 and the experimentally determined values of k_d, we were able to calculate the travel distances of E1162 and E1162 Δesp cells for any given removal efficiency (i.e., $1 - C/C_0$). Our results suggested that, for the ionic strength of 20 mM, the travel distances of E1162 and E1162 Δesp cells that corresponds to a removal efficiency of 99.9% (e.g., $C/C_0 = 0.001$) were 30 and 60 m, respectively.

Results of PCR assays indicated that the esp_{fm} gene was more prevalent in sewage and septic system samples than livestock, wild animal and bird samples (Scott, Jenkins et al. 2005, Whitman, Przybyla-Kelly et al. 2007, Ahmed, Goonetilleke et al. 2009). It was proposed that the esp_{fm} gene could potentially be used as a molecular marker for the identification of human sources of fecal pollution (Scott, Jenkins et al. 2005, Ahmed, Goonetilleke et al. 2009, Scott, Harwood et al. 2009). For the groundwater system, the potential usefulness of this emerging technique can be complicated by the effects of Esp on the transport of *E. faecium* cells. As

previously discussed, *E. faecium* strains without Esp could travel over longer distances than *E. faecium* strains with Esp. If the pathogenic microorganisms of interest from both sources do not have similar patterns in terms of transport behavior (e.g., if the mobility of the pathogens from both sources is comparable), this esp_{fm} gene technique may underestimate the contribution from fecal sources that tend to contain high levels of Esp (i.e., human sources).

Several recent publications highlighted the important impacts of outer membrane proteins (OMP) on the transport of Gram-negative bacteria (e.g., *E. coli*) within saturated sand packs (Lutterodt, Basnet et al. 2009, Walczak, Bardy et al. 2011, Walczak, Bardy et al. 2011). Lutterodt et al. (2009), for instance, reported that OMP Ag43 could enhance the attachment of *E. coli* cells to the surface of quartz sands. It was hypothesized that the positive charges of the α-domain of Ag43, which extends from the cell surface to the environment, facilitated the attachment of *E. coli* cells to the negatively charged quartz surfaces (Lutterodt, Basnet et al. 2009). Additionally, it was suggested that the OMP TolC could decrease the attachment of *E. coli* from various environmental sources on the surface of quartz sands (Walczak, Bardy et al. 2011, Walczak, Bardy et al. 2011). Findings from this research indicated that cell wall protein such as Esp could also have significant impact on the transport of Gram-positive bacteria such as *E. faecium* within saturated quartz sands. There is a growing body of evidence suggesting that bacterial isolates obtained from various environmental sources could display marked variations in their transport behavior within saturated porous media (Bolster, Walker et al. 2006, Bolster, Cook et al. 2010, Foppen, Lutterodt et al. 2010, Walczak, Bardy et al. 2011).) Findings from previous studies (Lutterodt, Basnet et al. 2009, Walczak, Bardy et al. 2011, Walczak, Bardy et al. 2011) as well as from this research suggested that cell surface proteins

may be an important factor behind the observed mobility variations. Future studies are warranted to elucidate the relationship between the abundance, structure, and properties of cell surface proteins and bacterial transport within aquifer materials.

Acknowledgment

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CHAPTER 6. Transport of *E. coli* K-12 and *B. Fragilis* within Sand Packs

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6.1. Introduction

More than 40% of the U.S. population depend on groundwater as the primary source of drinking water, and approximately 72% of the groundwater used for drinking water purposes is not disinfected (Yates 1994, Hutson, Barber et al. 2004). Groundwater, however, is susceptible to microbial contamination, and it was estimated that pathogens in groundwater have caused ~50% of the outbreaks of waterborne illness in the United States (Craun, Brunkard et al. 2010, Craun 2012). The fast and reliable detection of groundwater microbial contamination and the identification of the contamination sources are of critical importance to the protection of public health.

Currently, public groundwater systems are commonly tested for microbial contamination by analyzing for the presence of total coliforms, and if detected, further tested using the indicator microorganisms such as *E. coli* and enterococci (USEPA 2007). However, the use of these common indicator microorganisms to distinguish the original sources of the contamination, known as microbial source tracking (MST), has found only limited success (Whitlock, Jones et al. 2002, Cimenti, Biswas et al. 2005, Scott, Jenkins et al. 2005, Graves, Hayedorn et al. 2007, Ahmad, Tourlousse et al. 2009).

Fecal anaerobes, such as Bacteroides, are several orders of magnitude more abundant in gastrointestinal tracts than fecal coliforms and have been long suggested as alternative indicators for fecal contamination (Fiksdal, Maki et al. 1985). Experiments performed in aerobic surface water environments showed that detectable *B. fragilis* can survive over extended periods over aerobic conditions, the detection of Bacteroides spp. can be more sensitive than the enumeration of *E. coli*, and more importantly, host-specific *Bacteriodes* markers can be used to determine the origin of fecal pollution (Fiksdal, Maki et al. 1985, Bower, Scopel et al. 2005, Layton, McKay et al. 2006, Gawler, Beecher et al. 2007, Ahmad, Tourlousse et al. 2009, Mieszkin, Furet et al. 2009, Reott, Parker et al. 2009, Sauer, VandeWalle et al. 2011). *Bacteriodes* spp. thus represents a promising groundwater fecal contamination indicator that can be used for MST. The use of *Bacteriodes* spp. for groundwater MST, however, can be complicated by their mobility within the aquifer matrix (Johanson, Feriancikova et al. 2012). If the mobility of *Bacteriodes* spp. In the subsurface system is high, they will be able to spread far from the source zone and thus can be used as an effective tool for the detection of fecal contamination and identification of microbial sources.

The primary goal of this research is to quantify the mobility of *B. fragilis* within sandy aquifer materials under different water chemistry conditions through a series of column transport experiments. Parallel experiments were performed for *E. coli* K-12, which allowed for the comparison of the mobility of *B. fragilis* and *E. coli* K-12. Our results showed that the mobility of *B. fragilis* within saturated sandy materials is comparable to or higher than the mobility of *E. coli* K-12. *B. fragilis*, therefore, can potentially be utilized for the identification of sources for groundwater microbial contamination.

6.2. Materials and Methods

6.2.1. Preparation of Bacterial Suspensions

Transport experiments were performed on both *B. fragilis* and *E. coli* K-12 under 4 different ionic strength conditions. Buffered electrolyte solutions were prepared with 0.2 mM $NaHCO_3$ (Fisher Scientific) to adjust the pH to ~7.2, and total ionic strength was adjusted to 1 mM, 5 mM, 20 mM, and 50 mM, respectively, using NaCl (Fisher Scientific). These background electrolyte solutions were used to prepare bacterial suspensions, and to equilibrate and flush columns before and after the transport experiments. *B. fragilis* (ATCC 25285) used in this research was cultured from dried, preserved pellets provided by MicroBioLogics (St. Cloud, Minnesota USA). One dry pellet was introduced to two milliliters sterile deionized water in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) with an atmosphere of 10% H_2, 5% CO_2 and 85% N_2. One half mL of the suspension was used to inoculate 50 mL sterile Tryptic Soy (TS) broth (Difco Laboratories) in 100 mL serum vials (Fisher Scientific). The serum vials were clamp sealed and incubated at 37°C for 48 hours with 90 rpm shaking. The *B. fragilis* cells were then harvested through centrifugation (4000 g, 10 minutes, 4°C). The fresh bacterial pellets were rinsed four times using the appropriate electrolyte solution to remove the growth media (Johanson, Feriancikova et al. 2012, Feriancikova, Bardy et al. 2013). The *E. coli* K-12 (ATCC 10798) cells preserved at −80°C were streaked onto TS agar (Difco Laboratories) plates, and incubated at 37°C overnight. One colony from the plate was transferred by sterile transfer loop into a sterile culture tube containing 15 mL sterile TS broth (Difco Laboratories). This starter culture was incubated six hours at 37°C with shaking at 90 rpm. Then 0.5 mL of the

starter culture was transferred into 250 mL sterile TS broth in an Erlenmeyer flask (Fisher Scientific), and incubated at 37°C with 90 rpm shaking for 18 hours. The *E. coli* K-12 cells were harvested through centrifugation in the same manner as *B. fragilis*. The rinsed bacterial cell pellets were used to prepare cell suspensions ($\sim 4 \times 10^7$ cells/mL as determined by plate counting) for the column transport experiments, which were initiated within three hours after harvesting.

6.2.2. Column Transport Experiments

Quartz sands used for the column transport experiments (size range: 0.211–0.297 mm; U.S. Silica) were cleaned following previously reported protocols (Xu, Liao et al. 2008). This size fraction was selected because it represents a major portion of natural porous media and clean quartz sands have been frequently utilized to investigate microbial transport within the subsurface system (Redman, Walker et al. 2004, Walczak, Wang et al. 2012, Yang, Kim et al. 2012). Following each cleaning step, the sands were thoroughly rinsed with deionized water. The clean sands were dried and then stored in high density polyethylene (HDPE) containers until used for the column experiments. The porosity of the sand was measured using the bulk density method (Weight 2008) and equaled 0.369.

Vertically-oriented duplicate glass chromatography columns (Kontes) measuring 2.5 cm diameter and 15 cm length were wet packed with the quartz sands using the background electrolyte solution, and equilibrated by pumping >30 pore volumes (PV) of the bacteria-free electrolyte solution using peristaltic pumps (MasterFlex). The flow rate was maintained at a specific discharge of 0.31 cm/min. The column effluent was connected to flow-through quartz

cells in a spectrophotometer (Shimadzu UV-1700), so that bacterial cell concentration in the effluent could be determined by measuring the absorbance at a wavelength of 220 nm. The bacterial cell suspension was injected into the top of the column for 60 minutes (~3.5 PV). Following injection, the column was flushed with bacteria-free electrolyte solution until the effluent returned to background absorbance values.

6.2.3. Deposition Rate Kinetics

Hydrodynamic dispersion of colloid-sized particles (such as the bacterial cells) has been previously shown to be negligible for uniform sands such as were used in this experiment (Johanson, Feriancikova et al. 2012, Feriancikova, Bardy et al. 2013). To facilitate the comparison of the mobility of *E. coli* and *B. fragilis* within the saturated sand packs, the clean-bed deposition rate coefficients (k_d), which measure the rates at which bacterial cells were being removed from the aqueous phase under pristine conditions (i.e., the sand surfaces are free of bacterial cells), were estimated from the early cell breakthrough concentrations in the effluent (Kretzschmar, Barmettler et al. 1997, Walker, Redman et al. 2005, Castro and Tufenkji 2007):

$$k_{d=-\frac{v}{\varepsilon L}\ln\left(\frac{C}{C_o}\right)} \quad (6.1)$$

where ε is porosity (0.37), v is the specific discharge (0.31 cm/min), L is the column length (15 cm) and C/C_0 is the normalized break-through concentration relevant to clean-bed conditions. Consistent with many previous studies, the C/C_0 values were obtained from the average bacterial breakthrough concentrations between 1.8–2.0 PV (Walker, Redman et al. 2005, Castro

and Tufenkji 2007). According to colloid filtration theory (Yao, Habibian et al. 1971, Rajagopalan and Tien 1976, Tufenkji and Elimelech 2004), the clean bed deposition rate coefficient (k_d) is a function of the frequency at which the suspended cells strike the surface of the sands (i.e., the single-collector contact efficiency η) and the probability that a cell that strikes the surface of the sand will get attached (i.e., attachment efficiency α):

$$k_d = \frac{3(1-\varepsilon)v}{2d_g}\alpha\eta \tag{6.2}$$

where d_g is sand-grain diameter. The value of η depends on parameters such as fluid velocity and viscosity as well as cell size and density. To estimate the values of η, the equations of Tufenkji and Elimelech (2004)(Equations 9-17) were used. The value of α was then calculated using equation 6.2.

6.2.4. XDLVO Interactions between Bacterial Cells and Sand Surfaces

The cell attachment efficiency (α) is determined by the energy interactions between the bacterial cells and the surface of the quartz sands. The extended Derjaguin-Landau-Verweu-Overbeek (XDLVO) theory accounts for cell-sand interactions occurring over short separation distances resulting from Lifshitz-van der Waals (LW) forces, electrostatic double layer (EDL) forces, and Lewis acid-base (AB) forces:

$$\Phi^{\mathrm{Total}} = \Phi^{\mathrm{LW}} + \Phi^{\mathrm{EDL}} + \Phi^{\mathrm{AB}} \tag{6.3}$$

The LW interaction ΦLW represents induced dipole forces that occur when a bacterium is very close to the grain surface. The EDL interaction Φ^{EDL} is caused by electrostatic charges on the cell

and the sand. Although the EDL interaction can work at distances greater than the LW interaction, the double layer can also be compressed by greater ionic concentration. The AB force Φ^{AB} can be attractive or repulsive, as it is caused by hydrophobic or hydrophilic interactions between the bacterium and the sand. For the cell-sand system (sphere-plate geometry), the forces can be calculated using the following equations (Kretzschmar, Barmettler et al. 1997, Ong, Razatos et al. 1999, Tendolkar, Baghdayan et al. 2004, Walker, Redman et al. 2005, Castro and Tufenkji 2007):

$$\Phi^{\mathrm{LW}} = \frac{Aa_b}{6h} \tag{6.4}$$

$$A = 24\pi h_0^2 \left(\sqrt{\gamma_b^{\mathrm{LW}}} - \sqrt{\gamma_w^{\mathrm{LW}}}\right)\left(\sqrt{\gamma_s^{\mathrm{LW}}} - \sqrt{\gamma_w^{\mathrm{LW}}}\right) \tag{6.5}$$

$$\Phi^{\mathrm{EDL}} = \pi\varepsilon_0\varepsilon_w a_b \left\{2\psi_b\psi_s \ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + (\psi_b^2 + \psi_s^2)\ln[1-\exp(-2\kappa h)]\right\} \tag{6.6}$$

$$\Phi^{\mathrm{AB}} = 2\pi a_b \lambda_w \Delta G_{h_0}^{AB} \exp\left(\frac{h_0 - h}{\lambda_w}\right) \tag{6.7}$$

$$\Delta G_{h_0}^{AB} = 2\left[\sqrt{\gamma_w^+}\left(\sqrt{\gamma_b^-} + \sqrt{\gamma_s^-} - \sqrt{\gamma_w^-}\right) + \sqrt{\gamma_w^-}\left(\sqrt{\gamma_b^+} + \sqrt{\gamma_s^+} - \sqrt{\gamma_w^+}\right) - \sqrt{\gamma_b^-\gamma_s^+} - \sqrt{\gamma_b^+\gamma_s^-}\right] \tag{6.8}$$

where A represents the Hamaker constant; a_b is the equivalent radius of the bacterial cells; h is the separation distance between the bacterium and sand surface; h_0 is the minimum equilibrium distance between the bacterium and sand surface; the subscripts b, w, and s refer to bacteria, water, and sand, respectively; γ^{LW} is the LW interfacial tension parameter for a given material; ε_0 and ε_w are the dielectric permittivity for vacuum and water, respectively; ψ is the surface potential of a given material; κ is the inverse of Debye length; λ_w is the characteristic decay length of AB interactions in water; $\Delta G_{h_0}^{AB}$ is the hydrophobicity interaction

free energies per unit area corresponding to h_0, and Υ^+ and Υ^- are the electron-accepting and electron-donating interfacial tension parameters respectively, for a given material.

Previously reported values for parameters used in this research include the following: h_0=0.157 nm; interfacial tension parameters for water (γ_w^{LW}=21.8 mJ m^{-2}, $\gamma_w^+ = \gamma_w^-$ = 25.5 mJ m^{-2})(van Oss 1993, van Oss 1995) ; interfacial tension parameters for quartz sand (γ_s^{LW} =39.2 mJ m^{-2}, γ_s^+ =1.4 mJ m^{-2}, and γ_s^- =47.8 mJ m^{-2} , and AB interaction decay length λ_w = 0.6 nm.

The remaining values required to calculate interaction energy were determined through measuring cell size a_b, measuring zeta potential as a substitute for ψ_b and ψ_s (Johanson, Feriancikova et al. 2012), and measuring contact angle to determine γ_b^{LW}, γ_b^+, and γ_b^- .

6.2.5 Cell Characterization

The equivalent bacteria radius a_b was determined by capturing images of the cells in each electrolyte solution using a Nikon Eclipse 50i microscope equipped with a Photometric Coolsnap ES digital camera and Metamorph software. The length and width of at least 30 bacterial cells suspended in each electrolyte solution were determined using ImageJ software, and the equivalent radii of the cells were calculated as

$$\left(\sqrt{\frac{L_c W_c}{\pi}}\right) \tag{6.9}$$

where L_c and W_c represent the length and width of the cell, respectively.

Zeta potentials for bacterial solutions were measured using cell suspensions prepared with the background electrolyte solution similar to the column transport experiments. For the quartz

sand, colloid size particles were prepared by pulverizing the sand, which was then suspended in the background electrolyte solutions. The zeta potential of the bacterial cells and the sand particles suspended in each solution was measured a minimum of five times using a Zeta-PALS analyzer (Brookhaven Instruments Corporation).The contact angles (Θ) of three probe liquids with known surface tension parameters were determined on bacterial lawns created by filtering bacterial cells onto a filter using a Rame-Hart goniometer. The three probe liquids were water (Υ^L= 72.8, Υ^{LW}= 21.8, Υ^+= Υ^-= 25.5 mJ m^{-2}), glycerol (Υ^L= 64.0, Υ^{LW}= 34.0, Υ^+= 3.92, Υ^-= 57.4 mJ m^{-2}), and diiodomethane (Υ^L= 50.8, Υ^{LW}= 50.8, Υ^+= Υ^-= 0 mJ m^{-2}). The interfacial tension values for bacterial cells (Υ_b^{LW}, Υ_b^+, and Υ_b^-) were determined from the following equation (one equation for each liquid)(Johanson, Feriancikova et al. 2012):

$$\gamma_i^L(1+\cos\theta) = 2\sqrt{\gamma_i^{LW}\gamma^{LW}} + 2\sqrt{\gamma_i^+\gamma^-} + 2\sqrt{\gamma_i^-\gamma^+} \qquad (6.10)$$

Where the subscript *i* represents the probe liquid used.

6.3 Results and Discussion

6.3.1 Transport of *B. fragilis* and *E. coli* K-12 within Sand Packs.

Results from the column transport experiments showed that as the ionic strength of the background solution increased, breakthrough concentrations of both *B. fragilis* and *E. coli* K-12 in the effluent decreased (Figure 6.1), indicating that cell retention within the sand pack increased with increasing ionic solution strength. The *E. coli* K-12 breakthrough concentration decreased from 97% to <10% and *B. fragilis* breakthrough concentrations decreased from 97%

to 63% as ionic strength increased from 1 mM to 50 mM. At low ionic strength (1 and 5 mM), breakthrough concentrations were similar for both *E. coli* K-12 and *B. fragilis* with >87% recovery in the effluent; however at higher ionic strength (20 and 50 mM) the different species had markedly different breakthrough concentrations. At 20 mM total ionic strength, the breakthrough concentration for *B. fragilis* was 72% compared to 47% for *E. coli* K-12, and at 50 mM *B. fragilis* had 63% breakthrough; compared to <10% for *E. coli* K-12. This indicates greater mobility of *B. fragilis* and greater retention of *E. coli* K-12 on the quartz sand at higher ionic strength. These results suggest that the *B. fragilis* is more likely to be transported with groundwater flow than the *E. coli* K-12.

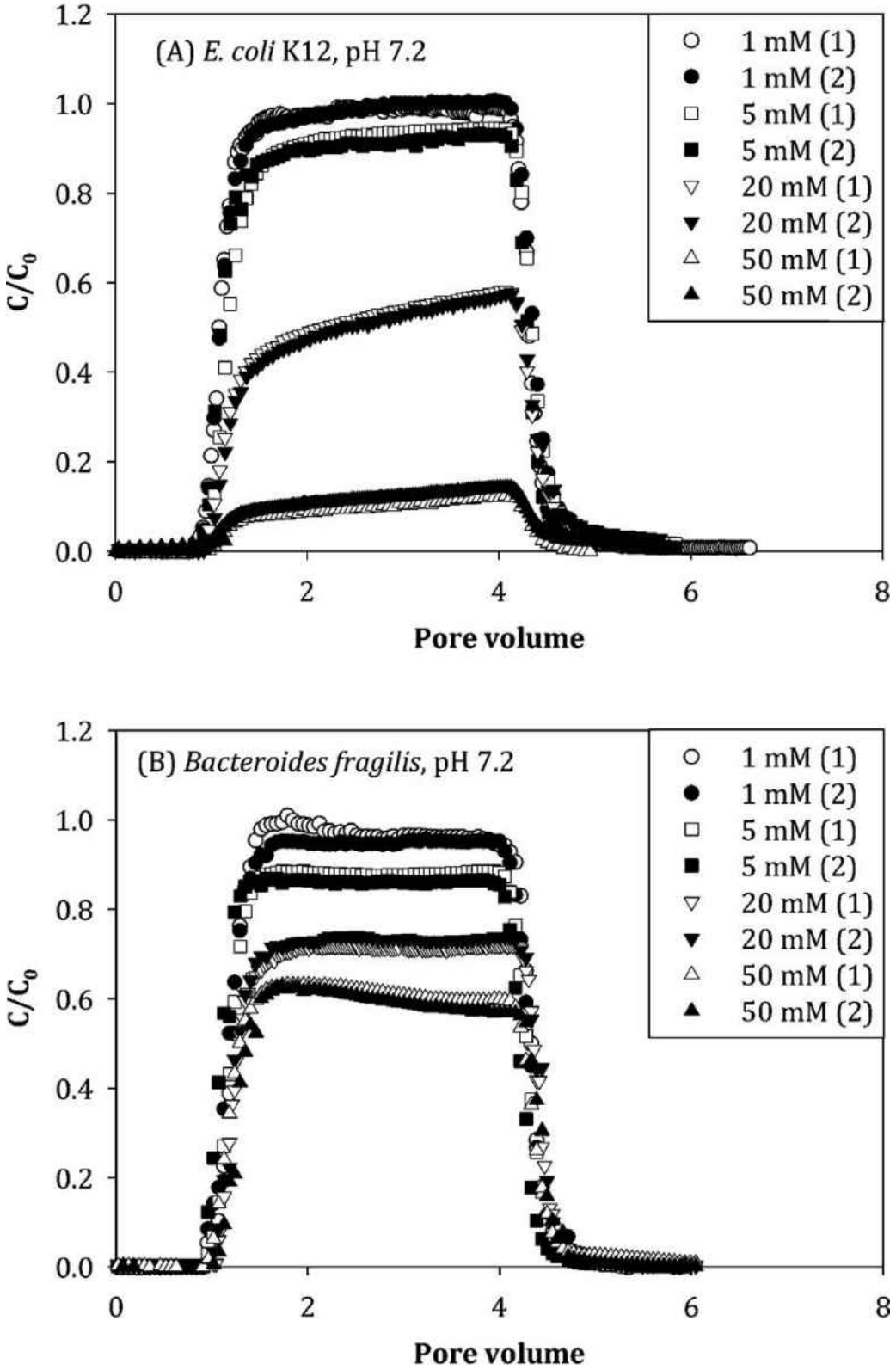

Figure 6.1. Breakthrough concentrations of (A) *E. coli* K-12 and (B) *B. fragilis* under ionic strength conditions of 1–50 mM. Same symbols (open and closed) represent the results from duplicate experiments.

Deposition rate coefficients (k_d) were calculated from equation 6.1 (Figure 6.2). At low ionic strengths the k_d values were very similar for *E. coli* K-12 and *B. fragilis.* At 1 mM total ionic strength both rates were 0.002 min^{-1}; at 5 mM they were 0.006 $min^{-1}$and 0.008 min^{-1}, respectively. The k_d generally increased with increasing ionic strength for both bacteria, however the *E. coli* K-12 had much greater k_d increase than the *B. fragilis*, and at higher ionic strength (20 mM and 50 mM) the deposition rates were significantly different. (*t*-test, $p < 0.05$) (0.018 (±0.001) min^{-1} and 0.026 (±0.002) min^{-1} for *B. fragilis*, and 0.042 (±0.001) min^{-1} and 0.132(±0.008) min^{-1} for *E. coli*, respectively). The use of equation 6.1 assumed that the values of C/C_0 remained stable within the selected pore volume range (i.e., 1.8–2 PV). Under 20 and 50 mM ionic strength conditions, the breakthrough concentrations of *E. coli* increased slightly over this pore volume range. The increase, however, was generally small (relative change < 5%) and the effects on the calculated K_d values should be insignificant.

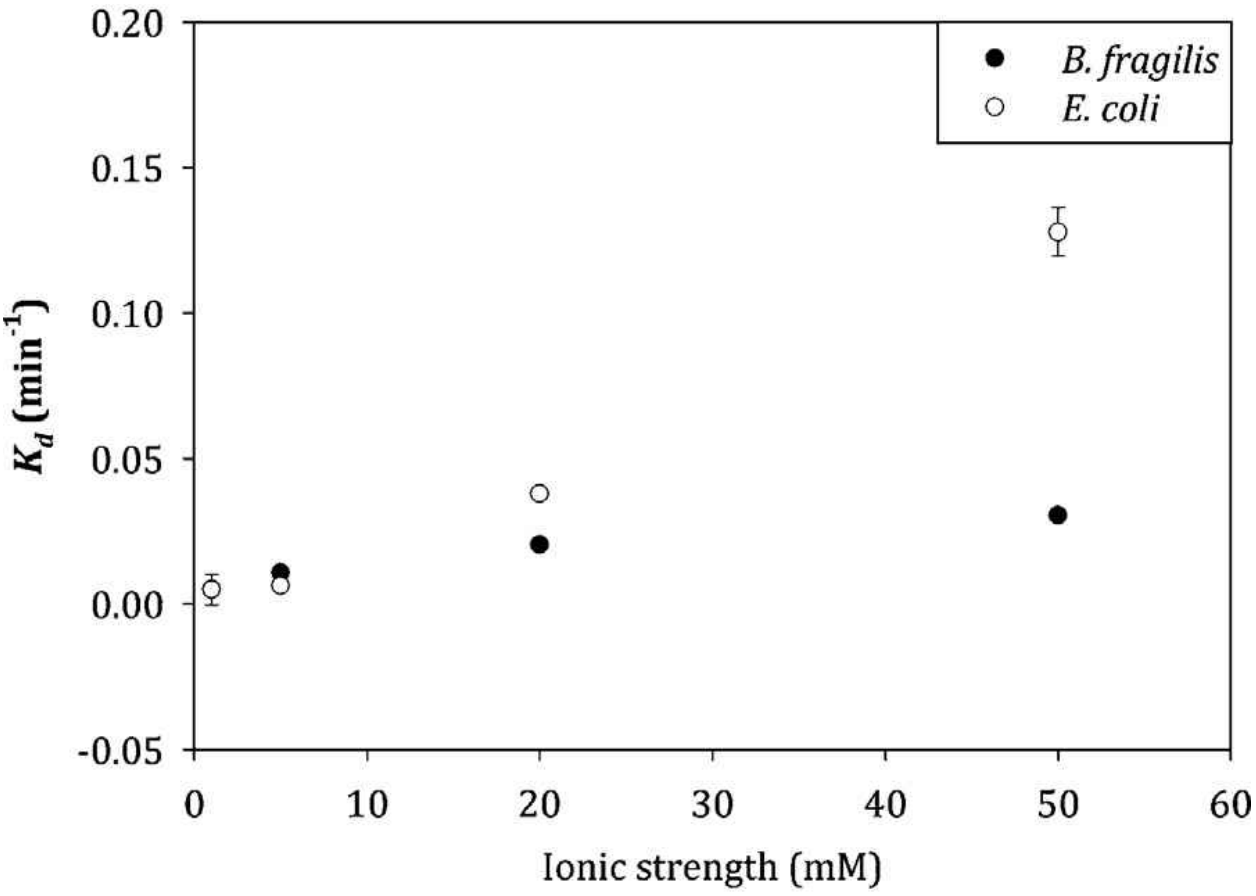

Figure 6.2. Comparison of the deposition rate coefficients (K_d) for *E. coli* K-12 and *B. fragilis*. The error bars, which are usually smaller than the symbols, represent the standard deviation of duplicate experiments.

As noted in equation 6.2, k_d is a function of the attachment efficiency α, which is strongly dependent on the energy interactions between the bacterial cells and the sand grains. Comparing α at various ionic strengths (Figure 6.3) showed a trend similar to the k_d trend, indicating that the attachment efficiency is likely the major controlling factor in the deposition rate for these bacteria.

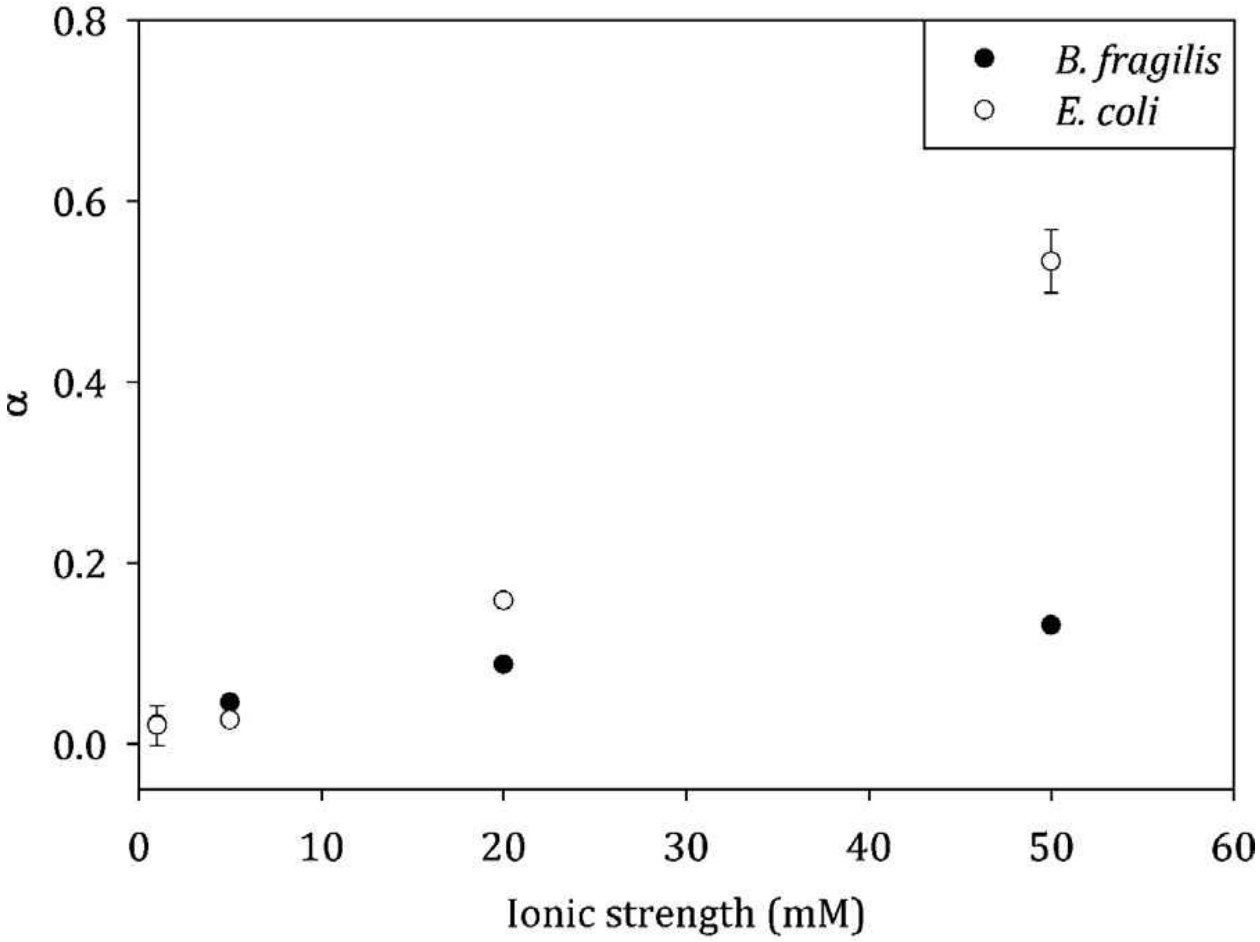

Figure 6.3. Comparison of cell attachment efficiency (*α*) for *E. coli* K-12 and *B. fragilis*. The error bars, which represent the standard deviation of duplicate experiments, are often smaller than the symbols.

6.3.2 XDLVO Energy Interactions.

Microscopic measurements were made of at least 30 cells for each ionic strength condition for each of the bacteria tested. For both *B. fragilis* and *E. coli* K-12 there was little variation in average cell size between various ionic strengths. The average equivalent cell diameter was 1.44±0.17 µm for *B. fragilis* and 1.94±0.25 µm for *E. coli* K-12. These corresponding sizes were used for the calculation of XDLVO interaction energies.

As shown in Table 6.1, water had the smallest contact angle for both bacteria (<30°); the largest contact angles were formed from diiodomethane (>54°). The measured angles for *B. fragilis* were consistently greater than for *E. coli* K-12. The interfacial tension parameters were calculated using equation 6.10, which in turn were used to evaluate *A* (equation 6.5; 2.09×10^{-21} J for *B. fragilis*, and 2.83×10^{-21} J for *E. coli* K-12, respectively) and to calculate $\Delta G_{h_0}^{AB}$ (equation 6.8; 24.95 and 25.84, respectively). The positive calculated values of $\Delta G_{h_0}^{AB}$ suggest a repulsive AB interaction with quartz sand.

Table 6.1. Contact Angle Measurements, Surface Tension Components, Hamaker Constant, ΔG_{h0}^{AB} and ΔG_{iwi} for *B. fragilis* and *E. coli* K-12

properties		***B. fragilis***	***E. coli*** K-12
contact angle (°) ($n \geq 4$)	water	27.6 (±4.1)	16.0 (±3.9)
	glycerol	34.2 (±7.8)	19.4 (±0.3)
	diiodomethane	59.5 (±3.8)	54.7 (±05.2)
surface tension components (mJ/m^2)	γ^{LW}	0.500	0.912
	γ^{+}	3.24	4.37
	γ^{-}	46.6	46.9
Hamaker constant ($x10^{-21}$ J), *A*		2.09	2.83
$\Delta G_{h0}^{AB}(mJ/m^2)$		25.84	24.95

Zeta potential measurements indicate that sand, *B. fragilis* and *E. coli* K-12 surfaces were all were negatively charged (Figure 6.4). Sand was more negative than either of the bacteria, and *E. coli* K-12 was more negative than *B. fragilis* at all ionic strengths. All three became less

negative with increasing ionic strength due to compression of the electric double layer, which would lead to lower repulsive forces. However, only a very small change occurred in *B. fragilis*, an increase of 5 mV, whereas both *E. coli* K-12 and sand experienced a much larger change in zeta potential, 14 mV and 18 mV, respectively.

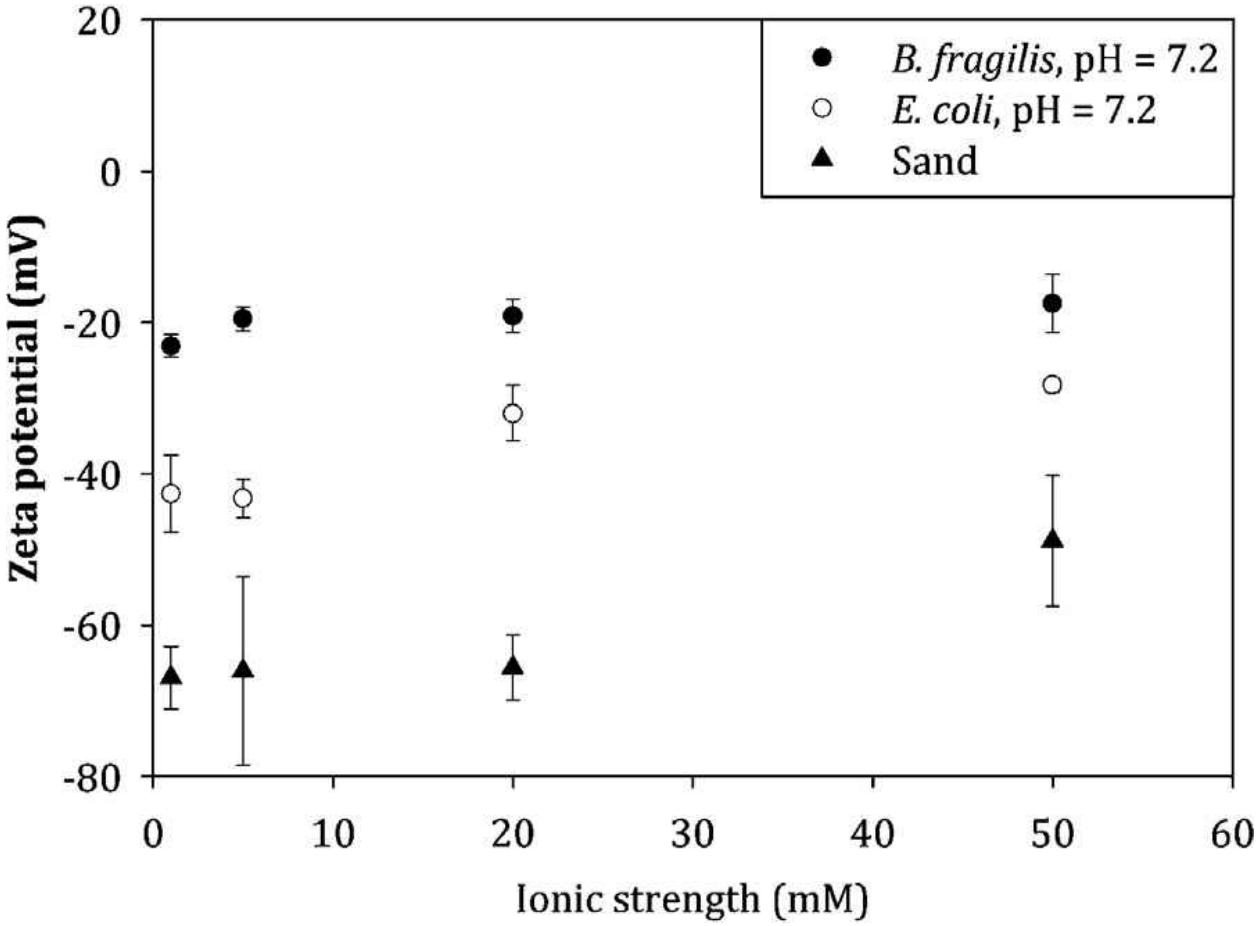

Figure. 6.4. The zeta potential values of *E. coli* K-12, *B. fragilis* and the quartz sand under ionic strength of 1 to 50 mM. The error bars represent the standard deviation of a minimum of 5 measurements.

Figure 6.5 shows the calculated XDLVO energy interaction profiles for each ionic strength condition. In every case there is a significant repulsive energy barrier between the bacterial cells and the surface of the quartz sand. Total energy barriers ranged from 17,100 *k*T to 26,800

*k*T, which would preclude deposition of the bacteria on the quartz grains under the tested conditions. Inspection of LW, EDL, and AB components show that the AB force has the largest influence on the extreme height of the energy barrier.

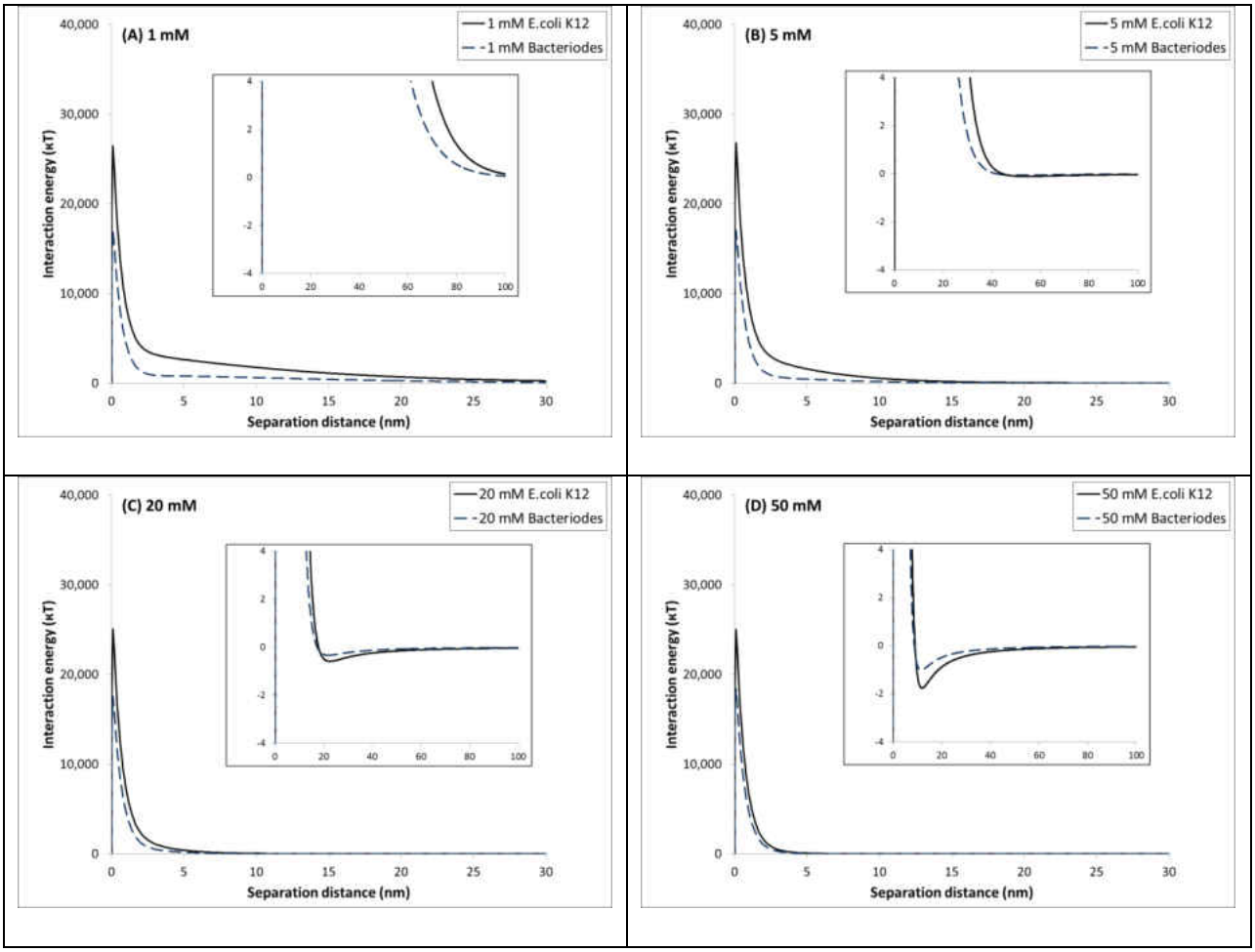

Figure 6.5. The calculated XDLVO interaction energy profiles between *E. coli* K-12 or *B. fragilis* cells and the quartz sand under ionic strength of 1 to 50 mM, respectively.

As noted in previous studies the EDL and LW forces have their strongest effects at different separation distances (Redman, Walker et al. 2004, Johanson, Feriancikova et al. 2012). This can lead to the presence of a secondary energy minimum at distances greater than the primary energy minimum. In general, a bacteria has a the thermal energy of about 0.5 *k*T, so if a

secondary energy minima exists, and has an energy level with depths greater than 0.5 *k*T, bacterial cells can be trapped in this secondary energy minimum (Redman, Walker et al. 2004). In this study, no secondary energy minimum was present at a total ionic strength of 1 mM, and the depth of the secondary minimum at 5 mM total ionic strength was smaller than 0.5 *k*T for both types of bacteria tested, indicating that few bacteria would be held due to this small secondary energy minimum. However, the depth of the calculated secondary minimum predicted by the XDLVO for 20 mM and 50 mM total ionic strength is greater than 0.5 *k*T. In addition, the calculated secondary minimum depth for the *E. coli* was significantly deeper than for the *B. fragilis* for both 20 mM and 50 mM total ionic strengths (Figure 6.5). This coincides with the greater attachment of *E. coli* K-12 at higher total ionic strengths.

The XDLVO theory considers the LW, EDL, and AB forces (equation 6.3). While the LW (equation 6.4) and AB (equation 6.7) forces are independent of ionic strength, the repulsive EDL force (equation 6.6) varies with ionic strength as both cell surface potential (Figure 6.4) and the Debye length (Equation 6.9) are dependent on water chemistry. When ionic strength increases, the surface potential of the sand, *E.coli*, and *B. fragilis* cells become slightly less negative and tend to decrease the magnitude of the EDL force. Additionally, the decrease in Debye length further lowers the EDL force. The decreased EDL force with increasing ionic strength in turn led to the formation of the secondary energy minimum under high ionic strength conditions.

Further inspection of the individual XDLVO forces showed that as *E. coli* cells are more negatively charged than the *B. fragilis* cells at a given ionic strength level (Figure 6.4), the EDL force was thus more repulsive for *E. coli*. The attractive LW force, however, was stronger for *E. coli* than for *B. fragilis* because *E. coli* had higher Hamaker constant (2.83×10^{-21}J vs. 2.09 x

10^{-21}J) and larger size (1.94 μm vs. 1.44 μm). Equation 6.7 suggested that the AB force decreased more rapidly with the separation distance than the LW and EDL forces and approached zero at separation distances of 7 nm or greater. Because the secondary energy minimum was generally located 7 nm or further away from the sand surface, the AB force has very small effects on the depth of the secondary energy minimum. When the LW (more attractive for *E. coli*), EDL (more repulsive for *E. coli*) and AB (~0 when h > 7 nm) were combined, the secondary energy minimum was deeper for *E. coli* than for *B. fragilis*.

The secondary energy minimum plays the largest role in determining bacterial attachment at higher ionic strength. This explains the deposition rate and attachment efficiency results (Figures 6.2 and 6.3), which showed nearly identical results for both types of bacteria at 1 mM and 5 mM total ionic strength, and significantly higher deposition rates and attachment efficiencies for *E. coli* K-12 at 20 mM and 50 mM total ionic strengths. In addition, the distance to the secondary energy minimum decreases with increasing ionic strength, corresponding to the compression of the EDL.

It is well known that many factors, such as surface macro-molecules, cell and grain roughness, and surface charge heterogeneity that are not considered by the XDLVO theory, can play important roles in cell immobilization (Kim, Bradford et al. 2009, Kim, Hong et al. 2009, Wang, Xu et al. 2011, Bradford and Torkzaban 2012). As suggested by several recent studies (Sang, Morales et al. 2013, Treumann, Torkzaban et al. 2014), further research that aims at quantifying and distinguishing the effects of such factors is warranted.

6.3.3 Environmental Implications

Groundwater can be tested for the presence of indicator microorganisms such as *E. coli* to detect if a groundwater source is contaminated with fecal bacteria (USEPA 2007). However, the use of *E. coli* for microbial source tracking (MST) has found only limited success (Whitlock, Jones et al. 2002, Cimenti, Biswas et al. 2005, Morrow, Stratton et al. 2005, Graves, Hayedorn et al. 2007, Ahmad, Tourlousse et al. 2009). Fecal anaerobes such as *Bacteroides* are several orders of magnitude more abundant than fecal coliforms, and host-specific *Bacteriodes* markers are present that can be used to determine the origin of fecal pollution (Fiksdal, Maki et al. 1985, Bower, Scopel et al. 2005, Layton, McKay et al. 2006, Gawler, Beecher et al. 2007, Ahmad, Tourlousse et al. 2009, Mieszkin, Furet et al. 2009). The results from this research indicate that *E coli* K-12 and *B. fragilis* displayed comparable mobility within packed sands under low ionic strength conditions, and in cases of high ionic strength groundwater, such as may be found near concentrated sources of fecal contamination, *B. fragilis* has a higher breakthrough concentration and a lower attachment efficiency than *E coli* K-12, suggesting that at higher ionic strength *B. fragilis* may be more readily transported within the groundwater system.

The greater attachment of *E. coli* K-12 compared *B. fragilis* implies the latter would potentially be detectable earlier and at a greater distance from the originating source of contamination than the former, providing an earlier warning of source contamination, and therefore being more protective of human health and the environment. As host-specific *Bacteriodes* markers are being explored to determine the origin of fecal pollution, this research supports the potential for *Bacteriodes spp*., which have been long suggested as alternative indicators for

fecal contamination (Fiksdal, Maki et al. 1985, Cimenti, Biswas et al. 2005) to serve as a promising groundwater fecal contamination indicator that can potentially be used for MST. Our experimental results also suggested that the *E. coli* breakthrough concentrations increased with time (a phenomenon often referred to as the blocking effect) under high ionic strength conditions. In contrast, the breakthrough concentration of *B. fragilis* decreased over time under the 50 mM ionic strength condition. It is likely, therefore, that the mobility of *E. coli* and *B. fragilis* will converge under long-term transport scenarios. Such temporal development in their transport behavior can have significant implications for the usefulness of *B. fragilis* as a microbial contamination indicator with source tracking capabilities, and therefore more research is warranted.

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APPENDICES

Appendix A: Column Test Breakthrough Curve Data

Notes: PV = pore volumes that have passed through the sand packed column
C/Co = normalized breakthrough concentrations
Numbers in parentheses indicates duplicate columns (1) and (2)

A.1. Breakthrough concentrations for *E. faecium* E1162

Table A.1 Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
-0.1500	7.93E-03	-3.97E-03	-6.52E-04	1.96E-03	0	0	0	0	7.30E-03	-4.37E-04
-0.1224	-2.41E-03	-4.76E-03	-2.28E-03	1.57E-03	0	0	-5.57E-03	1.90E-03	3.32E-03	-8.74E-04
-0.0947	-3.45E-03	-3.18E-03	-2.93E-03	1.96E-03	0	0	-7.86E-03	1.14E-03	1.99E-03	-4.37E-04
-0.0671	2.76E-03	-7.94E-04	-2.28E-03	1.57E-03	0	0	-5.57E-03	7.59E-04	1.66E-03	-8.74E-04
-0.0395	6.89E-04	-1.59E-03	-2.93E-03	3.14E-03	0	0	-5.57E-03	-3.79E-04	1.66E-03	-8.74E-04
-0.0119	6.89E-04	1.19E-03	-2.93E-03	1.18E-03	0	0	-5.57E-03	-1.14E-03	1.66E-03	-1.75E-03
0.0158	5.51E-03	2.38E-03	-2.93E-03	1.57E-03	0	0	-6.88E-03	-1.14E-03	1.99E-03	-8.74E-04
0.0434	5.86E-03	3.97E-03	6.52E-04	1.18E-03	0	0	-4.26E-03	-3.03E-03	2.66E-03	-8.74E-04
0.0710	4.13E-03	4.76E-03	-3.58E-03	1.18E-03	0	0	-5.57E-03	-1.52E-03	1.99E-03	-4.37E-04
0.0986	4.13E-03	4.76E-03	-2.93E-03	1.96E-03	0	0	-6.22E-03	-1.52E-03	1.99E-03	-8.74E-04
0.1263	5.17E-03	4.76E-03	-1.96E-03	1.57E-03	0	0	-7.20E-03	-1.52E-03	1.99E-03	-1.75E-03
0.1539	5.51E-03	6.35E-03	-3.91E-03	1.18E-03	0	0	-6.22E-03	-1.90E-03	2.66E-03	-4.37E-04
0.1815	5.17E-03	0.0111	-2.93E-03	-7.84E-04	0	0	-3.93E-03	-3.41E-03	2.32E-03	-4.37E-04
0.2091	4.13E-03	6.35E-03	-4.24E-03	0	0	0	-4.26E-03	-3.41E-03	3.32E-03	-8.74E-04
0.2368	6.20E-03	4.37E-03	-3.91E-03	-7.84E-04	0	0	-8.84E-03	-3.03E-03	2.66E-03	-8.74E-04
0.2644	4.13E-03	5.56E-03	-3.91E-03	-7.84E-04	0	0	-7.53E-03	-1.52E-03	1.99E-03	-8.74E-04
0.2920	3.79E-03	5.56E-03	-3.58E-03	-7.84E-04	0	0	-5.24E-03	-1.14E-03	1.99E-03	-1.75E-03
0.3197	2.76E-03	4.76E-03	-1.96E-03	1.96E-03	0	0	-7.20E-03	-1.90E-03	3.98E-03	-8.74E-04
0.3473	3.45E-03	4.37E-03	4.89E-03	3.14E-03	0	0	-4.91E-03	-3.03E-03	3.32E-03	4.37E-04
0.3749	2.76E-03	4.76E-03	6.84E-03	5.88E-03	0	0	-5.24E-03	-3.03E-03	1.99E-03	0
0.4025	3.79E-03	4.37E-03	5.54E-03	3.14E-03	0	0	-8.84E-03	-3.03E-03	1.66E-03	-4.37E-04
0.4302	2.76E-03	3.97E-03	2.93E-03	1.96E-03	0	0	-7.53E-03	-1.90E-03	1.99E-03	-4.37E-04
0.4578	2.76E-03	3.57E-03	9.78E-04	1.57E-03	0	0	-5.57E-03	-1.52E-03	2.32E-03	4.37E-04
0.4854	4.13E-03	3.57E-03	-6.52E-04	1.57E-03	0	0	-7.20E-03	-1.52E-03	2.66E-03	0
0.5130	2.41E-03	3.57E-03	-1.63E-03	1.57E-03	0	0	-7.20E-03	-3.03E-03	1.66E-03	4.37E-04
0.5407	2.76E-03	3.57E-03	-2.93E-03	1.96E-03	9.83E-03	7.17E-03	-2.62E-03	-3.03E-03	1.66E-03	0

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
0.5683	2.41E-03	2.38E-03	-2.28E-03	4.31E-03	6.88E-03	5.18E-03	-3.93E-03	-3.03E-03	0	0
0.5959	1.72E-03	1.98E-03	-2.93E-03	1.18E-03	3.60E-03	3.98E-03	-6.22E-03	-3.03E-03	-3.32E-04	-4.37E-04
0.6235	2.41E-03	1.98E-03	-2.28E-03	1.18E-03	3.28E-03	3.19E-03	-4.26E-03	-3.03E-03	3.32E-04	-4.37E-04
0.6512	1.38E-03	1.59E-03	-2.28E-03	1.57E-03	1.97E-03	1.59E-03	-8.84E-03	-1.52E-03	0	-8.74E-04
0.6788	6.89E-04	1.98E-03	-3.58E-03	3.92E-04	9.83E-04	1.59E-03	-5.24E-03	-3.03E-03	3.32E-04	-8.74E-04
0.7064	3.45E-04	1.98E-03	-3.91E-03	1.18E-03	2.62E-03	1.59E-03	-4.26E-03	-3.03E-03	0	-1.75E-03
0.7341	0	1.19E-03	-2.28E-03	0	2.62E-03	1.59E-03	-5.24E-03	-1.90E-03	0	-4.37E-04
0.7617	3.45E-04	1.19E-03	0	1.57E-03	6.55E-04	1.19E-03	-8.84E-03	-3.03E-03	3.32E-04	-8.74E-04
0.7893	0	0	-3.58E-03	1.18E-03	3.60E-03	7.97E-04	-7.53E-03	-1.52E-03	0	0
0.8169	0	0	0	0	0	0	-4.26E-03	-1.90E-03	0	0
0.8446	-1.38E-03	0	-4.24E-03	0	-9.83E-04	7.97E-04	-5.57E-03	-1.90E-03	1.99E-03	-4.37E-04
0.8722	-1.38E-03	-3.97E-04	-4.56E-03	3.53E-03	-1.31E-03	-7.97E-04	-7.20E-03	-3.41E-03	1.66E-03	-8.74E-04
0.8998	-1.72E-03	-3.97E-04	-4.24E-03	7.84E-03	-2.29E-03	0	-3.93E-03	-3.41E-03	6.64E-04	-8.74E-04
0.9274	3.45E-04	-7.94E-04	-4.24E-03	1.96E-03	0	0	-6.88E-03	-1.90E-03	0	-4.37E-04
0.9551	-2.07E-03	1.98E-03	-2.93E-03	1.57E-03	6.55E-04	7.97E-04	-4.91E-03	-1.90E-03	0	-8.74E-04
0.9827	-2.07E-03	1.98E-03	-1.96E-03	1.57E-03	9.83E-04	4.38E-03	-6.22E-03	-3.79E-04	3.32E-04	-8.74E-04
1.0103	3.45E-04	6.35E-03	9.78E-04	9.80E-03	1.97E-03	0.0115	-4.26E-03	7.59E-04	3.32E-04	-4.37E-04
1.0379	6.20E-03	0.0167	8.15E-03	0.0227	5.24E-03	0.0235	-4.26E-03	3.79E-03	3.32E-04	-8.74E-04
1.0656	0.019	0.0377	0.0176	0.0188	0.0118	0.0402	-3.27E-04	6.07E-03	2.66E-03	-4.37E-04
1.0932	0.0462	0.0655	0.0303	0.0945	0.0285	0.0621	-1.64E-03	9.48E-03	1.99E-03	-4.37E-04
1.1208	0.0799	0.1155	0.0864	0.1454	0.04	0.0868	5.24E-03	0.0133	1.99E-03	-8.74E-04
1.1484	0.1451	0.1921	0.0984	0.1932	0.0868	0.1159	7.53E-03	0.0171	1.66E-03	4.37E-04
1.1761	0.1923	0.2846	0.174	0.2681	0.1209	0.1497	0.0111	0.0197	3.32E-04	0
1.2037	0.3377	0.3748	0.2249	0.3131	0.1278	0.1816	0.0131	0.0228	1.99E-03	4.37E-04
1.2313	0.4245	0.4692	0.3073	0.35	0.2182	0.217	0.016	0.0258	2.32E-03	4.37E-04
1.2590	0.4917	0.5554	0.3575	0.5075	0.2585	0.2525	0.0174	0.0285	2.32E-03	1.75E-03

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
1.2866	0.5954	0.6272	0.3976	0.5291	0.3028	0.2871	0.0216	0.0296	2.66E-03	2.18E-03
1.3142	0.6678	0.6868	0.4859	0.5052	0.3421	0.3186	0.0265	0.0311	4.32E-03	2.62E-03
1.3418	0.7039	0.7312	0.5534	0.6377	0.3666	0.3477	0.0229	0.0319	4.32E-03	3.06E-03
1.3695	0.767	0.6407	0.5921	0.6475	0.3902	0.3716	0.0259	0.0322	4.65E-03	3.93E-03
1.3971	0.7815	0.8218	0.6202	0.6741	0.3997	0.3931	0.0275	0.0338	4.65E-03	4.81E-03
1.4247	0.8342	0.8444	0.6345	0.6823	0.4312	0.4058	0.0239	0.0341	6.97E-03	5.68E-03
1.4523	0.8487	0.8547	0.6524	0.6968	0.441	0.4194	0.0269	0.0341	6.97E-03	4.81E-03
1.4800	0.848	0.8607	0.672	0.7012	0.4525	0.4289	0.0265	0.0341	6.31E-03	5.68E-03
1.5076	0.8611	0.867	0.6896	0.707	0.444	0.4345	0.0269	0.0341	7.30E-03	5.24E-03
1.5352	0.8687	0.8714	0.6948	0.7121	0.4561	0.4401	0.0255	0.0341	6.97E-03	6.55E-03
1.5628	0.8704	0.8734	0.6971	0.7133	0.461	0.4405	0.0239	0.0345	9.30E-03	5.68E-03
1.5905	0.8735	0.8742	0.7043	0.7149	0.4581	0.4444	0.0239	0.0341	9.30E-03	6.55E-03
1.6181	0.8642	0.8789	0.7114	0.7145	0.459	0.4436	0.0269	0.0334	8.96E-03	5.24E-03
1.6457	0.8814	0.8805	0.7117	0.7184	0.4561	0.4464	0.0236	0.0334	8.96E-03	6.55E-03
1.6734	0.8976	0.8833	0.7121	0.72	0.4518	0.4476	0.0255	0.0338	7.30E-03	6.55E-03
1.7010	0.899	0.8817	0.7111	0.7211	0.4558	0.448	0.0246	0.0338	6.97E-03	6.55E-03
1.7286	0.9	0.8825	0.7091	0.7258	0.4561	0.4484	0.0265	0.0334	6.97E-03	6.55E-03
1.7562	0.8966	0.8853	0.7153	0.7231	0.4577	0.4512	0.0255	0.0338	6.97E-03	6.55E-03
1.7839	0.9021	0.8845	0.7137	0.72	0.459	0.452	0.0259	0.0338	7.30E-03	6.55E-03
1.8115	0.9059	0.8849	0.7147	0.7219	0.4574	0.452	0.0255	0.0322	6.31E-03	5.68E-03
1.8391	0.9041	0.8865	0.7144	0.7184	0.4597	0.452	0.0232	0.0334	7.30E-03	6.55E-03
1.8667	0.9035	0.8865	0.7085	0.7211	0.4541	0.4524	0.0236	0.0322	7.64E-03	6.55E-03
1.8944	0.8941	0.8884	0.7137	0.7192	0.4558	0.452	0.0252	0.0322	6.97E-03	6.55E-03
1.9220	0.9035	0.8896	0.7098	0.7192	0.4528	0.452	0.0239	0.0322	7.30E-03	6.55E-03
1.9496	0.9165	0.8908	0.7144	0.7192	0.459	0.4552	0.0259	0.0322	7.30E-03	5.68E-03
1.9772	0.9162	0.8884	0.7091	0.7153	0.4574	0.4568	0.0232	0.0322	8.96E-03	5.68E-03

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
2.0049	0.9134	0.8904	0.7091	0.727	0.4554	0.4568	0.0252	0.0338	7.30E-03	6.99E-03
2.0325	0.9155	0.8904	0.7049	0.7235	0.4548	0.4576	0.0252	0.0334	8.96E-03	5.68E-03
2.0601	0.9031	0.892	0.7108	0.7258	0.4489	0.4576	0.0239	0.0338	7.30E-03	6.55E-03
2.0878	0.9035	0.8896	0.7091	0.7231	0.46	0.4572	0.0265	0.0341	6.97E-03	6.55E-03
2.1154	0.9055	0.8884	0.7049	0.7227	0.4541	0.46	0.0236	0.0345	6.97E-03	6.55E-03
2.1430	0.8938	0.8908	0.7	0.7258	0.4561	0.4592	0.0252	0.0341	6.31E-03	5.68E-03
2.1706	0.9014	0.8912	0.702	0.7235	0.4548	0.4604	0.0252	0.0341	6.97E-03	6.55E-03
2.1983	0.901	0.8865	0.6994	0.7227	0.4548	0.4604	0.0269	0.0341	5.64E-03	8.74E-03
2.2259	0.8886	0.9015	0.6974	0.7227	0.4541	0.4596	0.0252	0.0341	6.97E-03	6.55E-03
2.2535	0.911	0.8956	0.6977	0.7231	0.46	0.4624	0.0282	0.0345	6.97E-03	6.55E-03
2.2811	0.9134	0.8956	0.6974	0.7219	0.4604	0.4604	0.0288	0.0345	8.96E-03	5.68E-03
2.3088	0.9124	0.896	0.6974	0.7239	0.4561	0.4624	0.0246	0.0345	7.30E-03	5.24E-03
2.3364	0.9035	0.8952	0.7003	0.7235	0.463	0.4616	0.0255	0.0345	6.97E-03	6.99E-03
2.3640	0.9072	0.894	0.6968	0.7235	0.4604	0.4624	0.0269	0.036	6.97E-03	6.55E-03
2.3916	0.911	0.8956	0.6968	0.7211	0.4604	0.4636	0.0269	0.0345	7.64E-03	6.55E-03
2.4193	0.9066	0.894	0.6958	0.7231	0.4584	0.4648	0.0255	0.0353	7.30E-03	6.99E-03
2.4469	0.9072	0.8936	0.6951	0.7235	0.4646	0.4636	0.0285	0.036	6.31E-03	6.55E-03
2.4745	0.9169	0.892	0.6994	0.7227	0.4636	0.4656	0.0255	0.0364	6.97E-03	6.55E-03
2.5022	0.9148	0.8928	0.6912	0.7235	0.4653	0.4679	0.0285	0.0364	7.30E-03	6.55E-03
2.5298	0.9079	0.8936	0.7013	0.7172	0.462	0.4663	0.0282	0.036	6.97E-03	6.99E-03
2.5574	0.9131	0.8952	0.7013	0.7192	0.4663	0.4679	0.0285	0.0364	7.30E-03	6.99E-03
2.5850	0.9124	0.8928	0.6994	0.72	0.4626	0.4663	0.0282	0.0364	7.30E-03	6.55E-03
2.6127	0.9097	0.894	0.6948	0.7184	0.463	0.4679	0.0269	0.0364	6.97E-03	6.99E-03
2.6403	0.9079	0.8956	0.6994	0.7172	0.4721	0.4679	0.0295	0.0364	6.31E-03	6.55E-03
2.6679	0.9107	0.8952	0.702	0.7168	0.4656	0.4663	0.0269	0.036	7.30E-03	6.55E-03
2.6955	0.9138	0.8932	0.701	0.7192	0.4636	0.4691	0.0288	0.0368	6.97E-03	6.55E-03

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
2.7232	0.9165	0.8928	0.6971	0.7184	0.4663	0.4679	0.0265	0.0379	6.31E-03	6.55E-03
2.7508	0.9079	0.894	0.7003	0.7192	0.4653	0.4683	0.0282	0.0368	6.97E-03	6.55E-03
2.7784	0.9066	0.8952	0.699	0.7153	0.4676	0.4683	0.0265	0.0376	7.64E-03	6.99E-03
2.8060	0.9069	0.8952	0.6955	0.7172	0.4676	0.4719	0.0288	0.0364	7.97E-03	6.99E-03
2.8337	0.9041	0.8956	0.6987	0.7149	0.4672	0.4743	0.0288	0.0368	7.30E-03	6.55E-03
2.8613	0.8976	0.8932	0.699	0.7133	0.4718	0.4719	0.0308	0.0368	7.64E-03	6.55E-03
2.8889	0.9	0.8952	0.7065	0.7227	0.4728	0.4723	0.0331	0.0376	7.64E-03	7.86E-03
2.9165	0.9069	0.8956	0.7023	0.7211	0.4669	0.4731	0.0282	0.0364	7.30E-03	6.99E-03
2.9442	0.9059	0.8984	0.7003	0.7231	0.4669	0.4711	0.0305	0.0368	7.30E-03	6.99E-03
2.9718	0.9035	0.8956	0.6922	0.7227	0.4663	0.4723	0.0288	0.0376	7.30E-03	6.99E-03
2.9994	0.8952	0.898	0.7003	0.7239	0.4672	0.4735	0.0288	0.0368	6.97E-03	6.99E-03
3.0271	0.9014	0.8964	0.702	0.7208	0.4672	0.4743	0.0301	0.0368	7.64E-03	6.99E-03
3.0547	0.9072	0.8972	0.6958	0.7208	0.4685	0.4751	0.0295	0.0368	7.30E-03	6.99E-03
3.0823	0.9069	0.8956	0.6987	0.7208	0.4725		0.0318	0.0387	7.64E-03	7.86E-03
3.1099	0.8983		0.7049	0.7231	0.4741		0.0301	0.0379	6.97E-03	6.99E-03
3.1376	0.9035	0.9127	0.6971	0.7227	0.4741	0.4851	0.0295	0.0379	7.30E-03	7.86E-03
3.1652	0.9066	0.9127	0.7023	0.7211	0.4718	0.4819	0.0305	0.0387	8.96E-03	7.86E-03
3.1928	0.8976	0.9127	0.7023	0.7239	0.4708	0.4974	0.0301	0.0387	7.64E-03	6.55E-03
3.2204		0.9131		0.7239	0.4761	0.4839	0.0327	0.0376		7.86E-03
3.2481	0.9035	0.9	0.7039	0.7208	0.4764	0.4863	0.0305	0.0379	8.96E-03	6.55E-03
3.2757	0.9066	0.8992	0.7003	0.7231	0.4764	0.4839	0.0265	0.0387	7.30E-03	6.99E-03
3.3033	0.9131	0.8992	0.6951	0.7235	0.4767	0.4819	0.0269	0.0379	7.30E-03	7.86E-03
3.3309	0.9221	0.898	0.7026	0.7235	0.4728	0.4839	0.0285	0.0376	7.30E-03	7.86E-03
3.3586	0.9207	0.8964	0.701	0.7258	0.4748	0.4819	0.0305	0.0406	6.97E-03	7.86E-03
3.3862	0.919	0.8964	0.702	0.7196	0.4764	0.4831	0.0288	0.0398	6.97E-03	6.99E-03
3.4138	0.9134	0.8952	0.7003	0.7235	0.4725	0.4839	0.0318	0.0402	7.30E-03	6.99E-03

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
3.4415	0.9148	0.8956	0.7036	0.7227	0.4774	0.4819	0.0295	0.0398	7.30E-03	7.86E-03
3.4691	0.9069	0.8972	0.7003	0.7251	0.4744	0.4851	0.0305	0.0395	6.97E-03	6.99E-03
3.4967	0.921	0.898	0.7039	0.7231	0.4784	0.4839	0.0318	0.0398	7.30E-03	6.55E-03
3.5243	0.9193	0.896	0.7039	0.7211	0.479	0.4851	0.0321	0.0402	6.97E-03	6.99E-03
3.5520	0.9193	0.898	0.7043	0.7235	0.4784	0.4859	0.0305	0.0395	7.30E-03	7.86E-03
3.5796	0.9093	0.8956	0.7013	0.7227	0.479	0.4879	0.0318	0.0398	7.30E-03	6.99E-03
3.6072	0.9172	0.8956	0.7023	0.7239	0.4826	0.4894	0.0327	0.0398	6.97E-03	6.99E-03
3.6348	0.9124	0.898	0.7062	0.7235	0.4794	0.4966	0.0301	0.0406	6.97E-03	6.99E-03
3.6625	0.9172	0.898	0.7091	0.7341	0.4777	0.4994	0.0318	0.0406	7.64E-03	6.99E-03
3.6901	0.9093	0.8972	0.7082	0.7251	0.4826	0.4974	0.0341	0.0398	7.97E-03	7.86E-03
3.7177	0.919	0.9008	0.7065	0.7255	0.4813	0.495	0.0318	0.0406	8.96E-03	6.99E-03
3.7453	0.9214	0.8972	0.7003	0.7255	0.4767	0.4942	0.0318	0.0398	7.30E-03	8.74E-03
3.7730	0.9214	0.8972	0.7065	0.7235	0.4872	0.4974	0.0318	0.0406	7.30E-03	7.86E-03
3.8006	0.919	0.9	0.7059	0.7239	0.482	0.4942	0.0331	0.0402	6.97E-03	8.74E-03
3.8282	0.911	0.9008	0.7062	0.7235	0.4797	0.4966	0.0305	0.0421	6.31E-03	6.55E-03
3.8559	0.9066	0.9	0.7059	0.7235	0.4794	0.4974	0.0327	0.0406	7.97E-03	9.17E-03
3.8835	0.9169	0.9	0.7046	0.7294	0.482	0.4974	0.0321	0.0406	7.97E-03	6.99E-03
3.9111	0.9145	0.9008	0.7059	0.7239	0.4784	0.4978	0.0347	0.0406	7.30E-03	8.74E-03
3.9387	0.9066	0.8992	0.7082	0.7235	0.4882	0.4978	0.0324	0.0421	7.30E-03	7.86E-03
3.9664	0.9097	0.9	0.7072	0.7235	0.4869	0.499	0.0318	0.0425	7.64E-03	7.86E-03
3.9940	0.9138	0.8992	0.7043	0.7255	0.4908	0.4978	0.0331	0.0425	6.97E-03	7.86E-03
4.0216	0.9097	0.9015	0.7108	0.7255	0.4898	0.4966	0.0341	0.0425	7.30E-03	6.99E-03
4.0492	0.9072	0.9015	0.7091	0.7251	0.4817	0.4978	0.0331	0.0425	7.64E-03	6.55E-03
4.0769	0.9134	0.9027	0.7072	0.7239	0.4898	0.4998	0.0331	0.0417	8.96E-03	6.99E-03
4.1045	0.909	0.9019	0.7003	0.7251	0.4921	0.4998	0.0354	0.0425	7.97E-03	7.86E-03
4.1321	0.9286	0.9015	0.7072	0.7227	0.4869	0.5018	0.0327	0.0425	7.64E-03	7.86E-03

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
4.1597	0.9262	0.9023	0.7085	0.7145	0.4846	0.5006	0.0327	0.0425	8.96E-03	7.86E-03
4.1874	0.9214	0.9	0.7059	0.7227	0.4862	0.5006	0.0318	0.0421	7.97E-03	8.74E-03
4.2150	0.919	0.8984	0.7023	0.7227	0.4849	0.5006	0.0331	0.0406	7.97E-03	8.74E-03
4.2426	0.9221	0.8972	0.7023	0.7141	0.4905	0.491	0.0318	0.0395	7.64E-03	6.99E-03
4.2703	0.9172	0.8896	0.6955	0.707	0.4797	0.4851	0.0327	0.0376	8.96E-03	8.74E-03
4.2979	0.9462	0.8678	0.6808	0.6863	0.4702	0.4691	0.0265	0.0341	7.30E-03	8.74E-03
4.3255	0.8976	0.8388	0.6573	0.6322	0.4702	0.4476	0.0255	0.03	7.30E-03	8.74E-03
4.3531	0.8676	0.79	0.6159	0.5808	0.4197	0.4178	0.0255	0.0269	6.97E-03	6.99E-03
4.3808	0.8256	0.7245	0.5749	0.5173	0.384	0.3883	0.0138	0.0239	7.30E-03	8.74E-03
4.4084	0.777	0.6439	0.5055	0.4464	0.345	0.3501	0.0144	0.0197	7.30E-03	6.99E-03
4.4360	0.716	0.5554	0.4647	0.3927	0.2969	0.3082	9.50E-03	0.0156	7.30E-03	6.55E-03
4.4636	0.7126	0.4736	0.3774	0.303	0.2844	0.27	0.0102	0.0129	6.97E-03	5.68E-03
4.4913	0.533	0.453	0.3138	0.2363	0.1936	0.2302	6.88E-03	0.0102	5.64E-03	6.55E-03
4.5189	0.45	0.63	0.2682	0.2583	0.1494	0.1912	5.57E-03	9.10E-03	4.65E-03	4.81E-03
4.5465	0.3618	0.2243	0.1998	0.1576	0.1186	0.1593	2.95E-03	6.45E-03	3.98E-03	3.93E-03
4.5741	0.2863	0.187	0.1545	0.0858	0.1317	0.1318	3.27E-03	6.07E-03	3.32E-03	2.62E-03
4.6018	0.225	0.1532	0.1183	0.0737	0.0623	0.1079	-3.27E-04	3.79E-03	2.66E-03	2.62E-03
4.6294	0.1768	0.1274	0.0929	0.0478	0.0505	0.0912	-6.55E-04	3.79E-03	2.66E-03	2.18E-03
4.6570	0.1378	0.1072	0.1424	0.0325	0.0396	0.0765	-1.64E-03	2.28E-03	2.66E-03	4.37E-04
4.6846	0.1106	0.0905	0.0385	0.0247	0.0364	0.0677	-1.64E-03	2.66E-03	1.99E-03	0
4.7123	0.0858	0.0762	0.0261	0.0317	0.0269	0.0581	9.82E-04	1.90E-03	1.99E-03	4.37E-04
4.7399	0.0803	0.0655	0.0261	0.0172	0.0246	0.0522	1.96E-03	2.28E-03	6.64E-04	0
4.7675	0.0655	0.0568	0.0143	3.14E-03	0.0226	0.0478	3.27E-04	2.28E-03	3.32E-04	-4.37E-04
4.7952	0.0438	0.0484	8.80E-03	1.57E-03	0.0177	0.043	-6.55E-04	1.90E-03	6.64E-04	-4.37E-04
4.8228	0.0382	0.0409	6.84E-03	-1.18E-03	0.019	0.039	-4.26E-03	2.28E-03	3.32E-04	-8.74E-04
4.8504	0.0327	0.0357	9.78E-04	-1.96E-03	0.0177	0.0346	-3.27E-04	0	3.32E-04	-4.37E-04

Table A.1 (Continued) Breakthrough concentrations for *E. faecium* E1162

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
4.8780	0.0255	0.0298	-6.52E-04	-2.35E-03	0.0118	0.0307	-2.29E-03	1.14E-03	3.32E-04	0
4.9057	0.0231	0.0258	-1.96E-03	3.14E-03	0.0115	0.0291	-2.29E-03	7.59E-04	2.66E-03	-8.74E-04
4.9333	0.0162	0.0214	-3.58E-03	-4.31E-03	9.83E-03	0.0263	-6.55E-04	1.14E-03	6.64E-04	0
4.9609	0.0138	0.0167	-3.58E-03	-5.10E-03	8.85E-03	0.0251	-1.96E-03	7.59E-04	3.32E-04	-4.37E-04
4.9885	9.30E-03	0.0143	-5.87E-03	-6.66E-03	7.86E-03	0.0235	0	1.14E-03	1.66E-03	-4.37E-04
5.0162	7.93E-03	0.0123	-6.19E-03	-5.49E-03	6.88E-03	0.0215	1.96E-03	1.14E-03	6.64E-04	0
5.0438	6.20E-03	9.92E-03	-6.19E-03	-5.10E-03	6.88E-03	0.0215	-2.62E-03	1.14E-03	3.32E-04	-4.37E-04
5.0714	7.58E-03	0.0107	-6.52E-03	-5.10E-03	6.23E-03	0.0207	-2.29E-03	0	3.32E-04	-8.74E-04
5.0990	3.79E-03		-7.17E-03	-5.49E-03	5.24E-03	0.0203	-4.26E-03	7.59E-04	1.66E-03	-4.37E-04
5.1267	2.41E-03	5.56E-03	-7.82E-03	-2.74E-03	8.19E-03	0.0203	-3.27E-04	0	0	-8.74E-04
5.1543	6.89E-04	2.38E-03	-8.15E-03	-6.66E-03	7.21E-03	0.0187	-1.96E-03	7.59E-04	2.66E-03	-8.74E-04
5.1819	3.45E-04	1.19E-03	-2.93E-03	-6.66E-03	4.91E-03	0.0187	-3.93E-03	2.28E-03	1.66E-03	-4.37E-04
5.2096	-2.07E-03	-7.94E-04	-9.45E-03	-6.66E-03	6.88E-03	0.0175	-1.64E-03	1.14E-03	6.64E-04	-8.74E-04
5.2372	-2.41E-03	-7.94E-04	-0.0108	-6.66E-03	8.19E-03	0.0183	-3.27E-03	1.90E-03	1.99E-03	-4.37E-04
5.2648	-3.79E-03	-3.97E-03	-0.0108	-7.05E-03	4.59E-03	0.0163	-1.64E-03	0	6.64E-04	-4.37E-04
5.2924	-5.17E-03	-3.97E-03	-9.45E-03	-6.66E-03	3.28E-03	0.0147	-4.91E-03	7.59E-04	6.64E-04	-8.74E-04
5.3201	-5.86E-03	-3.18E-03	-0.0111	-7.45E-03	6.23E-03	0.0147	-3.27E-03	7.59E-04	3.32E-04	-8.74E-04
5.3477	-6.20E-03	-5.95E-03	-0.0114	-7.05E-03	8.85E-03	0.0143	-3.93E-03	7.59E-04	1.99E-03	-8.74E-04
5.3753	-7.93E-03	-6.35E-03	-0.0114	-7.45E-03	3.60E-03	0.0143	-2.29E-03	7.59E-04	1.66E-03	-8.74E-04
5.4029	-7.24E-03	-6.35E-03	-0.0117	-7.45E-03	3.28E-03	0.0131	-2.62E-03	7.59E-04		
5.4306	-7.24E-03	-6.35E-03	-0.0127	-7.84E-03	2.95E-03	0.0127	-4.26E-03	7.59E-04		
5.4582	-0.017	-3.56E-03	-0.0121	-9.42E-03			-4.91E-03	7.59E-04		

Table A.2.Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
-0.15	-2.37E-03	4.63E-03	5.92E-03	7.34E-03	0	0	0	0	-1.30E-03	2.91E-03
-0.1224	-2.76E-03	0	6.71E-03	7.69E-03	0	0	3.60E-04	-6.98E-04	-4.34E-04	2.18E-03
-0.0947	-2.37E-03	1.99E-03	7.89E-03	0.0101	0	0	0	-1.40E-03	-1.74E-03	1.45E-03
-0.0671	-3.95E-04	1.99E-03	6.71E-03	0.0122	0	0	0	0	-1.74E-03	1.82E-03
-0.0395	-3.95E-04	1.65E-03	5.92E-03	0.0154	0	0	-3.60E-04	-6.98E-04	-1.30E-03	1.45E-03
-0.0119	0	1.65E-03	6.31E-03	0.0224	0	0	-3.60E-04	-1.74E-03	-1.30E-03	1.09E-03
0.0158	1.58E-03	2.65E-03	6.71E-03	0.0259	0	0	-3.60E-04	3.49E-04	-1.30E-03	1.45E-03
0.0434	2.37E-03	2.65E-03	6.31E-03	0.0276	0	0	0	1.74E-03	-1.74E-03	1.82E-03
0.071	3.16E-03	3.31E-03	5.92E-03	0.0297	0	0	0	3.14E-03	-1.74E-03	1.82E-03
0.0986	3.55E-03	3.97E-03	5.52E-03	0.0301	0	0	0	2.44E-03	-1.30E-03	2.18E-03
0.1263	4.34E-03	3.31E-03	5.52E-03	0.0276	0	0	-3.60E-04	6.98E-04	-1.30E-03	2.18E-03
0.1539	3.55E-03	2.65E-03	5.13E-03	0.0262	0	0	-3.60E-04	6.98E-04	-4.34E-04	2.18E-03
0.1815	4.34E-03	2.65E-03	3.95E-03	0.0224	0	0	-3.60E-04	0	-1.30E-03	1.45E-03
0.2091	3.16E-03	4.30E-03	3.55E-03	0.0199	0	0	-2.16E-03	3.49E-04	-4.34E-04	1.45E-03
0.2368	3.16E-03	5.29E-03	3.55E-03	0.0164	0	0	-1.08E-03	0	-1.74E-03	0
0.2644	3.55E-03	1.65E-03	3.55E-03	0.0206	0	0	-1.08E-03	-1.40E-03	0	1.45E-03
0.292	4.34E-03	1.99E-03	3.95E-03	0.0122	0	0	-3.60E-04	3.49E-04	-1.74E-03	1.09E-03
0.3197	4.34E-03	1.99E-03	3.16E-03	0.0105	0	0	-3.60E-04	0	-4.34E-04	-3.63E-04
0.3473	2.37E-03	1.65E-03	9.87E-03	8.74E-03	0	0	-1.08E-03	-6.98E-04	-1.30E-03	1.09E-03
0.3749	2.37E-03	2.65E-03	8.29E-03	8.39E-03	0	0	-1.44E-03	0	-4.34E-04	0
0.4025	3.55E-03	0	0.0205	6.29E-03	0	0	-1.08E-03	-6.98E-04	-4.34E-04	1.09E-03
0.4302	3.16E-03	1.65E-03	5.13E-03	4.89E-03	0	0	-1.44E-03	-6.98E-04	1.74E-03	0
0.4578	2.37E-03	3.31E-04	3.95E-03	4.54E-03	0	0	-1.08E-03	0	1.30E-03	1.09E-03
0.4854	2.37E-03	0	3.55E-03	4.89E-03	0	0	-1.44E-03	0	1.30E-03	1.09E-03
0.513	1.97E-03	3.31E-04	5.52E-03	7.69E-03	0	0	-1.08E-03	-1.40E-03	1.30E-03	-7.27E-04
0.5407	3.16E-03	6.62E-04	3.16E-03	3.49E-03	0	0	-1.08E-03	0	1.30E-03	-7.27E-04

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
0.5683	2.37E-03	1.65E-03	2.76E-03	3.15E-03	0	0	-1.44E-03	0	8.68E-04	-7.27E-04
0.5959	2.37E-03	0	2.76E-03	2.45E-03	0	0	-1.44E-03	0	0	-1.09E-03
0.6235	1.97E-03	0	2.76E-03	2.45E-03	8.93E-03	0	-1.08E-03	-6.98E-04	0	-7.27E-04
0.6512	1.97E-03	-3.31E-04	1.97E-03	3.15E-03	5.95E-03	-7.68E-04	-1.44E-03	-6.98E-04	1.30E-03	-3.63E-04
0.6788	7.90E-04	0	1.97E-03	2.45E-03	3.83E-03	-3.84E-04	-2.16E-03	-6.98E-04	0	-7.27E-04
0.7064	0	0	1.97E-03	0	2.13E-03	-1.54E-03	-3.60E-04	-6.98E-04	0	-7.27E-04
0.7341	7.90E-04	6.62E-04	7.89E-04	0	2.13E-03	-1.15E-03	-3.60E-04	-6.98E-04	0	-1.09E-03
0.7617	7.90E-04	-3.31E-04	1.18E-03	1.05E-03	1.28E-03	-1.15E-03	-2.16E-03	-6.98E-04	0	-7.27E-04
0.7893	7.90E-04	-9.93E-04	7.89E-04	6.99E-04	4.25E-04	1.54E-03	-2.16E-03	0	0	-7.27E-04
0.8169	0	0	0	0	0	0	-1.44E-03	0	0	0
0.8446	7.90E-04	0	-3.95E-04	4.54E-03	-4.25E-04	0	-1.44E-03	-6.98E-04	0	-7.27E-04
0.8722	-3.95E-04	-9.93E-04	-1.58E-03	-3.49E-04	-4.25E-04	0	-2.16E-03	0	8.68E-04	-7.27E-04
0.8998	0	0	-1.58E-03	0	-8.50E-04	1.54E-03	-1.08E-03	6.98E-04	0	-7.27E-04
0.9274	-3.95E-04	-1.65E-03	-3.95E-04	-3.49E-04	-8.50E-04	1.54E-03	-1.08E-03	-6.98E-04	0	-7.27E-04
0.9551	7.90E-04	3.31E-04	-3.95E-04	-3.49E-04	4.25E-04	1.54E-03	-3.60E-04	6.98E-04	-4.34E-04	-1.82E-03
0.9827	3.55E-03	2.32E-03	1.18E-03	1.40E-03	4.25E-03	-7.68E-04	2.16E-03	1.74E-03	-4.34E-04	-1.09E-03
1.0103	0.0122	0.0109	6.71E-03	4.54E-03	0.0132	1.15E-03	5.76E-03	4.19E-03	-4.34E-04	-1.09E-03
1.0379	0.0284	0.0261	0.0154	0.0133	0.0298	7.30E-03	0.0122	4.19E-03	0	-7.27E-04
1.0656	0.0557	0.0546	0.0324	0.0262	0.0548	0.0211	0.022	0.014	1.74E-03	-7.27E-04
1.0932	0.0963	0.0807	0.0588	0.0433	0.0918	0.0446	0.036	0.0314	4.77E-03	-1.09E-03
1.1208	0.1504	0.1519	0.0951	0.0559	0.1386	0.0799	0.0515	0.0467	9.54E-03	2.91E-03
1.1484	0.2203	0.1479	0.1425	0.1209	0.162	0.1268	0.0702	0.0638	0.0165	5.45E-03
1.1761	0.2921	0.3282	0.1985	0.1601	0.2547	0.1844	0.0904	0.0813	0.0204	9.08E-03
1.2037	0.3853	0.4073	0.262	0.2114	0.3248	0.2493	0.1084	0.1012	0.026	0.012
1.2313	0.4686	0.4781	0.3295	0.2729	0.3967	0.2328	0.1263	0.1182	0.0321	0.0156
1.259	0.5555	0.5327	0.3994	0.3302	0.4613	0.4222	0.1422	0.1339	0.0373	0.0203

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
1.2866	0.6348	0.6065	0.4692	0.3949	0.5234	0.4802	0.1555	0.1479	0.0408	0.0193
1.3142	0.7031	0.6776	0.5359	0.4554	0.5761	0.5344	0.1652	0.1594	0.0438	0.0258
1.3418	0.7635	0.7282	0.5987	0.4711	0.6212	0.5786	0.1746	0.1657	0.0464	0.0283
1.3695	0.8101	0.7732	0.6504	0.548	0.656	0.6181	0.1814	0.1713	0.0477	0.0313
1.3971	0.8504	0.7398	0.6957	0.6318	0.6824	0.6477	0.1857	0.1758	0.0495	0.0313
1.4247	0.8752	0.8529	0.7356	0.6811	0.7045	0.6727	0.189	0.1789	0.0503	0.0342
1.4523	0.9064	0.8738	0.768	0.7182	0.7215	0.6857	0.1901	0.1807	0.0503	0.0345
1.48	0.9206	0.8897	0.7932	0.7339	0.7313	0.6965	0.194	0.1807	0.0503	0.0345
1.5076	0.9345	0.9045	0.8133	0.6968	0.7436	0.7065	0.1929	0.1814	0.0499	0.0331
1.5352	0.9467	0.9151	0.8295	0.8059	0.7504	0.7119	0.1933	0.1782	0.0512	0.0352
1.5628	0.9538	0.9065	0.8441	0.8171	0.753	0.7196	0.1911	0.1807	0.0503	0.0345
1.5905	0.9601	0.9383	0.8496	0.8324	0.7551	0.7265	0.1926	0.1828	0.0503	0.0352
1.6181	0.966	0.9446	0.8599	0.8496	0.7632	0.7299	0.1926	0.1793	0.0486	0.0331
1.6457	0.9688	0.9433	0.8639	0.8614	0.767	0.7342	0.1908	0.1789	0.0499	0.0334
1.6734	0.9708	0.9542	0.8717	0.8649	0.7687	0.7368	0.1908	0.1789	0.0473	0.0345
1.701	0.9759	0.9588	0.8749	0.8709	0.7725	0.7411	0.1915	0.1779	0.0477	0.0323
1.7286	0.9779	0.9624	0.8753	0.8789	0.7725	0.7415	0.1901	0.1772	0.0477	0.0323
1.7562	0.9822	0.9661	0.8788	0.8803	0.7764	0.7472	0.189	0.1768	0.0469	0.0323
1.7839	0.9826	0.9687	0.8828	0.8842	0.7785	0.7468	0.189	0.1761	0.0469	0.0323
1.8115	0.9878	0.9624	0.8828	0.8859	0.7802	0.7491	0.1879	0.1754	0.0464	0.0313
1.8391	0.9886	0.9697	0.8856	0.8842	0.7815	0.7514	0.1879	0.1758	0.0443	0.0305
1.8667	0.9909	0.9701	0.8863	0.8828	0.7853	0.7549	0.1886	0.1758	0.0443	0.0305
1.8944	0.9909	0.9763	0.8856	0.8943	0.7861	0.7553	0.1908	0.1758	0.0443	0.0302
1.922	0.9937	0.977	0.8871	0.8943	0.7891	0.7564	0.1886	0.174	0.0443	0.0298
1.9496	0.9933	0.9787	0.8863	0.8929	0.7891	0.7584	0.1879	0.174	0.043	0.0291
1.9772	0.9949	0.9849	0.8891	0.8957	0.7917	0.7572	0.189	0.1734	0.043	0.0283

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
2.0049	0.9953	0.9813	0.8883	0.8925	0.7925	0.7595	0.1897	0.1744	0.0416	0.0291
2.0325	0.9953	0.9843	0.8883	0.8957	0.7955	0.7706	0.1908	0.174	0.0421	0.028
2.0601	0.9968	0.9793	0.8875	0.8943	0.7955	0.7745	0.1908	0.174	0.0416	0.0283
2.0878	0.9949	0.9859	0.8903	0.8967	0.7963	0.7733	0.1908	0.1754	0.043	0.0283
2.1154	0.9921	0.9883	0.8871	0.8953	0.7989	0.771	0.1908	0.1744	0.0421	0.0283
2.143	0.9921	0.9849	0.8883	0.8925	0.7989	0.771	0.1908	0.1744	0.0408	0.0276
2.1706	0.9921	0.9813	0.8891	0.8908	0.7997	0.7706	0.1908	0.1758	0.0395	0.028
2.1983	0.9913	0.9853	0.8883	0.8999	0.7993	0.7768	0.1908	0.1754	0.0408	0.0273
2.2259	0.9893	0.9902	0.8915	0.8838	0.7993	0.7795	0.1915	0.1744	0.0403	0.0262
2.2535	0.9909	0.9926	0.8919	0.8789	0.8014	0.7756	0.1933	0.1758	0.0403	0.0262
2.2811	0.9909	0.9959	0.8899	0.8852	0.8014	0.7768	0.1915	0.1744	0.0416	0.0273
2.3088	0.9893	0.9926	0.8891	0.8814	0.8031	0.7772	0.1926	0.1761	0.0416	0.0273
2.3364	0.9889	0.9859	0.8923	0.8807	0.804	0.7749	0.194	0.1779	0.0416	0.0273
2.364	0.9921	0.9916	0.8923	0.88	0.8061	0.7768	0.1933	0.1782	0.0408	0.0273
2.3916	0.9913	0.9932	0.8938	0.8814	0.8031	0.7733	0.1937	0.1807	0.0416	0.0276
2.4193	0.9949	1.0008	0.8938	0.8929	0.8048	0.7818	0.194	0.1782	0.0395	0.0273
2.4469	0.9968	0.9952	0.8986	0.8929	0.807	0.7876	0.1951	0.18	0.0403	0.0273
2.4745	0.9953	0.9926	0.895	0.8985	0.807	0.7876	0.1955	0.1817	0.0408	0.0258
2.5022	0.9953	0.9939	0.8958	0.8953	0.807	0.786	0.1958	0.1828	0.0403	0.0273
2.5298	0.9968	0.9945	0.8978	0.889	0.8087	0.7849	0.1976	0.1814	0.0408	0.0273
2.5574	0.9953	0.9922	0.8946	0.889	0.8104	0.7841	0.1958	0.1814	0.0416	0.0273
2.585	0.9953	0.9935	0.895	0.8908	0.8108	0.7826	0.1969	0.1831	0.0408	0.0273
2.6127	0.9968	0.9969	0.897	0.8901	0.8108	0.7772	0.1969	0.1814	0.0416	0.0273
2.6403	0.9953	0.9975	0.895	0.8904	0.8104	0.7876	0.1991	0.1817	0.0416	0.0276
2.6679	0.9968	1.0015	0.8942	0.9006	0.8091	0.7872	0.198	0.1831	0.0408	0.0254
2.6955	0.9953	0.9982	0.8978	0.8943	0.8112	0.7876	0.1976	0.1838	0.0421	0.0276

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
2.7232	0.9953	0.9975	0.895	0.8918	0.8091	0.786	0.1991	0.1835	0.0408	0.0276
2.7508	0.9933	0.9926	0.895	0.8929	0.8116	0.786	0.1994	0.1835	0.0416	0.028
2.7784	0.9949	1.0025	0.8982	0.8925	0.8134	0.786	0.1994	0.1838	0.0421	0.0273
2.806	0.9953	0.9932	0.8982	0.8901	0.8104	0.7849	0.198	0.1838	0.0416	0.0273
2.8337	0.9949	0.9916	0.897	0.8939	0.8116	0.7856	0.1994	0.1835	0.0416	0.0283
2.8613	0.9953	0.9889	0.8966	0.8943	0.8125	0.786	0.2012	0.1838	0.0421	0.0276
2.8889	0.9945	0.9916	0.8966	0.8939	0.8159	0.7876	0.2009	0.1838	0.043	0.0276
2.9165	0.9972	0.9906	0.9006	0.8925	0.8151	0.7876	0.2016	0.1866	0.0416	0.028
2.9442	0.9992	0.9902	0.9006	0.8939	0.8159	0.7883	0.2016	0.1856	0.0438	0.028
2.9718	0.9968	0.9972	0.8978	0.8842	0.8159	0.7876	0.2019	0.1866	0.0438	0.0291
2.9994	0.998	0.9959	0.8986	0.8789	0.8138	0.7899	0.2016	0.1877	0.043	0.028
3.0271	1	0.9959	0.8994	0.8929	0.8189	0.7895	0.2037	0.1877	0.0438	0.0283
3.0547	0.9972	0.9959	0.897	0.8925	0.8159	0.7876	0.2034	0.1877	0.0421	0.0291
3.0823	0.998	0.9959	0.8958	0.8876	0.8197	0.7914	0.2048	0.189	0.043	0.028
3.1099	0.9996	0.9952	0.9002	0.889	0.8168	0.791	0.2045	0.1894	0.0443	0.0283
3.1376	1.0032	0.9853	0.8966	0.8915	0.8142	0.7883	0.2055	0.1894	0.0421	0.0276
3.1652	0.998	0.9952	0.8986	0.8915	0.8197	0.7902	0.2059	0.1908	0.0438	0.0283
3.1928	1.0016	0.9975	0.8978	0.888	0.8197	0.7902	0.2066	0.1904	0.0443	0.0291
3.2204	1.0118		0.8978	0.8901	0.8223	0.7899	0.2059	0.1894	0.0443	0.0283
3.2481	0.9992	0.9926	0.8986	0.8908	0.8223	0.7852	0.2066	0.1911	0.0438	0.0298
3.2757	0.998	0.9959	0.8982	0.8901	0.8223	0.8095	0.2073	0.1911	0.0443	0.0283
3.3033	1.0016	0.9945	0.8994	0.8943	0.8227	0.8006	0.207	0.1932	0.0447	0.0291
3.3309	0.998	0.9959	0.8986	0.8957	0.8223	0.7987	0.207	0.1953	0.0447	0.0291
3.3586	0.9976	0.9945	0.8986	0.8904	0.8231	0.8083	0.2091	0.1957	0.0447	0.0291
3.3862	1	0.9926	0.9006	0.8925	0.8231	0.8091	0.2095	0.1953	0.0443	0.0283
3.4138	0.9976	0.9926	0.8986	0.8901	0.8253	0.8106	0.2099	0.1985	0.0447	0.0305

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
3.4415	0.998	0.9916	0.8986	0.8908	0.8261	0.8083	0.2091	0.1967	0.0447	0.0302
3.4691	1	0.9939	0.8978	0.8915	0.8236	0.8102	0.2099	0.1988	0.0469	0.0305
3.4967	1.0004	0.9939	0.897	0.889	0.8257	0.8068	0.2113	0.1988	0.0451	0.0291
3.5243	1.0016	0.9916	0.8978	0.8925	0.827	0.8083	0.2113	0.1985	0.0464	0.0302
3.552	1.0016	0.9982	0.9013	0.8929	0.8274	0.8045	0.2109	0.1995	0.0464	0.0305
3.5796	1.002	0.9952	0.8978	0.8925	0.8291	0.8095	0.2127	0.1992	0.0464	0.0305
3.6072	1.002	0.9982	0.8986	0.8939	0.827	0.8133	0.2127	0.2013	0.0464	0.0305
3.6348	1.0036	0.9932	0.9002	0.8925	0.8295	0.8137	0.2138	0.2006	0.0464	0.0305
3.6625	1.002	0.9945	0.9013	0.8883	0.8295	0.816	0.2138	0.2009	0.0469	0.0302
3.6901	1.0032	0.9926	0.8994	0.8772	0.8321	0.8175	0.2138	0.2009	0.0464	0.0313
3.7177	1.0036	0.9883	0.9002	0.8915	0.8304	0.8175	0.2149	0.203	0.0464	0.0313
3.7453	1.0047	0.9899	0.9013	0.8939	0.8316	0.8152	0.2138	0.204	0.0473	0.0302
3.773	1.0004	0.9952	0.9029	0.8915	0.8291	0.8137	0.2156	0.2068	0.0477	0.0313
3.8006	1.0032	0.9955	0.9002	0.8967	0.8316	0.8137	0.2167	0.2079	0.0473	0.0302
3.8282	1.0036	0.9959	0.8986	0.896	0.8325	0.8133	0.2156	0.2058	0.0473	0.0313
3.8559	1.0004	0.9952	0.9009	0.8943	0.8329	0.8102	0.2167	0.2068	0.0477	0.0323
3.8835	1.0016	0.9972	0.9025	0.8957	0.8329	0.811	0.2174	0.2086	0.0486	0.0313
3.9111	1.002	0.9972	0.9002	0.8957	0.8325	0.8171	0.2174	0.2079	0.0477	0.0323
3.9387	1.002	0.9922	0.9002	0.8918	0.835	0.8187	0.2185	0.2065	0.0473	0.0323
3.9664	1.0016	0.9975	0.9002	0.8915	0.8329	0.8152	0.2192	0.2061	0.0477	0.0323
3.994	1.0032	0.9972	0.8986	0.8908	0.8376	0.816	0.2189	0.2058	0.0486	0.0323
4.0216	1.002	0.9975	0.9002	0.8925	0.8389	0.8179	0.2199	0.2061	0.0495	0.0313
4.0492	1	0.9969	0.8982	0.8929	0.8397	0.8202	0.2196	0.2065	0.0495	0.0323
4.0769	1.0032	0.9926	0.897	0.8901	0.8418	0.8125	0.221	0.2079	0.0499	0.0342
4.1045	1.0032	0.9969	0.9002	0.8915	0.8482	0.8229	0.2214	0.2079	0.0499	0.0323
4.1321	1.0032	0.9906	0.9002	0.8918	0.8486	0.8233	0.2217	0.2079	0.0499	0.0331

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
4.1597	0.998	0.9932	0.8994	0.8904	0.8512	0.8187	0.2235	0.2065	0.0495	0.0331
4.1874	0.9968	0.9939	0.8986	0.8978	0.8482	0.8171	0.2214	0.2058	0.0499	0.0331
4.215	0.9937	0.9909	0.8915	0.8925	0.8423	0.8156	0.2185	0.204	0.0503	0.0331
4.2426	0.9826	0.9916	0.8812	0.8866	0.8321	0.8125	0.2138	0.1988	0.0495	0.0331
4.2703	0.9633	0.9906	0.8615	0.8709	0.8082	0.8052	0.2066	0.1915	0.0477	0.0334
4.2979	0.9337	0.9879	0.835	0.8555	0.7751	0.8083	0.194	0.1807	0.0469	0.0331
4.3255	0.8942	0.981	0.7944	0.8303	0.7317	0.7745	0.18	0.1643	0.043	0.0313
4.3531	0.8445	0.9671	0.7466	0.7922	0.6769	0.7388	0.1631	0.1461	0.0382	0.0298
4.3808	0.7762	0.936	0.6855	0.7514	0.6144	0.6942	0.1429	0.1266	0.033	0.0276
4.4084	0.7019	0.8963	0.6184	0.693	0.5472	0.635	0.122	0.1074	0.0286	0.0243
4.436	0.6238	0.8331	0.5489	0.6234	0.4741	0.567	0.1037	0.09	0.023	0.0218
4.4636	0.5389	0.7603	0.4775	0.5581	0.4018	0.4883	0.0846	0.0729	0.0187	0.0182
4.4913	0.458	0.6759	0.4092	0.5543	0.3363	0.4107	0.0691	0.0576	0.0156	0.0153
4.5189	0.3861	0.5932	0.3441	0.4057	0.2742	0.3358	0.0547	0.0443	0.0108	0.0138
4.5465	0.3202	0.575	0.2877	0.3446	0.2236	0.2816	0.0457	0.0363	8.24E-03	9.08E-03
4.5741	0.2645	0.4208	0.2376	0.2904	0.1862	0.2121	0.0364	0.029	6.94E-03	6.90E-03
4.6018	0.2187	0.3514	0.1985	0.252	0.1565	0.1663	0.0302	0.0237	4.34E-03	5.45E-03
4.6294	0.1777	0.2968	0.1614	0.2055	0.1318	0.1348	0.0248	0.0206	4.34E-03	2.91E-03
4.657	0.1441	0.2488	0.1361	0.1642	0.1156	0.111	0.022	0.0181	1.74E-03	1.82E-03
4.6846	0.1188	0.2074	0.1129	0.1331	0.1033	0.0945	0.0198	0.0167	1.74E-03	1.09E-03
4.7123	0.0991	0.1727	0.0951	0.1087	0.0944	0.0826	0.0173	0.0153	1.74E-03	0
4.7399	0.0809	0.1479	0.0817	0.0902	0.0872	0.0761	0.0144	0.015	1.30E-03	0
4.7675	0.0679	0.1949	0.0706	0.1349	0.0795	0.1122	0.0144	0.014	8.68E-04	-1.09E-03
4.7952	0.0557	0.0953	0.0631	0.0552	0.0744	0.0622	0.014	0.0136	1.30E-03	-7.27E-04
4.8228	0.0474	0.0821	0.0576	0.0451	0.0702	0.0553	0.0122	0.0129	1.30E-03	-1.09E-03
4.8504	0.0403	0.0691	0.0537	0.0374	0.0663	0.0526	0.0115	0.0122	8.68E-04	-1.09E-03

Table A.2. (Continued) Breakthrough concentrations for E. faecium E1162ΔEsp

PV	1 mM (1)	1 mM (2)	2.5 mM (1)	2.5 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
4.878	0.0336	0.0596	0.0493	0.0384	0.0616	0.0507	0.0119	0.0112	1.30E-03	-1.82E-03
4.9057	0.0292	0.0784	0.0458	0.0287	0.0591	0.0488	0.0101	0.0105	1.30E-03	-7.27E-04
4.9333	0.0249	0.0427	0.0438	0.0206	0.0561	0.0442	9.72E-03	0.0112	0	-1.82E-03
4.9609	0.0205	0.0371	0.0426	0.0182	0.0544	0.0438	9.36E-03	0.0105	8.68E-04	-1.09E-03
4.9885	0.0186	0.0318	0.0403	0.0161	0.0514	0.0419	9.72E-03	0.0105	-4.34E-04	-1.09E-03
5.0162	0.015	0.0281	0.0395	0.0154	0.0493	0.0403	8.28E-03	0.0112	-4.34E-04	-1.82E-03
5.0438	0.0134	0.0301	0.0371	0.0126	0.0472	0.0392	8.28E-03	0.0115	1.30E-03	-1.09E-03
5.0714	0.0111	0.0278	0.0355	0.0126	0.0463	0.0376	7.92E-03	0.0105	1.30E-03	-1.82E-03
5.099	9.08E-03	0.0189	0.0324	0.0129	0.0451	0.0361	8.28E-03	9.77E-03	-4.34E-04	-1.82E-03
5.1267	7.50E-03	0.0165	0.0324	0.0101	0.0429	0.0353	7.92E-03	9.77E-03	-4.34E-04	-1.82E-03
5.1543	5.92E-03	0.0146	0.0316	0.0154	0.0412	0.0334	8.28E-03	9.07E-03	0	-1.82E-03
5.1819	4.74E-03	0.0152	0.0296	9.44E-03	0.0395	0.033	6.48E-03	9.07E-03	-4.34E-04	-1.09E-03
5.2096	3.16E-03	0.0116	0.0268	8.39E-03	0.0391	0.0327	6.48E-03	8.37E-03	-4.34E-04	-1.82E-03
5.2372	2.37E-03	0.0106	0.0253	8.74E-03	0.0357	0.0307	6.48E-03	8.02E-03	-1.30E-03	-1.82E-03
5.2648	1.58E-03	9.26E-03	0.0233	7.34E-03	0.0344	0.0303	6.84E-03	8.02E-03	-4.34E-04	-1.82E-03
5.2924	-3.95E-04	8.93E-03	0.0213	7.34E-03	0.0327	0.0296	5.76E-03	9.07E-03	-4.34E-04	-2.54E-03
5.3201	-3.95E-04	8.27E-03	0.0205	9.44E-03	0.031	0.0288	6.48E-03	8.02E-03	0	-1.82E-03
5.3477	-2.76E-03	7.28E-03	0.0193	7.34E-03	0.0302	0.0277	5.76E-03	0.0101	0	-2.54E-03
5.3753	-3.16E-03	6.29E-03	0.017	6.29E-03	0.0302	0.0261	5.76E-03	8.37E-03	0	-1.82E-03
5.4029	-2.76E-03	4.30E-03	0.0174	5.24E-03	0.0276	0.0265	5.76E-03	9.07E-03		
5.4306	-4.34E-03	6.29E-03	0.0154	5.24E-03	0.0272	0.0246	6.48E-03	8.37E-03		
5.4582	-1.94E-03	-3.32E-04	8.57E-03	-2.17E-03			5.76E-03	9.07E-03		

A.3. Breakthrough concentrations for *E. coli K-12*

Table A3. Breakthrough concentrations of *E.coli* K-12 at pH 7.2 and various ionic strengths.								
	PV = pore volumes that have passed through the sand packed column							
	C/Co = normalized breakthrough concentrations							
	E. coli							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
-0.15	0.0155	0	0	0	0	0	0	0
-0.1224	0.0217	-1.17E-03	-3.84E-04	-1.41E-03	-1.58E-03	2.20E-03	-0.011	1.43E-03
-0.0947	0.0197	0	0	-3.18E-03	-2.37E-03	4.40E-03	-0.0102	3.57E-03
-0.0671	0.0201	0	0	-3.18E-03	-2.37E-03	4.03E-03	-7.67E-03	5.00E-03
-0.0395	0.0236	7.77E-04	1.92E-03	-2.83E-03	-2.37E-03	2.57E-03	-0.0106	5.72E-03
-0.0119	0.0201	0	7.68E-04	-1.41E-03	-1.58E-03	3.67E-04	-0.0124	7.86E-03
0.0158	0.0181	1.17E-03	7.68E-04	-1.77E-03	-1.97E-03	2.57E-03	-0.0128	8.93E-03
0.0434	0.0178	1.17E-03	1.92E-03	-3.18E-03	-1.97E-03	4.77E-03	-0.0124	0.01
0.071	0.0175	0	7.68E-04	-1.77E-03	-1.97E-03	2.57E-03	-0.0121	0.0111
0.0986	0.0175	2.33E-03	7.68E-04	-7.07E-04	-1.97E-03	3.67E-04	-0.0121	0.0129
0.1263	0.0181	1.17E-03	0	-7.07E-04	-1.97E-03	2.93E-03	-0.0124	0.0136
0.1539	0.0162	1.94E-03	1.15E-03	-1.06E-03	-1.97E-03	5.50E-03	-6.57E-03	0.0154
0.1815	0.0158	1.17E-03	1.15E-03	-1.06E-03	-1.97E-03	4.77E-03	-6.94E-03	0.0193
0.2091	0.0181	1.94E-03	1.15E-03	-1.06E-03	-1.97E-03	0	-9.50E-03	0.0236
0.2368	0.0236	1.94E-03	1.15E-03	-7.07E-04	-1.58E-03	2.20E-03	-0.0121	0
0.2644	0.034	1.17E-03	0	-1.41E-03	-2.37E-03	4.77E-03	-0.0128	-7.95E-04
0.292	0.0517	1.94E-03	0	-1.06E-03	-2.37E-03	2.57E-03	-0.011	-1.19E-03
0.3197	0.0893	1.17E-03	0	-1.41E-03	-1.97E-03	-1.10E-03	-0.0121	0
0.3473	0.1449	1.17E-03	0	-1.77E-03	-2.37E-03	3.67E-04	-0.0102	-7.95E-04
0.3749	0.2128	1.17E-03	0	-1.06E-03	-2.37E-03	2.57E-03	-8.40E-03	-7.95E-04
0.4025	0.2701	7.77E-04	0	-2.83E-03	-1.58E-03	2.57E-03	-0.0106	0
0.4302	0.3406	1.17E-03	1.15E-03	-2.83E-03	-1.97E-03	3.67E-04	-0.011	-1.19E-03
0.4578	0.4994	1.94E-03	7.68E-04	-3.18E-03	-2.37E-03	-2.93E-03	-0.011	0
0.4854	0.5873	1.17E-03	1.15E-03	-2.83E-03	-2.37E-03	-1.10E-03	-9.86E-03	-7.95E-04
0.513	0.6507	1.94E-03	7.68E-04	-3.54E-03	-3.15E-03	1.47E-03	-9.86E-03	-7.95E-04
0.5407	0.7251	2.72E-03	7.68E-04	-3.54E-03	-2.76E-03	2.93E-03	-0.0106	0
0.5683	0.7717	2.33E-03	0	-2.83E-03	-2.76E-03	1.47E-03	-4.75E-03	-7.95E-04
0.5959	0.7354	2.72E-03	7.68E-04	-2.83E-03	-2.37E-03	-1.47E-03	-5.48E-03	-7.95E-04
0.6235	0.8693	1.94E-03	7.68E-04	-3.18E-03	-2.76E-03	1.10E-03	-8.04E-03	-1.19E-03
0.6512	0.892	1.94E-03	7.68E-04	-3.89E-03	-3.15E-03	2.93E-03	-0.0106	0
0.6788	0.9069	1.17E-03	7.68E-04	-3.18E-03	-2.76E-03	1.10E-03	-9.86E-03	-1.19E-03
0.7064	0.9001	1.17E-03	7.68E-04	-3.18E-03	-2.37E-03	3.67E-04	-0.0102	-7.95E-04
0.7341	0.9243	1.94E-03	7.68E-04	-3.18E-03	-2.76E-03	-7.33E-04	-9.86E-03	-1.19E-03
0.7617	0.9324	1.94E-03	0	-3.89E-03	-2.76E-03	2.20E-03	-0.0106	-1.19E-03
0.7893	0.9386	1.17E-03	7.68E-04	-3.89E-03	-2.37E-03	2.57E-03	-0.0106	-1.19E-03

Table A.3 (cont). Breakthrough concentrations of *E.coli* K-12 at pH 7.2 and various ionic strengths.

	E. coli							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
0.8169	0.9392	1.94E-03	0	-3.89E-03	-2.76E-03	1.47E-03	-0.0102	-1.19E-03
0.8446	0.9324	1.17E-03	0	-1.41E-03	-4.34E-03	-1.47E-03	-7.67E-03	-7.95E-04
0.8722	0.9586	1.17E-03	0	-2.83E-03	-2.76E-03	1.47E-03	-0.0106	-1.19E-03
0.8998	0.9635	1.17E-03	0	-2.83E-03	-2.37E-03	1.47E-03	-0.011	0
0.9274	0.9657	7.77E-04	1.15E-03	-3.54E-03	-2.76E-03	3.67E-04	-0.0106	-1.19E-03
0.9551	0.9696	1.17E-03	7.68E-04	-3.89E-03	-2.76E-03	-1.47E-03	-5.84E-03	-1.19E-03
0.9827	0.9702	4.27E-03	7.68E-04	-2.83E-03	-2.37E-03	-7.33E-04	-9.50E-03	-1.19E-03
1.0103	0.9732	9.32E-03	1.92E-03	1.41E-03	0	2.20E-03	-0.0102	-7.95E-04
1.0379	0.9677	0.0218	3.84E-03	0.0166	3.94E-03	1.10E-03	-9.86E-03	-1.19E-03
1.0656	0.9699	0.047	0.0104	0.0396	0.0126	1.47E-03	-9.86E-03	-1.19E-03
1.0932	0.9774	0.0855	0.0219	0.0792	0.026	0.011	-1.83E-03	-1.19E-03
1.1208	0.9745	0.1399	0.0441	0.1036	0.0469	0.0275	2.19E-03	0
1.1484	0.9677	0.2137	0.0779	0.2348	0.0733	0.0502	6.21E-03	-7.95E-04
1.1761	0.9699	0.2976	0.1244	0.3115	0.106	0.0741	0.0113	-1.19E-03
1.2037	0.9635	0.3873	0.1835	0.3939	0.1419	0.0631	0.0281	-7.95E-04
1.2313	0.9696	0.4747	0.2541	0.4816	0.1805	0.1492	0.0336	-1.19E-03
1.259	0.9719	0.5625	0.3305	0.5587	0.2176	0.1859	0.0387	0
1.2866	0.9732	0.6391	0.4096	0.6266	0.2531	0.2215	0.0431	-1.19E-03
1.3142	0.9709	0.7055	0.4833	0.6874	0.2814	0.2215	0.0471	-7.95E-04
1.3418	0.9745	0.7572	0.5516	0.7316	0.3106	0.2868	0.0562	-7.95E-04
1.3695	0.9719	0.798	0.61	0.7641	0.3327	0.3146	0.0625	-7.95E-04
1.3971	0.9745	0.8318	0.661	0.7903	0.3516	0.3352	0.0639	1.59E-03
1.4247	0.9745	0.8594	0.7029	0.8041	0.3685	0.3271	0.0654	1.99E-03
1.4523	0.9751	0.8722	0.7374	0.7641	0.3827	0.3561	0.0687	4.37E-03
1.48	0.9774	0.885	0.7655	0.838	0.395	0.3825	0.073	8.74E-03
1.5076	0.9774	0.9071	0.7893	0.8416	0.4028	0.3905	0.073	0.0147
1.5352	0.9767	0.9196	0.8061	0.8494	0.4115	0.3898	0.0789	0.0207
1.5628	0.9774	0.9289	0.8207	0.8363	0.4198	0.4045	0.0782	0.0286
1.5905	0.9709	0.9351	0.8357	0.8624	0.4229	0.4118	0.076	0.0362
1.6181	0.9787	0.9386	0.8445	0.8639	0.43	0.4107	0.0752	0.0441
1.6457	0.9796	0.9421	0.8545	0.8635	0.4359	0.4118	0.0752	0.0517
1.6734	0.9819	0.9483	0.8622	0.8709	0.4399	0.4221	0.0785	0.0572
1.701	0.9916	0.9487	0.8699	0.8723	0.4426	0.4279	0.0782	0.0636
1.7286	0.9903	0.9495	0.8737	0.8748	0.4474	0.432	0.0782	0.0692
1.7562	0.9909	0.9518	0.8787	0.8685	0.4517	0.4334	0.0807	0.0727
1.7839	0.9916	0.9545	0.8841	0.8773	0.4533	0.4367	0.08	0.0759
1.8115	0.9916	0.9557	0.8868	0.8787	0.4568	0.4422	0.0793	0.0787
1.8391	0.9916	0.9557	0.8902	0.8815	0.4572	0.4463	0.08	0.0831
1.8667	0.9893	0.9588	0.8925	0.8815	0.4375	0.4437	0.0844	0.0835
1.8944	0.9887	0.958	0.8952	0.8861	0.4687	0.451	0.0825	0.0851

Table A.3 (cont). Breakthrough concentrations of *E.coli* K-12 at pH 7.2 and various ionic strengths.

	E. coli							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
1.922	0.9851	0.9584	0.8975	0.8925	0.4722	0.4547	0.0822	0.0866
1.9496	0.9851	0.9631	0.899	0.8946	0.4742	0.4547	0.0822	0.0882
1.9772	0.9874	0.9604	0.9017	0.8946	0.4758	0.4536	0.0888	0.0894
2.0049	0.9948	0.9604	0.9033	0.8929	0.4789	0.4613	0.0873	0.0898
2.0325	0.9871	0.9588	0.904	0.8957	0.4809	0.4657	0.0866	0.091
2.0601	0.9929	0.967	0.9056	0.8992	0.4821	0.4631	0.0844	0.0926
2.0878	0.9929	0.9666	0.9094	0.8978	0.4856	0.4639	0.084	0.0926
2.1154	0.9913	0.9681	0.9098	0.9003	0.4852	0.4712	0.0906	0.0938
2.143	0.9893	0.9701	0.9098	0.8967	0.4903	0.4719	0.0891	0.0946
2.1706	0.9822	0.9716	0.9117	0.895	0.4903	0.4727	0.0869	0.0954
2.1983	0.9916	0.974	0.9144	0.8992	0.4919	0.4793	0.0866	0.0958
2.2259	0.9893	0.9716	0.9152	0.8989	0.4939	0.4807	0.0862	0.097
2.2535	0.9871	0.9701	0.9159	0.8999	0.4943	0.4796	0.092	0.0982
2.2811	0.9851	0.972	0.9179	0.9017	0.4966	0.4818	0.0902	0.0982
2.3088	0.9913	0.9763	0.9179	0.8982	0.499	0.4862	0.0891	0.0986
2.3364	0.9916	0.9767	0.9213	0.9031	0.499	0.4877	0.0888	0.0986
2.364	0.9867	0.9755	0.9205	0.9049	0.5014	0.4877	0.0891	0.1002
2.3916	0.9909	0.9767	0.9209	0.9021	0.5034	0.4914	0.0928	0.1014
2.4193	0.9916	0.9798	0.9209	0.9049	0.5034	0.4932	0.092	0.101
2.4469	0.9932	0.9798	0.9225	0.9031	0.5061	0.4928	0.0946	0.1014
2.4745	0.9909	0.9806	0.9232	0.9045	0.5077	0.4936	0.0975	0.1029
2.5022	0.9916	0.9806	0.9228	0.9059	0.5085	0.4983	0.0942	0.1069
2.5298	0.9913	0.9829	0.9248	0.907	0.5104	0.502	0.0946	0.1045
2.5574	0.988	0.9848	0.9251	0.9134	0.5128	0.5031	0.0942	0.1045
2.585	0.9893	0.9837	0.9236	0.9148	0.5136	0.498	0.0928	0.1053
2.6127	0.9903	0.9841	0.9286	0.9134	0.5144	0.502	0.095	0.1065
2.6403	0.988	0.9852	0.9274	0.9098	0.5171	0.5057	0.095	0.1069
2.6679	0.9871	0.988	0.9267	0.9105	0.5183	0.5042	0.0961	0.1069
2.6955	0.9851	0.9895	0.9294	0.912	0.5199	0.5064	0.0986	0.1069
2.7232	0.9887	0.9887	0.9274	0.9049	0.5191	0.5097	0.0972	0.1073
2.7508	0.989	0.988	0.9313	0.9105	0.5215	0.5097	0.0986	0.1089
2.7784	0.9893	0.9915	0.9313	0.9116	0.5231	0.5046	0.0961	0.1093
2.806	0.9887	0.9915	0.9309	0.9102	0.5246	0.5101	0.0997	0.1101
2.8337	0.9867	0.9934	0.929	0.9098	0.5258	0.5123	0.1008	0.1101
2.8613	1.0032	0.9907	0.9332	0.9077	0.5274	0.516	0.0993	0.1113
2.8889	0.9906	0.9926	0.9332	0.9105	0.5298	0.5141	0.0982	0.1125
2.9165	0.9816	0.9969	0.9317	0.9102	0.5282	0.5196	0.0982	0.1121
2.9442	0.9835	0.9981	0.9332	0.9116	0.529	0.5222	0.1026	0.1125
2.9718	0.9809	0.9953	0.9332	0.9116	0.5309	0.52	0.1015	0.1121
2.9994	0.9822	0.9949	0.9359	0.9066	0.5333	0.5167	0.1008	0.1145

Table A.3 (cont). Breakthrough concentrations of *E.coli* K-12 at pH 7.2 and various ionic strengths.

	E. coli							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
3.0271	0.9799	0.9981	0.9351	0.9098	0.5337	0.52	0.1015	0.1145
3.0547	0.979	1	0.9351	0.9134	0.5365	0.5248	0.0997	0.1145
3.0823	0.979	1	0.9374	0.9141	0.5357	0.5226	0.1077	0.1145
3.1099	0.9751	0.9992	0.9351	0.9141	0.5376	0.524	0.107	0.1153
3.1376	0.9702	1	0.937	0.925	0.5369	0.5281	0.1052	0.1157
3.1652	0.9932	0.9996	0.9363	0.9148	0.5396	0.5314	0.1045	0.1169
3.1928	0.9942	1.0023	0.9374	0.9148	0.5412	0.5306	0.1045	0.1157
3.2204	0.9909	0.9996	0.9363	0.9176	0.542	0.5314	0.1045	0.1165
3.2481	0.9926	0.9996	0.9363	0.9165	0.5428	0.5358	0.1048	0.1169
3.2757	0.9942	0.9996	0.9386	0.9056	0.5467	0.5383	0.1048	0.118
3.3033	0.9916	1	0.9378	0.9194	0.5443	0.5325	0.1056	0.1196
3.3309	0.9913	0.9992	0.9374	0.9197	0.5475	0.535	0.1096	0.1184
3.3586	0.9903	0.9981	0.937	0.9219	0.5483	0.5376	0.1063	0.1196
3.3862	0.988	0.9953	0.9374	0.9204	0.5495	0.5365	0.1074	0.12
3.4138	0.9887	0.9973	0.9397	0.9222	0.5514	0.5336	0.1063	0.12
3.4415	0.9887	1.0035	0.9401	0.9165	0.5534	0.5383	0.1052	0.1204
3.4691	0.9819	0.9992	0.9397	0.9211	0.5534	0.5416	0.1103	0.1208
3.4967	0.9654	1.0019	0.9397	0.9197	0.5538	0.5442	0.1107	0.1224
3.5243	0.9486	1.0016	0.9386	0.9183	0.5558	0.5464	0.1103	0.1224
3.552	0.9159	1.0019	0.9397	0.9272	0.5593	0.546	0.1096	0.1212
3.5796	0.8535	1.0004	0.9393	0.9272	0.557	0.5504	0.1132	0.1228
3.6072	0.7794	1.0023	0.9401	0.9254	0.5581	0.5512	0.1129	0.1228
3.6348	0.6876	0.9992	0.9417	0.9204	0.5593	0.5508	0.1129	0.1244
3.6625	0.6679	1	0.9405	0.9226	0.5593	0.5464	0.1136	0.1244
3.6901	0.4796	1.0035	0.9397	0.9243	0.5621	0.5519	0.1107	0.124
3.7177	0.3745	1.0016	0.9417	0.9197	0.5629	0.5548	0.1165	0.1252
3.7453	0.3079	1.0019	0.942	0.9275	0.5652	0.5508	0.1154	0.1256
3.773	0.2455	1	0.9401	0.9303	0.566	0.5519	0.1165	0.1264
3.8006	0.1902	1.0023	0.9417	0.9335	0.5684	0.5559	0.1136	0.1272
3.8282	0.1523	1.0047	0.9424	0.931	0.5684	0.5585	0.1154	0.1288
3.8559	0.1746	1	0.9417	0.9282	0.5684	0.5589	0.1158	0.1284
3.8835	0.0941	1.0016	0.9443	0.9282	0.5708	0.557	0.1169	0.13
3.9111	0.0734	1.0016	0.9417	0.9296	0.5711	0.557	0.1209	0.1288
3.9387	0.0627	1.0016	0.9428	0.9332	0.5727	0.5622	0.1194	0.1292
3.9664	0.0566	1.0047	0.9424	0.9289	0.5735	0.5647	0.1194	0.13
3.994	0.0482	1.0004	0.9447	0.9289	0.5735	0.5603	0.1194	0.1312
4.0216	0.043	1.0019	0.9436	0.9303	0.5767	0.5589	0.1176	0.132
4.0492	0.0382	1.0054	0.9447	0.93	0.5767	0.5647	0.1238	0.132
4.0769	0.0362	1.0101	0.9424	0.9289	0.579	0.5658	0.1205	0.132
4.1045	0.0414	1.0074	0.9459	0.9314	0.5782	0.5669	0.1209	0.1343

Table A.3 (cont). Breakthrough concentrations of *E.coli* K-12 at pH 7.2 and various ionic strengths.

	E. coli							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
4.1321	0.031	1.0047	0.9455	0.931	0.5798	0.5669	0.1209	0.1316
4.1597	0.0288	1.0047	0.9443	0.931	0.5818	0.5702	0.1209	0.1343
4.1874	0.0268	1.0082	0.9443	0.93	0.5822	0.5743	0.1224	0.1343
4.215	0.0246	1.0016	0.9428	0.9254	0.5818	0.5743	0.1231	0.1359
4.2426	0.0236	0.9973	0.9417	0.9321	0.5814	0.5732	0.1224	0.1359
4.2703	0.0236	0.9883	0.9332	0.9049	0.5771	0.5724	0.1216	0.1371
4.2979	0.021	0.972	0.9202	0.8748	0.5684	0.568	0.1205	0.1371
4.3255	0.0184	0.9437	0.8937	0.8292	0.551	0.5563	0.1191	0.1379
4.3531	0.0178	0.9021	0.8545	0.7716	0.5258	0.5537	0.1096	0.1375
4.3808	0.0162	0.8411	0.8019	0.6899	0.4931	0.5057	0.0997	0.1383
4.4084	0.0178	0.7735	0.7355	0.5997	0.4659	0.469	0.0906	0.1387
4.436	0.0175	0.6993	0.6549	0.5131	0.4024	0.4283	0.0946	0.1403
4.4636	0.0158	0.6173	0.5708	0.5619	0.3516	0.4393	0.0665	0.1403
4.4913	0.0168	0.5303	0.4856	0.3105	0.3043	0.3267	0.0559	0.1403
4.5189	0.0184	0.4452	0.4061	0.2447	0.2605	0.2853	0.0504	0.1411
4.5465	0.0178	0.3722	0.3347	0.2005	0.2223	0.2501	0.0427	0.1411
4.5741	0.0155	0.3069	0.2745	0.151	0.1896	0.2178	0.0318	0.1411
4.6018	0.0136	0.2502	0.2242	0.1216	0.1628	0.2376	0.0245	0.1411
4.6294	0.0126	0.2075	0.1858	0.133	0.1419	0.1562	0.0215	0.1419
4.657	0.0113	0.1737	0.1547	0.0873	0.1253	0.1346	0.0179	0.1407
4.6846	0.0126	0.1465	0.1286	0.0714	0.1092	0.1228	0.0157	0.1379
4.7123	0.0126	0.1251	0.1117	0.0629	0.0993	0.1368	0.0135	0.1328
4.7399	0.0107	0.1084	0.0975	0.0573	0.0895	0.0913	0.0161	0.1256
4.7675	0.0103	0.0956	0.0864	0.0523	0.0808	0.0847	0.0135	0.1176
4.7952	0.0107	0.0859	0.0775	0.0499	0.0741	0.0803	7.67E-03	0.1065
4.8228	0.0103	0.0773	0.0714	0.0474	0.0694	0.0755	4.38E-03	0.0958
4.8504	0.011	0.0688	0.0645	0.0474	0.065	0.0737	4.38E-03	0.0847
4.878	0.011	0.0715	0.0591	0.0453	0.0623	0.0689	8.40E-03	0.0743
4.9057	9.06E-03		0.0564	0.04	0.0579	0.062	4.38E-03	0.0656
4.9333	8.73E-03	0.0482	0.0518	0.0382	0.0548	0.0601	1.83E-03	0.0572
4.9609	8.41E-03	0.0435	0.0484	0.0385	0.0524	0.0579	1.83E-03	0.0505
4.9885	9.06E-03	0.0416	0.0457	0.0357	0.0512	0.0568	1.83E-03	0.0457
5.0162	8.73E-03	0.0408	0.0445	0.0339	0.0497	0.0583	1.83E-03	0.0413
5.0438	7.44E-03	0.0373	0.0438	0.0339	0.0469	0.0491	0	0.0378
5.0714	7.44E-03	0.0369	0.0411	0.0318	0.0453	0.0499	-3.65E-04	0.0342
5.099	7.44E-03	0.0361	0.0395	0.0293	0.0449	0.0488	0	0.033
5.1267	7.76E-03	0.0346	0.0392	0.0269	0.0426	0.0477	3.65E-04	0.0306
5.1543	8.41E-03	0.0322	0.038	0.0265	0.0426	0.0462	-7.30E-04	0.0278
5.1819	8.73E-03	0.0307	0.0372	0.0269	0.041	0.0458	-1.83E-03	0.0278
5.2096	8.41E-03	0.0291	0.0357	0.0237	0.0398	0.0396	-3.65E-04	0.0274

Table A.3 (cont). Breakthrough concentrations of *E.coli* K-12 at pH 7.2 and various ionic strengths.

	E. coli							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
5.2372	8.41E-03	0.0268	0.0345	0.0219	0.0374	0.0411	-1.83E-03	0.0262
5.2648	8.41E-03	0.0249	0.0334	0.0209	0.0382	0.0392	-2.56E-03	0.025
5.2924	7.76E-03	0.0237	0.0315	0.0209	0.0371	0.0389	-2.56E-03	0.0238
5.3201	7.44E-03	0.0221	0.0311	0.0191	0.0367	0.04	-2.19E-03	0.0234
5.3477	7.44E-03	0.0214	0.0303	0.0187	0.0351	0.037	-2.92E-03	0.0234
5.3753	7.44E-03	0.0194	0.0288	0.0184	0.0351	0.0359	-2.92E-03	0.0231
5.4029	7.44E-03	0.0183	0.0276	0.017	0.0343	0.0356	-2.19E-03	0.0219
5.4306	7.76E-03	0.0175	0.0273	0.017	0.0343	0.0345	-2.19E-03	0.0219
5.4582	6.79E-03	0.0167	0.0261	0.0163	0.0327	0.0337	-4.38E-03	0.0211
5.4858	7.76E-03	0.0159	0.025	0.0149	0.0327	0.0359	-4.38E-03	0.0211
5.5134	7.44E-03	0.0155	0.0246	0.0163	0.0323	0.0315	-4.38E-03	0.0207
5.5411	7.44E-03	0.0155	0.0242	0.0163	0.0319	0.0319	-4.75E-03	0.0207
5.5687	7.44E-03	0.0136	0.023	0.0159	0.0311	0.0319	-5.48E-03	0.0191
5.5963	7.44E-03	0.0136	0.0242	0.0149	0.0319	0.0334	-4.38E-03	0.0191
5.6240	6.79E-03	0.0128	0.0226	0.0145	0.0307	0.029	-3.65E-03	0.0191
5.6516	7.44E-03	0.0132	0.0219	0.0138	0.03	0.029	-4.38E-03	0.0191
5.6792	8.73E-03	0.0124	0.0219	0.0124	0.0296	0.0286	-5.48E-03	0.0191
5.7068	8.73E-03	0.0124	0.0219	0.0138	0.0296	0.029	-6.57E-03	0.0179
5.7345	7.44E-03	0.0124	0.02	0.0124	0.0296	0.0257	-5.84E-03	0.0179
5.7621	6.79E-03	0.0117	0.02	0.0124	0.0284	0.0279	-4.75E-03	0.0179
5.7897	6.79E-03	0.0105	0.0192	0.0117	0.028	0.0279	-6.57E-03	0.0179
5.8173	7.44E-03	0.0105	0.0192	9.90E-03	0.028	0.0268	-6.94E-03	0.0171
5.8450	6.47E-03	9.71E-03	0.0188	9.19E-03	0.028		-6.57E-03	0.0179
5.8726	7.44E-03	9.71E-03	0.018	9.19E-03				0.0175
5.9002	7.76E-03	9.71E-03	0.018	8.84E-03				0.0171
5.9278	7.76E-03	9.71E-03	0.018	8.49E-03				0.0163
5.9555	7.44E-03	9.32E-03	0.0192	8.49E-03				0.0171
5.9831	6.79E-03	9.32E-03	0.0169	7.07E-03				0.0163
6.0107								0.0163
6.0384								0.0163
6.0660								0.0155
6.0936								0.0155

A.4. Breakthrough concentrations for *B. fragilis*

Table A4. Breakthrough concentrations of *B.fragilis* at pH 7.2 and various ionic strengths.

PV = pore volumes that have passed through the sand packed column

C/Co = normalized breakthrough concentrations

	Bacteroides							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
-0.15	0.054	0	0.045	0.0473	-6.79E-03	-7.64E-03	-7.83E-04	-1.42E-03
-0.1224	0.054	0	0.045	0.0455	0	-4.42E-03	-3.91E-04	-1.07E-03
-0.0947	0.054	0	0.043	0.0441	-6.79E-04	-8.04E-04	0	-1.07E-03
-0.0671	0.0543	0	0.0426	0.0416	1.70E-03	0	0	0
-0.0395	0.0543	0	0.0422	0.0405	2.04E-03	3.22E-03	0	-3.55E-04
-0.0119	0.0543	0	0.0415	0.0416	3.05E-03	3.22E-03	3.91E-04	0
0.0158	0.054	0	0.0415	0.0405	2.04E-03	5.63E-03	1.57E-03	1.42E-03
0.0434	0.0554	0	0.0415	0.0405	3.05E-03	4.42E-03	1.96E-03	-3.55E-04
0.071	0.0536	0	0.0415	0.0394	2.04E-03	6.03E-03	2.35E-03	-3.55E-04
0.0986	0.0536	0	0.0415	0.043	6.79E-04	7.64E-03	2.74E-03	0
0.1263	0.0536	0	0.0415	0.0394	6.79E-04	4.02E-03	2.74E-03	3.55E-04
0.1539	0.0526	0	0.0415	0.0383	6.79E-04	3.62E-03	1.96E-03	-3.55E-04
0.1815	0.0526	0	0.0387	0.0394	6.79E-04	4.02E-03	1.57E-03	-3.55E-04
0.2091	0.0536	0	0.0383	0.0366	3.39E-04	3.62E-03	1.57E-03	-3.55E-04
0.2368	0.0653	0	0.0375	0.0355	1.70E-03	3.22E-03	1.57E-03	3.55E-04
0.2644	0.0536	0	0.0363	0.034	0	3.22E-03	1.96E-03	-3.55E-04
0.292	0.0526	0	0.0355	0.0312	0	1.61E-03	1.57E-03	-1.07E-03
0.3197	0.0533	0	0.0331	0.0283	0	1.61E-03	0	-3.55E-04
0.3473	0.0526	0	0.0327	0.0276	3.39E-04	1.61E-03	1.57E-03	-3.55E-04
0.3749	0.0536	0	0.0299	0.0237	-3.39E-04	3.62E-03	0	-1.07E-03
0.4025	0.054	0	0.0275	0.0233	0	1.61E-03	0	-1.07E-03
0.4302	0.0536	0	0.0255	0.0204	-1.70E-03	4.02E-04	3.91E-04	-1.07E-03
0.4578	0.054	0	0.0243	0.0204	-3.39E-04	4.02E-04	-3.91E-04	-1.07E-03
0.4854	0.0533	0	0.0223	0.0197	-3.39E-04	1.61E-03	1.57E-03	-3.55E-04
0.513	0.0526	0	0.0203	0.0215	-6.79E-04	0	1.96E-03	-1.07E-03
0.5407	0.0519	0	0.0203	0.0176	-2.04E-03	-8.04E-04	0	-1.07E-03
0.5683	0.0519	0	0.0195	0.0172	-2.04E-03	1.21E-03	0	-1.07E-03
0.5959	0.0505	0	0.0183	0.0161	-2.37E-03	0	3.91E-04	-1.07E-03
0.6235	0.0505	0	0.0187	0.0183	-2.37E-03	0	3.91E-04	-1.07E-03
0.6512	0.0512	0	0.0175	0.0183	-3.05E-03	4.02E-04	0	-1.07E-03
0.6788	0.0584	0	0.0163	0.0158	-3.05E-03	1.21E-03	0	-1.07E-03
0.7064	0.0485	0	0.0163	0.0158	-3.05E-03	-1.21E-03	-3.91E-04	0
0.7341	0.0488	0	0.0171	0.0158	-4.07E-03	-1.21E-03	-3.91E-04	-1.07E-03
0.7617	0.0488	0	0.0163	0.0176	-4.07E-03	0	0	-3.55E-04
0.7893	0.0485	0	0.0159	0.0161	-3.39E-03	-1.21E-03	-3.91E-04	-1.07E-03

Table A.4 (cont). Breakthrough concentrations of *B.fragilis* at pH 7.2 and various ionic strengths.

	Bacteroides							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
0.8169	0.0478	0	0.0163	0.0158	-4.07E-03	-3.62E-03	-3.91E-04	-1.42E-03
0.8446	0.0485	0	0.0163	0.0172	-4.07E-03	-2.81E-03	-3.91E-04	-1.07E-03
0.8722	0.0478	0	0.0159	0.0179	-4.41E-03	-3.62E-03	-3.91E-04	-1.42E-03
0.8998	0.0485	0	0.0163	0.0215	-5.43E-03	-4.42E-03	-3.91E-04	-3.55E-04
0.9274	0.0468	0	0.0171	0.0323	-4.07E-03	-4.82E-03	-7.83E-04	-1.42E-03
0.9551	0.0485	0	0.0171	0.0405	-6.79E-03	-5.63E-03	0	-2.13E-03
0.9827	0.0495	0.0709	0.0227	0.0871	-5.09E-03	-3.62E-03	5.87E-03	-1.07E-03
1.0103	0.0536	0.0846	0.0283	0.1236	-5.43E-03	-1.21E-03	0.0172	-1.07E-03
1.0379	0.0591	0.1074	0.043	0.1842	-2.04E-03	0.0113	0.0364	1.78E-03
1.0656	0.0725	0.1419	0.067	0.244	7.12E-03	0.0322	0.0626	4.62E-03
1.0932	0.0946	0.1591	0.1004	0.3254	7.12E-03	0.0579	0.099	0.0163
1.1208	0.1028	0.1779	0.1455	0.4128	0.0594	0.0844	0.1409	0.0341
1.1484	0.1523	0.317	0.2025	0.4938	0.0852	0.1057	0.189	0.0597
1.1761	0.2255	0.3539	0.271	0.5673	0.1574	0.1953	0.2411	0.0948
1.2037	0.316	0.4493	0.3487	0.6203	0.1978	0.2335	0.292	0.1399
1.2313	0.3882	0.5222	0.432	0.5605	0.2785	0.2227	0.3436	0.1911
1.259	0.5023	0.5919	0.5145	0.7547	0.364	0.4144	0.391	0.2195
1.2866	0.5212	0.6372	0.5927	0.7941	0.3959	0.4642	0.4329	0.2085
1.3142	0.6866	0.7126	0.6612	0.8149	0.4638	0.4847	0.4712	0.3733
1.3418	0.7633	0.7523	0.717	0.83	0.5177	0.5269	0.5014	0.4124
1.3695	0.8179	0.7963	0.7617	0.845	0.5554	0.5856	0.5276	0.4358
1.3971	0.8465	0.8344	0.7955	0.8511	0.5954	0.6105	0.5499	0.4809
1.4247	0.895	0.8573	0.8198	0.8561	0.6141	0.6202	0.5656	0.5129
1.4523	0.8946	0.8689	0.8366	0.8615	0.593	0.6411	0.5773	0.5406
1.48	0.9565	0.8921	0.8497	0.8629	0.6473	0.666	0.5886	0.5534
1.5076	0.9541	0.9038	0.8597	0.8636	0.6514	0.6805	0.5973	0.5232
1.5352	0.974	0.9162	0.8653	0.8665	0.664	0.6885	0.6043	0.5939
1.5628	0.9789	0.9222	0.8717	0.854	0.6646	0.6953	0.6106	0.601
1.5905	0.9837	0.9234	0.8749	0.8647	0.6762	0.7014	0.6153	0.6052
1.6181	0.9875	0.9202	0.8764	0.8683	0.6796	0.6937	0.62	0.6095
1.6457	0.9892	0.9458	0.8792	0.8665	0.6846	0.7158	0.6219	0.6162
1.6734	0.9909	0.9438	0.88	0.8669	0.6887	0.7158	0.625	0.6184
1.701	0.9909	0.945	0.8804	0.8687	0.6857	0.7178	0.6258	0.6194
1.7286	0.9916	0.9498	0.8824	0.8683	0.6979	0.7174	0.6274	0.6223
1.7562	0.9919	0.9526	0.8836	0.8679	0.6992	0.7166	0.6301	0.623
1.7839	0.9947	0.9526	0.8848	0.8597	0.7013	0.7158	0.6297	0.6255
1.8115	1.0088	0.9526	0.8836	0.8647	0.7057	0.7158	0.6305	0.6262
1.8391	1.0088	0.9518	0.8844	0.8658	0.7057	0.7207	0.6313	0.6223
1.8667	1.004	0.9547	0.8844	0.8647	0.7057	0.7235	0.6313	0.6248
1.8944	0.9988	0.9522	0.8844	0.8658	0.7108	0.7243	0.6317	0.6248

Table A.4 (cont). Breakthrough concentrations of *B.fragilis* at pH 7.2 and various ionic strengths.

	Bacteroides							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
1.922	0.9936	0.9518	0.8828	0.8669	0.7101	0.7267	0.6329	0.6255
1.9496	0.9916	0.9502	0.8836	0.8665	0.7135	0.7271	0.6313	0.6248
1.9772	0.9912	0.9514	0.8836	0.8647	0.7152	0.7303	0.6325	0.623
2.0049	0.9895	0.9514	0.8852	0.8658	0.7155	0.7287	0.6313	0.6169
2.0325	0.9881	0.9498	0.8828	0.8636	0.7165	0.7279	0.6317	0.623
2.0601	0.9871	0.9478	0.882	0.8647	0.7138	0.7271	0.6313	0.6226
2.0878	0.9861	0.9446	0.8824	0.8629	0.7186	0.7271	0.6313	0.6212
2.1154	0.985	0.947	0.8816	0.8647	0.7206	0.7291	0.6301	0.6205
2.143	0.9909	0.9498	0.8824	0.864	0.7165	0.7295	0.6282	0.6187
2.1706	0.9916	0.9474	0.8804	0.8608	0.7182	0.7295	0.6282	0.6194
2.1983	0.9737	0.9446	0.882	0.8561	0.7152	0.7355	0.6282	0.6184
2.2259	0.974	0.9458	0.8804	0.8615	0.7165	0.7355	0.6297	0.6177
2.2535	0.9727	0.9478	0.8816	0.8636	0.7182	0.7359	0.6278	0.6177
2.2811	0.9747	0.9458	0.8796	0.8619	0.7182	0.7395	0.6278	0.6155
2.3088	0.9709	0.9414	0.8796	0.8619	0.7189	0.7383	0.627	0.6155
2.3364	0.9699	0.945	0.8792	0.8565	0.7172	0.7395	0.6274	0.6152
2.364	0.9685	0.9438	0.8796	0.8629	0.7186	0.7412	0.627	0.6134
2.3916	0.9727	0.9502	0.8792	0.8619	0.7182	0.7404	0.6282	0.613
2.4193	0.9665	0.9478	0.8764	0.8604	0.7182	0.7383	0.6286	0.6127
2.4469	0.9679	0.945	0.8764	0.8615	0.7172	0.7395	0.6254	0.6109
2.4745	0.9679	0.9422	0.8756	0.8615	0.7172	0.7347	0.625	0.6109
2.5022	0.9758	0.949	0.8764	0.8604	0.7182	0.7404	0.6239	0.6091
2.5298	0.9648	0.9518	0.876	0.8619	0.7165	0.7436	0.6243	0.6098
2.5574	0.9648	0.947	0.8776	0.8615	0.7176	0.7416	0.6219	0.607
2.585	0.9634	0.9458	0.8772	0.859	0.7131	0.7391	0.6219	0.6059
2.6127	0.9624	0.9446	0.8749	0.8568	0.7172	0.7395	0.62	0.6045
2.6403	0.9668	0.9502	0.876	0.8619	0.7162	0.7391	0.6192	0.6027
2.6679	0.9627	0.947	0.8764	0.859	0.7165	0.7391	0.6196	0.6017
2.6955	0.9593	0.9458	0.8749	0.8608	0.7162	0.7351	0.6184	0.601
2.7232	0.961	0.9466	0.8741	0.8597	0.7128	0.7347	0.6172	0.5999
2.7508	0.9589	0.947	0.8756	0.8629	0.7131	0.7347	0.6157	0.601
2.7784	0.961	0.947	0.8756	0.8619	0.7135	0.7323	0.6164	0.5999
2.806	0.9685	0.945	0.876	0.864	0.7131	0.7323	0.6153	0.5995
2.8337	0.9572	0.9446	0.8756	0.8604	0.7152	0.7323	0.6153	0.5971
2.8613	0.9572	0.947	0.8764	0.8629	0.7135	0.7323	0.6141	0.5967
2.8889	0.9589	0.9478	0.8764	0.8644	0.7152	0.7331	0.6114	0.5967
2.9165	0.9596	0.9446	0.8764	0.8636	0.7131	0.7331	0.6129	0.5956
2.9442	0.9627	0.9446	0.8772	0.8615	0.7131	0.7331	0.611	0.5942
2.9718	0.9572	0.9438	0.8764	0.8619	0.7148	0.7307	0.6106	0.5931
2.9994	0.9572	0.947	0.876	0.8629	0.7148	0.7335	0.609	0.5917

Table A.4 (cont). Breakthrough concentrations of *B.fragilis* at pH 7.2 and various ionic strengths.

	Bacteroides							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
3.0271	0.9527	0.9446	0.8764	0.8629	0.7247	0.7351	0.6082	0.5914
3.0547	0.9648	0.9522	0.8756	0.859	0.7152	0.7331	0.6082	0.5907
3.0823	0.9641	0.9518	0.8772	0.8608	0.7131	0.7323	0.6078	0.5896
3.1099	0.9651	0.9543	0.876	0.8608	0.7108	0.7351	0.6063	0.5892
3.1376	0.9641	0.9514	0.8772	0.8597	0.7118	0.7291	0.6055	0.5889
3.1652	0.9644	0.9543	0.8776	0.8579	0.7108	0.7315	0.6047	0.5878
3.1928	0.9634	0.9518	0.876	0.8615	0.7135	0.7315	0.6035	0.586
3.2204	0.9648	0.9522	0.8772	0.8608	0.7152	0.7307	0.6043	0.586
3.2481	0.9603	0.9518	0.8792	0.8597	0.7111	0.7307	0.6023	0.5828
3.2757	0.9634	0.9571	0.8792	0.8558	0.7108	0.7287	0.6023	0.5828
3.3033	0.9624	0.9518	0.8796	0.8579	0.7111	0.7295	0.602	0.5796
3.3309	0.9617	0.9522	0.88	0.8604	0.7115	0.7287	0.5996	0.5804
3.3586	0.9617	0.9526	0.882	0.8629	0.7138	0.7279	0.5992	0.5814
3.3862	0.9613	0.9534	0.88	0.8619	0.7128	0.7295	0.5996	0.5828
3.4138	0.9613	0.9526	0.8804	0.8629	0.7118	0.7315	0.6	0.5804
3.4415	0.9603	0.9543	0.8796	0.864	0.7118	0.7303	0.5996	0.5804
3.4691	0.9627	0.9514	0.8804	0.8636	0.7138	0.7307	0.5977	0.5779
3.4967	0.961	0.9522	0.8796	0.8608	0.7091	0.7303	0.5977	0.5789
3.5243	0.961	0.9518	0.8804	0.8636	0.7135	0.7307	0.5973	0.5793
3.552	0.961	0.9518	0.8804	0.8636	0.7138	0.7315	0.5973	0.5779
3.5796	0.9596	0.9518	0.8816	0.8629	0.7135	0.7307	0.5973	0.5765
3.6072	0.961	0.9514	0.8816	0.8644	0.7152	0.7295	0.5973	0.5754
3.6348	0.9617	0.9498	0.8836	0.8647	0.7162	0.7315	0.5969	0.575
3.6625	0.961	0.9502	0.8816	0.864	0.7138	0.7303	0.5969	0.575
3.6901	0.9603	0.9514	0.8828	0.8636	0.7162	0.7327	0.5977	0.5761
3.7177	0.9586	0.9498	0.8836	0.8647	0.7186	0.7323	0.5969	0.5743
3.7453	0.9617	0.9518	0.8828	0.8662	0.7176	0.7323	0.5969	0.5718
3.773	0.9586	0.9526	0.8836	0.8647	0.7182	0.7331	0.5961	0.5718
3.8006	0.9586	0.9526	0.8824	0.8644	0.7162	0.7323	0.5961	0.5718
3.8282	0.961	0.9534	0.8848	0.8647	0.7165	0.7351	0.5969	0.5725
3.8559	0.9572	0.9543	0.8836	0.859	0.7182	0.7323	0.5961	0.5718
3.8835	0.9572	0.9518	0.8848	0.8662	0.7199	0.7347	0.5969	0.574
3.9111	0.9596	0.9502	0.8852	0.8679	0.7162	0.7379	0.5977	0.5736
3.9387	0.9551	0.9518	0.8852	0.8644	0.7199	0.7331	0.5969	0.5718
3.9664	0.9462	0.9514	0.8848	0.8647	0.7199	0.7375	0.5969	0.5693
3.994	0.9472	0.9514	0.8848	0.8583	0.7186	0.7379	0.5996	0.5676
4.0216	0.9476	0.9498	0.8848	0.859	0.7203	0.7367	0.5969	0.5693
4.0492	0.9441	0.9518	0.8824	0.855	0.7186	0.7379	0.5992	0.5697
4.0769	0.9441	0.9358	0.8796	0.8461	0.7199	0.7355	0.5969	0.5693
4.1045	0.9424	0.9302	0.8737	0.8282	0.7209	0.7379	0.5973	0.5683

Table A.4 (cont). Breakthrough concentrations of *B.fragilis* at pH 7.2 and various ionic strengths.

	Bacteroides							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
4.1321	0.94	0.9266	0.8585	0.797	0.7182	0.7395	0.5977	0.5658
4.1597	0.928	0.9038	0.8386	0.7547	0.7199	0.7367	0.5961	0.5693
4.1874	0.919	0.8745	0.8071	0.7379	0.7189	0.7347	0.5914	0.5683
4.215	0.9056	0.8324	0.7637	0.6246	0.7203	0.7327	0.5808	0.5683
4.2426	0.8602	0.8056	0.711	0.5368	0.7186	0.7215	0.5636	0.5676
4.2703	0.83	0.733	0.6525	0.4605	0.7118	0.7078	0.5354	0.5615
4.2979	0.7512	0.6641	0.5851	0.3766	0.6894	0.6957	0.5014	0.5516
4.3255	0.6625	0.5923	0.5153	0.3315	0.6626	0.6917	0.4618	0.5459
4.3531	0.5804	0.5258	0.4428	0.2344	0.6433	0.6001	0.4114	0.4997
4.3808	0.4989	0.4501	0.3747	0.1777	0.5723	0.5535	0.3632	0.4621
4.4084	0.5271	0.4437	0.3117	0.1347	0.4862	0.5189	0.3155	0.4237
4.436	0.2843	0.2677	0.2563	0.1032	0.418	0.4425	0.2618	0.3733
4.4636	0.2555	0.2116	0.2069	0.1079	0.4163	0.3967	0.22	0.3207
4.4913	0.1788	0.1691	0.1682	0.062	0.269	0.4457	0.1777	0.3051
4.5189	0.1358	0.1451	0.1347	0.0491	0.2266	0.2395	0.1436	0.2181
4.5465	0.1083	0.1158	0.108	0.0401	0.192	0.1925	0.1186	0.1591
4.5741	0.087	0.0878	0.0877	0.0337	0.1316	0.1355	0.0935	0.136
4.6018	0.098	0.0753	0.0705	0.0305	0.1187	0.0993	0.0759	0.1041
4.6294	0.0557	0.0609	0.0594	0.0262	0.0841	0.0756	0.0642	0.0806
4.657	0.0444	0.0521	0.0486	0.0233	0.0692	0.0591	0.054	0.0629
4.6846	0.0413	0.0433	0.0415	0.0204	0.0662	0.0691	0.0477	0.0515
4.7123	0.0437	0.0385	0.0359	0.0444	0.0468	0.0446	0.0403	0.0764
4.7399	0.0347	0.0377	0.0307	0.0158	0.0431	0.0265	0.0384	0.0273
4.7675	0.0337	0.0673	0.0255	0.0133	0.0407	0.0201	0.0341	0.0224
4.7952	0.0337	0.0244	0.0227	0.0122	0.0312	0.0181	0.0309	0.021
4.8228	0.0313	0.0212	0.0203	0.0118	0.0285	0.0221	0.0301	0.0178
4.8504	0.0292	0.0208	0.0175	0.01	0.0268	0.0121	0.0286	0.0156
4.878	0.0289	0.0184	0.0163	0.01	0.0221	0.0121	0.0278	0.0139
4.9057	0.0261	0.018	0.0139	9.68E-03	0.0221	0.0129	0.0258	0.0142
4.9333	0.0248	0.0184	0.0132	8.24E-03	0.0173	0.0145	0.0258	0.0139
4.9609	0.0241	0.0172	0.0108	7.88E-03	0.0166	0.0145	0.0254	0.0103
4.9885	0.022	0.0148	9.57E-03	6.81E-03	0.0173	0.0157	0.0254	9.94E-03
5.0162	0.022	0.0124	8.77E-03	6.81E-03	0.0126	0.0149	0.0247	9.23E-03
5.0438	0.0217	0.014	8.37E-03	6.45E-03	0.0109	0.0145	0.0254	9.59E-03
5.0714	0.0193	0.0116	8.37E-03	6.45E-03	0.0119	0.0129	0.0254	8.52E-03
5.099	0.0175	0.0112	7.17E-03	6.81E-03	9.16E-03	0.0125	0.0247	7.81E-03
5.1267	0.0165	9.62E-03	5.58E-03	5.73E-03	7.80E-03	0.0121	0.0235	9.23E-03
5.1543	0.0158	9.62E-03	5.58E-03	4.66E-03	9.16E-03	0.01	0.0243	7.46E-03
5.1819	0.022	9.22E-03	5.18E-03	6.45E-03	7.46E-03	0.01	0.0223	7.46E-03
5.2096	0.0124	9.62E-03	4.78E-03	4.30E-03	7.12E-03	8.84E-03	0.0231	6.39E-03

Table A.4 (cont). Breakthrough concentrations of *B.fragilis* at pH 7.2 and various ionic strengths.

	Bacteroides							
PV	1 mM (1)	1 mM (2)	5 mM (1)	5 mM (2)	20 mM (1)	20 mM (2)	50 mM (1)	50 mM (2)
5.2372	0.0117	8.82E-03	3.99E-03	3.94E-03	7.12E-03	7.64E-03	0.0223	6.39E-03
5.2648	0.0113	7.61E-03	4.38E-03	3.94E-03	5.43E-03	5.63E-03	0.0219	6.04E-03
5.2924	0.0138	9.22E-03	3.99E-03	2.51E-03	3.05E-03	6.03E-03	0.0215	5.33E-03
5.3201	8.94E-03	9.22E-03	3.99E-03	2.15E-03	3.39E-03	8.44E-03	0.0215	4.62E-03
5.3477	8.60E-03	7.61E-03	3.99E-03	2.87E-03	1.70E-03	1.61E-03	0.0204	4.26E-03
5.3753	6.88E-03	7.21E-03	2.39E-03	2.51E-03	3.39E-04	4.02E-04	0.0204	4.26E-03
5.4029	8.25E-03	6.81E-03	1.99E-03	3.94E-03	3.39E-04	0	0.02	5.33E-03
5.4306	7.56E-03	6.01E-03	2.39E-03	2.87E-03	-3.39E-04	1.61E-03	0.0188	4.62E-03
5.4582	6.53E-03	6.01E-03	2.39E-03	3.94E-03	-6.79E-04	1.61E-03	0.0196	3.91E-03
5.4858	5.84E-03	5.61E-03	1.99E-03	2.51E-03	-1.70E-03	5.63E-03	0.0176	3.91E-03
5.5134	6.88E-03	7.21E-03	1.20E-03	2.51E-03	-3.39E-04	7.64E-03	0.0172	3.91E-03
5.5411	5.84E-03	5.61E-03	1.99E-03	2.51E-03	-2.04E-03	2.01E-03	0.0172	2.49E-03
5.5687	5.84E-03	3.61E-03	1.99E-03	2.51E-03	-1.70E-03	2.01E-03	0.0168	3.91E-03
5.5963	4.81E-03	4.41E-03	1.20E-03	1.79E-03	-3.05E-03	4.42E-03	0.016	3.55E-03
5.6240	4.47E-03	3.61E-03	2.39E-03	1.79E-03	-3.05E-03	4.42E-03	0.016	3.55E-03
5.6516	4.81E-03	3.61E-03	0	1.79E-03	-2.37E-03	3.22E-03	0.0149	2.49E-03
5.6792	4.47E-03	2.81E-03	7.97E-04	2.51E-03	-3.39E-03	4.02E-03	0.016	2.13E-03
5.7068	3.78E-03	1.60E-03	1.20E-03	3.94E-03	-2.37E-03	5.63E-03	0.0145	1.78E-03
5.7345	3.78E-03	4.41E-03	7.97E-04	2.15E-03	-3.39E-03	5.63E-03	0.0137	2.13E-03
5.7621	3.09E-03	8.02E-04	7.97E-04	1.43E-03	-4.41E-03	6.03E-03	0.0121	1.78E-03
5.7897	3.09E-03	4.01E-04	0	1.43E-03	-3.05E-03	5.63E-03	0.0129	2.13E-03
5.8173	3.78E-03	8.02E-04	0	3.58E-04	-4.41E-03	6.43E-03	0.0114	1.42E-03
5.8450	1.03E-03	4.01E-04	-3.99E-04	1.43E-03	-4.41E-03	4.42E-03	0.011	1.42E-03
5.8726	0	-1.20E-03	-3.99E-04	0	-3.39E-03	4.02E-03	0.0106	1.42E-03
5.9002	2.06E-03	-1.20E-03	0	1.79E-03	-5.77E-03	3.62E-03	0.0102	1.42E-03
5.9278	0	0	0	0	-5.43E-03	3.22E-03	9.00E-03	3.55E-04
5.9555	-6.88E-04	-1.60E-03	1.20E-03	-3.58E-04	-4.41E-03	3.22E-03	9.00E-03	3.55E-04
5.9831	-1.38E-03	-1.60E-03	7.97E-04	0	-5.77E-03	3.62E-03	8.61E-03	2.49E-03
6.0107					-5.43E-03	2.01E-03	8.22E-03	2.13E-03
6.0384					-5.77E-03	1.61E-03	8.61E-03	1.42E-03
6.0660					-5.77E-03	3.62E-03	8.22E-03	1.42E-03
6.0936					-5.43E-03	2.01E-03	8.61E-03	1.42E-03

Appendix B: Cell Size Measurements

B.1. Cell size measurements for *E. faecium* cells at various ionic strengths, pH 7.2

	E1162 buffered to pH 7.2					E1162 Δesp, buffered to pH 7.2				
	1 mM	2.5 mM	5 mM	20 mM	50 mM	1 mM	2.5 mM	5 mM	20 mM	50 mM
#	Length (pixels)*				-	Length (pixels)*				
1	23.142	17.678	20.025	21.000	20.125	22.804	17.720	19.416	17.263	20.000
2	19.379	18.000	21.932	20.224	19.105	19.000	23.707	20.616	21.587	19.821
3	16.465	17.357	21.471	18.682	21.378	20.000	21.024	22.472	19.026	24.477
4	19.345	17.500	19.416	20.100	20.616	21.095	19.416	17.029	22.023	20.870
5	18.049	19.416	18.868	21.587	20.809	23.601	20.248	18.601	22.561	21.990
6	20.100	21.024	21.024	17.692	19.105	21.954	20.616	23.345	19.313	18.950
7	20.352	18.561	17.263	20.616	19.849	19.313	22.561	24.207	19.925	19.090
8	19.889	23.505	21.000	19.799	21.095	21.260	21.932	23.707	20.518	23.739
9	19.889	22.192	21.024	19.000	21.024	20.224	21.401	21.633	21.213	18.135
10	21.499	21.219	22.023	18.788	20.000	20.025	19.416	17.804	16.492	21.375
11	21.499	18.007	15.811	20.396	18.028	23.022	20.000	21.095	21.378	19.686
12	20.276	20.006	21.541	19.105	21.000	21.471	21.260	19.849	17.464	17.075
13	21.838	22.006	19.235	22.023	21.024	23.431	18.000	20.591	23.409	18.135
14	22.161	22.699	20.025	20.125	17.029	17.263	21.840	21.213	22.361	20.870
15	19.799	21.219	19.105	21.633	19.416	17.117	20.809	18.974	20.025	22.784
16	21.082	16.800	21.095	16.553	20.100	19.416	19.313	20.616	19.416	16.275
17	21.868	18.062	20.000	17.000	21.024	19.313	19.416	19.849	22.804	19.821
18	22.010	22.204	12.000	20.396	19.647	20.616	22.023	22.627	22.804	17.075
19	22.667	25.812	18.868	18.682	21.190	21.095	20.000	19.209	17.464	19.230
20	21.715	20.125	18.028	18.111	20.616	20.616	18.439	20.616	17.088	19.230
21	23.027	18.035	20.224	24.166	21.024	19.723	21.401	23.324	20.000	18.135
22	23.570	22.209	18.439	20.396	17.720	21.378	20.881	17.889	23.022	21.868
23	21.333	23.324	19.235	20.125	21.024	21.378	17.263	23.601	22.204	23.851
24	20.667	21.006	20.000	22.361	20.224	20.000	18.028	17.088	20.100	20.309
25	19.889	22.389	21.378	20.100	18.028	21.541	19.000	17.000	21.932	19.686
26	20.000	22.147	21.260	18.000	18.028	18.788	20.000	19.105	23.022	22.706
27	23.561	21.477	22.023	22.000	19.026	20.248	17.000	18.000	19.647	19.821
28	22.959	19.906	20.248	18.028	18.682	18.788	18.358	21.633	22.804	23.739
29	19.978	18.111	21.095	21.024	19.235	19.026	20.224	18.028	20.000	17.075
30	21.592	22.411	19.105	20.616	21.024	20.025	20.591	19.723	20.591	22.667
31	20.580						18.682			22.667
32							22.023			
ave*	20.974	20.480	19.759	19.944	19.873	20.451	20.081	20.295	20.582	20.283
st.dev	1.609	2.273	2.075	1.701	1.230	1.605	1.638	2.157	2.014	2.251

B.1 (*continued*)

*From pixel measurements, 15.7 pixels = 1 µm. Length is equal to the cell diameter.

	E1162 buffered to pH 7.2					E1162 Δesp, buffered to pH 7.2				
	1 mM	2.5 mM	5 mM	20 mM	50 mM	1 mM	2.5 mM	5 mM	20 mM	50 mM
ave µm*	**1.336**	**1.304**	**1.259**	**1.270**	**1.266**	**1.303**	**1.279**	**1.293**	**1.311**	**1.292**
stddev µm*	0.102	0.145	0.132	0.108	0.078	0.102	0.104	0.137	0.128	0.143

overall average (µm)	1.29	1.30
Standard deviation (µm)	0.12	0.12

B.2. Cell size measurements for *E. coli* cells at various ionic strengths, pH 7.2

Summary of Average sizes

E coli **cell sizes**		average diameter (um) for all ionic strengths			
Average	1.941	**St.Dev**	0.245	n=	227

E coli **cell sizes; buffered to pH 7.2**				1mMBuffered	
	1 mM		effective diameter	**Average**	**St.Dev**
photo	size =	1392 x 1040		2.05	0.24
15.7	pixels =	1	µm	n=	49

E coli **cell sizes; buffered to pH 7.2**				5mMBuffered	
		5 mM	effective diameter	**Average**	**St.Dev**
photo	size =	1392 x 1040		1.90	0.25
15.7	pixels =	1	µm	n =	47

E coli **cell sizes; buffered to pH 7.2**				20mMBuffered	
		20 mM	effective diameter	**Average**	**St.Dev**
photo	size =	1392 x 1040		1.87	0.20
15.7	pixels =	1	µm	n=	51

E coli cell sizes				50mMBuffered-2	
		50 mM	effective diameter	**Average**	**St.Dev**
photo	size =	1392 x 1040		1.95	0.25
15.7	pixels =	1	µm	n=	80

Measured cell sizes follow

1mMBuffered *E. coli*

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)	photo
1	31.4	12.0	2.00	0.76	1.39	1mMbuff-1
2	50.7	17.4	3.23	1.11	2.14	
3	66.2	17.4	4.22	1.11	2.44	
4	26.6	18.4	1.69	1.17	1.59	
5	39.7	16.9	2.53	1.07	1.86	
6	49.4	17.6	3.15	1.12	2.12	
7	32.4	21.0	2.06	1.33	1.87	
8	42.1	18.6	2.68	1.18	2.01	
9	45.4	18.0	2.89	1.15	2.05	
10	44.1	18.0	2.81	1.15	2.03	
11	47.6	14.5	3.03	0.92	1.89	
12	39.4	18.0	2.51	1.15	1.92	
13	37.3	18.1	2.38	1.15	1.87	
14	41.3	19.3	2.63	1.23	2.03	
15	46.5	18.7	2.96	1.19	2.12	
16	55.0	19.1	3.50	1.22	2.33	
17	46.049	21.6	2.93	1.37	2.27	1mMbuff-2
18	44.033	17.6	2.80	1.12	2.00	
19	38.49	16.6	2.45	1.06	1.82	
20	30.162	18.7	1.92	1.19	1.71	
21	55.7	23.0	3.55	1.46	2.57	
22	40.387	20.7	2.57	1.32	2.08	
23	33.939	19.3	2.16	1.23	1.84	
24	47.558	21.0	3.03	1.33	2.27	
25	37.088	17.4	2.36	1.11	1.83	
26	31.176	20.5	1.99	1.31	1.82	
27	29.571	20.5	1.88	1.31	1.77	
28	52.786	18.0	3.36	1.15	2.22	
29	43.106	17.4	2.75	1.11	1.97	
30	50.955	17.3	3.25	1.10	2.13	
31	36.17	17.6	2.30	1.12	1.82	
32	56.88	21.3	3.62	1.35	2.50	
33	54.726	21.1	3.49	1.34	2.44	
34	47.98	19.1	3.06	1.22	2.18	
35	37.337	19.3	2.38	1.23	1.93	
36	34.916	19.8	2.22	1.26	1.89	
37	55.941	18.3	3.56	1.17	2.30	
38	30.384	21.3	1.94	1.35	1.83	
39	59.651	20.8	3.80	1.32	2.53	
40	36.609	18.0	2.33	1.15	1.85	
41	47.327	20.1	3.01	1.28	2.22	
42	50.987	16.9	3.25	1.08	2.11	

	43	39.694	17.4	2.53	1.11	1.89	
	44	36.124	22.6	2.30	1.44	2.06	
	45	47.558	18.6	3.03	1.18	2.14	
	46	46.971	17.1	2.99	1.09	2.03	
	47	50.357	18.9	3.21	1.20	2.22	
	48	48.676	21.1	3.10	1.34	2.30	
	49	42.773	21.4	2.72	1.36	2.17	
					ave	**2.05**	
1 mM					st dev	**0.24**	

5mMBuffered *E. coli*

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)	photo
						5mMbuff-1
1	32.1	16.5	2.04	1.05	1.65	
2	31.3	16.9	2.00	1.07	1.65	
3	41.2	15.7	2.62	1.00	1.83	
4	42.0	13.5	2.68	0.86	1.71	
5	24.5	19.5	1.56	1.24	1.57	
6	40.2	13.5	2.56	0.86	1.68	
7	33.7	14.0	2.15	0.89	1.56	
8	36.7	18.3	2.34	1.17	1.86	
9	41.2	18.0	2.62	1.15	1.96	
10	38.3	15.1	2.44	0.96	1.73	
11	34.9	18.4	2.22	1.17	1.82	
12	44.4	21.1	2.83	1.34	2.20	
13	29.4	16.6	1.87	1.06	1.59	
14	44.9	18.7	2.86	1.19	2.08	
15	38.9	16.9	2.48	1.08	1.84	
16	48.7	13.8	3.10	0.88	1.86	
17	28.1	16.9	1.79	1.08	1.57	
18	55.7	20.9	3.55	1.33	2.45	
19	43.6	19.3	2.78	1.23	2.08	
						5mMbuff-2
20	38.7	19.3	2.46	1.23	1.96	
21	35.3	20.3	2.25	1.29	1.92	
22	64.2	19.5	4.09	1.24	2.54	
23	39.2	17.4	2.50	1.11	1.88	
24	28.4	14.8	1.81	0.94	1.47	
25	37.4	18.6	2.38	1.18	1.89	
26	28.1	19.3	1.79	1.23	1.67	

	27	43.5	18.6	2.77	1.18	2.04	
	28	62.3	16.0	3.97	1.02	2.27	
	29	35.8	19.5	2.28	1.24	1.90	
	30	41.2	16.2	2.62	1.03	1.86	
	31	43.2	18.7	2.75	1.19	2.04	
	32	51.2	21.6	3.26	1.37	2.39	
	33	25.7	17.6	1.64	1.12	1.53	
							5mMbuff-
	34	51.152	15.2	3.26	0.97	2.00	3
	35	39.525	13.8	2.52	0.88	1.68	
	36	31.658	17.6	2.02	1.12	1.70	
	37	34.529	16.9	2.20	1.08	1.74	
	38	39.948	22.6	2.54	1.44	2.16	
	39	36.701	17.6	2.34	1.12	1.83	
	40	31.364	18.3	2.00	1.17	1.72	
	41	54.71	19.5	3.48	1.24	2.34	
	42	38.49	20.5	2.45	1.31	2.02	
	43	37.873	21.1	2.41	1.34	2.03	
	44	40.719	18.4	2.59	1.17	1.97	
	45	43.106	18.6	2.75	1.18	2.03	
	46	41.517	21.1	2.64	1.34	2.13	
	47	33.815	21.6	2.15	1.37	1.94	
						1.90	0.25
5 mM						ave	std dev

20mMBuffered *E. coli*

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)	photo
						20mMbuff-
1	46.9	19.8	2.98	1.26	2.19	1
2	36.4	15.8	2.32	1.00	1.72	
3	43.1	14.5	2.75	0.92	1.80	
4	29.9	15.6	1.90	0.99	1.55	
5	33.1	17.4	2.11	1.11	1.73	
6	56.0	18.6	3.57	1.18	2.32	
7	42.5	15.1	2.71	0.96	1.82	
8	34.0	15.7	2.17	1.00	1.66	
9	47.5	17.4	3.02	1.11	2.07	
10	47.8	13.0	3.05	0.83	1.79	
11	38.1	13.5	2.43	0.86	1.63	
12	57.6	15.8	3.67	1.01	2.17	

	13	31.6	15.2	2.01	0.97	1.57	
	14	50.4	15.6	3.21	0.99	2.02	
	15	51.2	16.6	3.26	1.06	2.09	
	16	44.8	16.9	2.85	1.08	1.98	
	17	41.5	16.9	2.64	1.08	1.90	
	18	53.9	14.5	3.44	0.92	2.01	
	19	36.9	12.2	2.35	0.78	1.53	
	20	50.1	18.2	3.19	1.16	2.17	
	21	50.5	13.4	3.21	0.85	1.87	20mMbuff-2
	22	29.6	21.5	1.89	1.37	1.81	
	23	42.2	13.5	2.69	0.86	1.72	
	24	32.4	16.6	2.06	1.06	1.67	
	25	38.5	14.0	2.45	0.89	1.67	
	26	26.3	16.9	1.67	1.08	1.51	
	27	33.1	17.4	2.11	1.11	1.73	
	28	33.0	15.6	2.10	0.99	1.63	
	29	37.9	15.6	2.41	0.99	1.75	
	30	44.7	18.7	2.85	1.19	2.08	
	31	64.7	14.8	4.12	0.94	2.22	
	32	37.1	18.3	2.36	1.17	1.87	
	33	45.7	16.6	2.91	1.06	1.98	
	34	40.7	16.6	2.59	1.06	1.87	
	35	43.0	17.6	2.74	1.12	1.98	
	36	42.3	18.4	2.70	1.17	2.01	20mMbuff-3
	37	43.6	16.9	2.78	1.08	1.95	
	38	48.7	15.6	3.10	0.99	1.98	
	39	31.9	16.2	2.03	1.03	1.64	
	40	34.3	15.8	2.18	1.01	1.67	
	41	50.7	16.9	3.23	1.08	2.10	
	42	32.7	16.0	2.08	1.02	1.64	
	43	39.6	18.0	2.52	1.15	1.92	
	44	40.5	16.5	2.58	1.05	1.86	
	45	50.6	18.3	3.23	1.17	2.19	
	46	39.4	14.8	2.51	0.94	1.74	
	47	42.1	15.1	2.68	0.96	1.81	
	48	43.9	15.2	2.79	0.97	1.85	
	49	35.8	18.4	2.28	1.17	1.85	
	50	55.0	17.4	3.50	1.11	2.23	
	51	39.4	16.9	2.51	1.07	1.85	1.89
						1.87	0.20
20 mM						ave	st dev

50mMBuffered *E. coli*

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)	photo
1	32.419	14.8	2.06	0.94	1.57	50mMbuff-1
2	36.861	19.3	2.35	1.23	1.92	
3	41.375	16.5	2.64	1.05	1.88	
4	56.256	17.6	3.58	1.12	2.26	
5	45.164	16.6	2.88	1.06	1.97	
6	29.627	16.9	1.89	1.08	1.61	
7	27.569	15.6	1.76	0.99	1.49	
8	39.439	18.6	2.51	1.18	1.94	
9	33.939	16.0	2.16	1.02	1.68	
10	31.338	17.4	2.00	1.11	1.68	
11	25.74	17.4	1.64	1.11	1.52	
12	45.847	19.3	2.92	1.23	2.14	
13	22.159	18.0	1.41	1.15	1.44	
14	38.315	17.4	2.44	1.11	1.86	
15	42.93	18.6	2.73	1.18	2.03	
16	34.038	16.6	2.17	1.06	1.71	50mMbuff-2
17	55.821	22.0	3.56	1.40	2.52	
18	28.322	20.3	1.80	1.29	1.72	
19	43.591	20.9	2.78	1.33	2.17	
20	45.294	18.6	2.88	1.18	2.08	
21	51.3	17.4	3.27	1.11	2.15	
22	48.416	15.2	3.08	0.97	1.95	
23	35.228	19.5	2.24	1.24	1.88	
24	35.867	16.5	2.28	1.05	1.75	
25	31.79	19.6	2.02	1.25	1.80	
26	30.273	22.3	1.93	1.42	1.87	
27	28.996	22.0	1.85	1.40	1.82	
28	32.419	19.3	2.06	1.23	1.80	
29	29.825	22.1	1.90	1.41	1.84	
30	59.382	18.0	3.78	1.15	2.35	
31	37.134	18.9	2.37	1.20	1.90	
32	50.987	19.5	3.25	1.24	2.26	
33	34.235	24.9	2.18	1.59	2.10	
34	41.375	22.0	2.64	1.40	2.17	50mMbuff-3
35	45.847	18.0	2.92	1.15	2.07	
36	41.759	20.3	2.66	1.29	2.09	
37	44.79	18.6	2.85	1.18	2.07	
38	38.924	18.2	2.48	1.16	1.91	

39	33.061	18.7	2.11	1.19	1.79	
40	31.122	18.2	1.98	1.16	1.71	
41	33.815	18.3	2.15	1.17	1.79	
42	36.724	20.3	2.34	1.29	1.96	
43	42.537	19.3	2.71	1.23	2.06	
44	47.681	21.0	3.04	1.33	2.27	
45	40.698	19.5	2.59	1.24	2.02	
46	56.495	18.2	3.60	1.16	2.30	
47	37.762	19.3	2.41	1.23	1.94	
48	44.013	18.7	2.80	1.19	2.06	50mMbuff-4
49	26.733	15.1	1.70	0.96	1.45	
50	34.892	25.7	2.22	1.64	2.15	
51	40.032	20.3	2.55	1.29	2.05	
52	40.387	22.3	2.57	1.42	2.16	
53	38.293	19.8	2.44	1.26	1.98	
54	29.627	16.2	1.89	1.03	1.58	
55	28.793	19.8	1.83	1.26	1.72	
56	40.283	22.1	2.57	1.41	2.14	
57	32.028	17.4	2.04	1.11	1.70	
58	40.698	21.6	2.59	1.37	2.13	
59	54.154	20.5	3.45	1.31	2.39	
60	34.796	18.7	2.22	1.19	1.83	
61	46.701	15.6	2.97	0.99	1.94	
62	34.333	21.4	2.19	1.36	1.95	
63	37.807	15.8	2.41	1.01	1.76	
64	40.698	18.6	2.59	1.18	1.98	
65	42.08	17.6	2.68	1.12	1.96	50mMbuff-5
66	45.015	17.6	2.87	1.12	2.03	
67	39.011	21.7	2.48	1.38	2.09	
68	38.315	18.6	2.44	1.18	1.92	
69	53.936	19.3	3.44	1.23	2.32	
70	37.606	18.3	2.40	1.17	1.89	
71	51.675	16.9	3.29	1.07	2.12	
72	34.235	14.0	2.18	0.89	1.57	
73	43.495	19.5	2.77	1.24	2.09	
74	32.419	18.6	2.06	1.18	1.76	
75	27.355	17.6	1.74	1.12	1.58	
76	54.479	22.0	3.47	1.40	2.49	
77	36.0	19.8	2.29	1.26	1.92	
78	58.6	21.5	3.73	1.37	2.55	
79	37.4	18.6	2.38	1.18	1.89	
80	32.4	16.9	2.06	1.08	1.68	
					1.95	0.25
50 mM *E coli*					Ave	St Dev

B.3. Cell size measurements for *B. fragilis* cells at various ionic strengths, pH 7.2

Summary of Average sizes

Bacteriodes cell sizes; buffered to pH 7.2				1mMBuffered-3.tiff	
				Average	**St.Dev**
photo	size =		1392 x 1040	1.52	0.15
15.7	pixels =	1	µm	n=	26.00

Bacteriodes cell sizes				5mMBuffered-2	
	5 mM	effective diameter		**Average**	**St.Dev**
photo	size =	1392 x 1040		1.48	0.40
15.7	pixels =	1	µm	n=	30.00

Bacteriodes cell sizes; buffered to pH 7.2				20mM buffered	
				Average	**St.Dev**
photo		size =	1392 x 1040	1.40	0.13
15.7	pixels =	1	µm	n=	30.00

Bacteriodes cell sizes; buffered to pH 7.2				50mM buffered	
	50 mM	effective diameter		**Average**	**St.Dev**
photo	size =	1392 x 1040		1.40	0.18
15.7	pixels =	1	µm	n=	32.00

Measured cell sizes follow

1mMBuffered B.fragilis

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)
1	48.8	12.2	3.11	0.78	1.75
2	42.5	8.5	2.71	0.54	1.37
3	38.8	13.6	2.47	0.87	1.65
4	34.9	12.6	2.22	0.81	1.51
5	43.4	8.6	2.77	0.55	1.39
6	50.5	11.2	3.22	0.71	1.71
7	40.7	11.7	2.59	0.74	1.57
8	51.9	7.2	3.30	0.46	1.39
9	48.5	12.8	3.09	0.82	1.79
10	50.9	10.6	3.24	0.68	1.67
11	44.3	12.2	2.82	0.77	1.67
12	45.2	12.0	2.88	0.77	1.68
13	37.3	13.0	2.38	0.83	1.58
14	36.4	11.7	2.32	0.75	1.48
15	40.3	12.2	2.57	0.78	1.59
16	39.6	11.3	2.52	0.72	1.52
17	25.7	10.2	1.64	0.65	1.16
18	52.3	8.6	3.33	0.55	1.52
19	41.0	10.8	2.61	0.69	1.51
20	41.7	10.6	2.66	0.68	1.51
21	33.0	12.0	2.10	0.76	1.43
22	32.7	10.6	2.08	0.68	1.34
23	41.3	9.0	2.63	0.57	1.39
24	38.9	10.8	2.48	0.69	1.47
25	35.8	10.3	2.28	0.66	1.38

5mM Buffered *B.fragilis*

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)
1	50.8	15.1	3.24	0.96	1.99
2	41.2	55.2	2.63	3.51	3.43
3	10.8	46.5	0.69	2.96	1.61
4	34.8	10.1	2.22	0.64	1.34
5	10.0	37.9	0.64	2.42	1.40
6	10.4	35.5	0.66	2.26	1.38
7	8.5	35.9	0.54	2.29	1.25
8	11.2	32.6	0.71	2.07	1.37
9	8.9	42.9	0.57	2.74	1.41
10	9.4	43.7	0.60	2.79	1.46
11	10.4	35.1	0.66	2.24	1.38
12	9.8	40.8	0.63	2.60	1.44
13	11.2	38.5	0.71	2.45	1.49
14	9.8	32.2	0.63	2.05	1.28
15	9.2	44.0	0.59	2.81	1.45
16	10.6	28.6	0.68	1.82	1.25
17	10.3	31.4	0.66	2.00	1.29
18	9.2	38.0	0.59	2.42	1.35
19	8.6	31.0	0.55	1.98	1.17
20	11.2	42.8	0.71	2.73	1.57
21	10.0	51.6	0.64	3.29	1.63
22	10.2	40.7	0.65	2.59	1.46
23	10.3	50.6	0.66	3.23	1.64
24	11.0	47.0	0.70	3.00	1.64
25	9.1	36.3	0.58	2.32	1.30
26	10.2	38.9	0.65	2.48	1.43
27	9.8	30.8	0.63	1.96	1.25
28	8.5	32.9	0.54	2.10	1.20
29	9.8	31.8	0.63	2.02	1.27
30	10.2	31.8	0.65	2.03	1.29

20mM buffered B.fragilis

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)
1	34.9	13.6	2.22	0.87	1.565548
2	30.5	10.6	1.94	0.68	1.294727
3	27.3	10.8	1.74	0.69	1.232267
4	34.2	11.0	2.18	0.70	1.397549
5	41.3	10.8	2.63	0.69	1.519827
6	34.5	13.0	2.20	0.83	1.522628
7	34.2	12.5	2.18	0.80	1.487276
8	45.6	9.5	2.90	0.60	1.494981
9	40.3	9.9	2.57	0.63	1.435697
10	46.0	11.0	2.93	0.70	1.620008
11	34.7	10.0	2.21	0.64	1.338235
12	38.3	9.2	2.44	0.59	1.350368
13	43.0	11.4	2.74	0.73	1.591625
14	38.3	10.0	2.44	0.64	1.406328
15	29.2	8.5	1.86	0.54	1.131403
16	39.1	10.0	2.49	0.64	1.420272
17	34.0	8.5	2.16	0.54	1.220213
18	43.7	8.1	2.78	0.51	1.348369
19	33.6	12.0	2.14	0.77	1.445709
20	32.6	11.0	2.07	0.70	1.360131
21	41.8	10.1	2.66	0.64	1.472605
22	34.7	10.6	2.21	0.68	1.379447
23	31.1	11.4	1.98	0.73	1.354379
24	37.1	10.8	2.36	0.69	1.437054
25	35.8	12.5	2.28	0.80	1.523201
26	27.9	9.8	1.77	0.63	1.190471
27	39.3	11.2	2.50	0.71	1.506876
28	41.7	11.2	2.65	0.71	1.551403
29	30.4	9.8	1.93	0.63	1.242885
30	33.4	9.5	2.13	0.60	1.27892

50mM buffered B.fragilis

Cell #	# pixels L	# pixels W	length (um)	width (um)	d_{eff} (um)
1	30.3	13.0	1.93	0.83	1.43
2	35.4	10.2	2.26	0.65	1.37
3	36.2	10.1	2.31	0.64	1.37
4	34.0	9.4	2.16	0.60	1.29
5	40.0	10.0	2.55	0.64	1.44
6	33.0	12.4	2.10	0.79	1.45
7	37.1	11.0	2.36	0.70	1.46
8	31.3	10.2	1.99	0.65	1.28
9	28.4	9.9	1.81	0.63	1.21
10	33.4	12.0	2.13	0.77	1.44
11	32.0	11.0	2.04	0.70	1.35
12	32.2	10.3	2.05	0.66	1.31
13	25.7	10.2	1.64	0.65	1.16
14	32.2	7.6	2.05	0.49	1.13
15	35.5	9.9	2.26	0.63	1.35
16	29.0	11.0	1.85	0.70	1.29
17	28.3	11.0	1.80	0.70	1.27
18	31.9	12.2	2.03	0.78	1.42
19	30.4	12.0	1.94	0.77	1.38
20	25.6	11.2	1.63	0.71	1.22
21	53.1	11.2	3.38	0.71	1.75
22	57.9	13.2	3.69	0.84	1.98
23	42.7	10.4	2.72	0.66	1.52
24	25.6	9.8	1.63	0.63	1.14
25	32.7	11.3	2.08	0.72	1.38
26	31.4	12.1	2.00	0.77	1.40
27	42.1	14.0	2.68	0.89	1.75
28	37.4	11.7	2.38	0.74	1.50
29	39.7	12.2	2.53	0.78	1.58
30	39.1	10.1	2.49	0.64	1.42
31	34.4	10.2	2.19	0.65	1.35
32	38.8	11.0	2.47	0.70	1.49

Appendix C. Zeta Potential Measurements

Table C.1 Sand Zeta Potential Measurements

Sand Zeta Potential Measurements, 4/6/2011
50-70 powdered sand in various ionic strength solutions.
minimum of 5 runs for each trial, 3 trials for each solution.

Sand zeta potential pH 7.2

ionic strength (mM)	zeta ave	st dev
1	-66.94	4.11
2.5	-63.30	10.00
5	-66.06	12.44
20	-65.60	4.28
50	-48.87	8.67

Table A.1 Sand Zeta Potential results (buffered with 0.2 mM $NaHCO_3$ to pH 7.2)

	mob.	zeta	cond (uS)
1 mM trial 1 buffered			284
1	-4.72	-62.69	
2	-4.72	-62.68	
3	-4.86	-64.56	
4	-5.73	-76.18	
5	-4.27	-56.83	
mean	-4.86	-64.59	
1 mM trial 3 buffered			287
1	-3.84	-51.1	
2	-5.24	-69.67	
3	-3.64	-48.35	
4	-5.62	-74.71	
5	-5.94	-78.92	
mean	-4.86	-64.55	

	mob.	zeta	cond (uS)
1 mM trial 2 buffered			285
1	-5.52	-73.35	
2	-4.83	-64.2	
3	-5.76	-76.62	
4	-6.31	-83.82	
5	-4.54	-60.41	
mean	-5.39	-71.68	

Table C.1 (cont) Sand Zeta Potential (buffered with 0.2 mM $NaHCO_3$ to pH 7.2)

	mob.	zeta	cond (uS)		mob.	zeta	cond (uS)
2.5 mM trial 1 buffered			623	2.5 mM trial 2 buffered			637
1	-5.3	-70.43		1	-4.12	-54.73	
2	-5.25	-69.76		2	-6.13	-81.48	
3	-5.16	-68.58		3	-5.87	-78.07	
4	-5.2	-69.07		4	-5.84	-77.69	
5	-6.54	-86.97		5	-4.45	-59.13	
mean	-5.49	-72.96		mean	-5.28	-70.22	
2.5 mM trial 3 buffered			640				
1	-5.32	-70.73					
2	-5.55	-73.77					
3	-4.49	-59.68					
4	-5.21	-69.21					
5	-4.95	-65.83					
mean	-5.1	-67.84					

	mob.	zeta	cond (uS)		mob.	zeta	cond (uS)
5 mM trial 1 buffered			1248	5 mM trial 2 buffered			1257
1	-4	-53.23		1	-5.26	-69.96	
2	-4.29	-57		2	-5.05	-67.17	
3	-4.88	-64.9		3	-5.8	-77.1	
4	-3.65	-48.5		4	-7.02		-93.31
5	-2.68	-35.6		5	-6.2		-82.35
mean	-3.9	-51.84		mean	-5.87	-71.41	
5 mM trial 3 buffered			1262				
1	-5.28	-70.22					
2	-5.92	-78.71					
3	-5.18	-68.86					
4	-5.65	-75.16					
5	-6.14	-81.69					
mean	-5.64	-74.93					

Table C.1 (cont) Sand Zeta Potential (buffered with 0.2 mM $NaHCO_3$ to pH 7.2)

	mob.	zeta	cond (uS)		mob.	zeta	cond (uS)
20 mM trial 1 buffered			4259	20 mM trial 2 buffered			4340
1	-4.29	-57.08		1	-4.91	-65.21	
2	-5.49	-72.93		2	-5.28	-70.15	
3	-5.34	-70.98		3	-4.45	-59.12	
4	-5.38	-71.5		4	-4.34	-57.72	
5	-6.03	-80.17		5	-4.65	-61.83	
mean	-5.31	-70.53		mean	-4.72	-62.81	
20 mM trial 3 buffered			4391				
1	-5.19	-68.99					
2	-4.54	-60.33					
3	-5.02	-66.47					
4	-4.09	-54.33					
5	-5.04	-66.95					
mean	-4.77	-63.47					

	mob.	zeta	cond (uS)		mob.	zeta	cond (uS)
50 mM trial 1 buffered			9320	50 mM trial 2 buffered			9767
1	-3.92	-52.17		1	-4.1	-54.56	
2	-3.47	-46.14		2	-4.27	-56.76	
3	-2.82	-37.48		3	-4.09	-54.38	
4	-2.08	-27.66		4	-3.82	-50.83	
5	-2.47	-32.88		5	-2.98	-39.66	
mean	-2.95	-39.26		mean	-3.85	-51.24	
50 mM trial 3 buffered			9758				
1	-4.22	-56.03					
2	-4.26	-56.58					
3	-3.63	-48.25					
4	-4.49	-59.65					
5	-4.51	-60					
mean	-4.22	-56.1					

Table C.2 Enterococcus zeta potential measurements

	Zeta Potential E1162					Zeta Potential KO39				
	1 mM	2.5 mM	5 mM	20 mM	50 mM	1 mM	2.5 mM	5 mM	20 mM	50 mM
trial 1	-43.11	-36.82	-21.53	12.66	-47.99	-36.65	-43.74	-24.87	-31.9	25.12
	-32.87	-40.59	-47.24	-10.01	-4.45	-41.7	-37.44	-13.62	-22.51	-12.07
	-44.92	-33.41	-32	-12.61	-43.18	-34.87	-50.69	-19.47	-13.87	-22.84
	-43.49	-50.94	-40.87	-45.67	46.36	-43.79	-44.75	-34.28	-27.81	-21.68
	-43.6	-42.5	-29.2	-39.07	40.9	-39.78	-38.82	-34.67	-19.74	-23.68
Ave	-41.598	-40.852	-34.168	-18.94	-1.672	-39.358	-43.088	-25.382	-23.166	-11.03
trial 2	-36.34	-41.9	-30.65	28.91	-19.84	-40.81	-30.1	-34.02	-13.36	-23.6
	-34.15	-37.51	-20.84	19.96	-51.65	-30.47	-53.58	-31.17	-30.85	-13.76
	-38.31	-30.55	-26.54	16.48	-23.35	-42.54	-37.87	-43.2	-18.23	-20.58
	-35.17	-45	-39.51	-27.48	-11.71	-39.81	-28	-52.72	35.98	-7.39
	-30.8	-33.15	-24.78	-34.15	10.17	-50.71	-38.9	-37.11	-39.95	-18.44
Ave	-34.954	-37.622	-28.464	0.744	-19.276	-40.868	-37.69	-39.644	-13.282	-16.754
trial 3	-36.73	-39.09	-38.34	-17.9	-41.31	-33.75	-39.26	-26.64	-32.37	-14.08
	-27.56	-40.13	-50.87	40.94	-25.41	-35.27	-44.12	-31.67	-32.26	-20.72
	-33.96	-35.95	-40.32	-15.34	17.66	-37.5	-29.62	-34.21	-36.63	-9.76
	-35.29	-50.17	-33.54	-20.07	-15.33	-38.26	-11.95	-30.33	-18.99	-19.67
	-35.41	-39.44	-32.03	-51.3	-26.31	-44.17	-47.44	-33.28	-28.57	-14.38
Ave	-33.79	-40.956	-39.02	12.734	-18.14	-37.79	-34.478	31.226	29.764	15.722
trial 4	-32.92	-49.85	-32.45	-14.48	-18.58	-37.12	-23.09	-25.52	-15.95	-25.82
	-30.6	-33.09	-28.95	-9.77	-43.01	-41.69	-40.72	-40.88	-22.24	-12.97
	-35.34	-37.22	16.04	15.74	-13.09	-31.3	-32.56	-32.97	-17.58	-28.45
	-37.98	-43.28	-32.58	-76.71	-26.07	-45.89	-28.49	-55	-34.39	-25.49
	-30.35	-39.88	-19.79	13.76	-26.47	-37.3	-42.88	-30.88	-28.9	-25.9
Ave	-33.438	-40.664	-19.546	-14.292	-25.444	-38.66	-33.548	-37.05	-23.812	-23.726
trial 5	36.91	-32.85	-25.97	-46.12	-8.03	-36.98	-38.18	-38.19	-15.5	-16.33
	-28.33	-31.37	-28.34	-25.7	46.09	-36.75	-25.23	-25.15	-26.61	-14.5
	-53.08	-32	-20.15	26.88	13.54	-31.49	-43.88	-28.57	-14.24	-14.91
	-16.74	-40.25	-35.1	-29.03	23.77	-42.88	-20.05	-31.25	-31.22	-12.86
	-40.61	-37.66	-39.34	-30.07	-10.66	-47.83	-20.84	-34.47	-25.48	-12.87
Ave	-20.37	-34.826	-29.78	20.808	12.942	39.186	-29.636	31.526	-22.61	14.294
Overall Ave	**-32.83**	**-38.98**	**-30.20**	**-13.21**	**-10.32**	**-39.17**	**-35.90**	**-32.97**	**-22.53**	**-16.31**

Table C.3 E.coli zeta potential measurements

E. coli Zeta potential (mV), pH 7.2

Ionic strength (mM)	Zeta ave	st dev
1 mM	-42.61	5.08
5 mM	-43.24	2.55
20 mM	-32.02	3.69
50 mM	-28.24	0.95

E. coli Zeta Potential Measurements

E. coli in various ionic strength solutions, pH 7.2. (buffered with 0.2 mM $NaHCO_3$)
Solutions have an absorbance of approximately 0.36 at 220 nm

Table A.3 *E coli* zeta potential results (mV) 4/5/2011

	mob.	zeta	cond (uS)		mob.	zeta	cond (uS)
1 mM trial 1 buffered			268	trial 2 buffered			268
1	-3.05	-40.53		1	-0.91		-12.1
2	-3.75	-49.91		2	-3.62	-48.09	
3	-2.8	-37.21		3	-3.43	-45.64	
4	-2.96	-39.31		4	-3.75	-49.85	
5	-3.33	-44.27		5	2.82		37.5
mean	-3.18	-42.25		mean	-1.78	-47.86	
trial 2 buffered (redone)			269	trial 3 buffered			266
1	-3.21	-42.73		1	-3.17	-42.12	
2	-1.15		-15.22	2	-3.14	-41.78	
3	1.65		21.88	3	-3.17	-42.12	
4	-2.51	-33.38		4	-2.78	-36.98	
5	-2.79	-37.05		5	-1.53	-20.32	
mean	-1.6	-37.72		mean	-2.76	-36.66	

Table C.3 (cont) *E coli* zeta potential results (mV) 4/5/2011

		mob.	zeta	cond (uS)			mob.	zeta	cond (uS)
5 mM trial 1 buffered				1219	5 mM trial 2 buffered				1278
	1	-2.88	-38.3			1	-3.26	-43.39	
	2	-2.48	-32.97			2	-3.39	-45.09	
	3	-3.82	-50.81			3	-2.99	-39.69	
	4	-3.9	-51.88			4	-3.15	-41.92	
	5	-3.69	-49.02			5	-4.06	-53.99	
mean		-3.35	-44.6		mean		-3.37	-44.82	
5 mM trial 3 buffered				1247					
	1	-1.93	-25.59						
	2	-2.84	-37.79						
	3	-4.43	-58.92						
	4	-2.74	-36.41						
	5	-3.22	-42.79						
mean		-3.03	-40.3						
20 mM trial 1 buffered				4296	20 mM trial 2 buffered				4382
	1	-2.58	-34.35			1	-2.65	-35.23	
	2	-2.52	-33.46			2	-2.44	-32.37	
	3	-2.11	-28.08			3	-1.99	-26.42	
	4	-2.91	-38.62			4	-2.77	-36.89	
	5	-0.33	-4.35			5	-2.86	-38.07	
mean		-2.09	-27.77		mean		-2.54	-33.79	
20 mM trial 3 buffered				4375					
	1	-2.68	-35.63						
	2	-2.35	-31.18						
	3	-3.15	-41.86						
	4	-2.74	-36.44						
	5	-2.06	-27.34						
mean		-2.59	-34.49						

Table C.3 (cont)*E coli* zeta potential results (mV)

			4/5/2011 cond (uS)				cond (uS)
	mob.	zeta			mob.	zeta	
50 mM trial 1 buffered			9370	50 mM trial 2 buffered			9666
	1	-2.42	-32.19		1	-2.73	-36.26
	2	-2.59	-34.38		2	-1.36	-18.13
	3	-2.26	-30		3	-1.64	-21.8
	4	-1.5	-19.97		4	-1.21	-16.14
	5	-2.01	-26.75		5	-3.27	-43.46
mean		-2.16	-28.66	mean		-2.04	-27.16
50 mM trial 3 buffered			9583				
	1	-3.09	-41.05				
	2	-1.74	-23.18				
	3	-2.39	-31.74				
	4	-1.66	-22.12				
	5	-1.99	-26.49				
mean		-2.18	-28.91				

Table C.4 *B. fragilis* zeta potential measurements (mV), pH 7.2

Bacteriodes fragilis in various ionic strength solutions.

Solutions have an absorbance of approximately 0.36 at 220 nm

B. fragilis zeta potential (mV), pH 7.2

ionic strength (mM)	zeta	st dev
1 mM	-23.0867	1.466026
5 mM	-19.4947	1.600408
20 mM	-19.1267	2.180031
50 mM	-17.4533	3.816613

Table C.4 (continued) ***B. fragilis*** **Zeta Potential Measurements (mV), 3/31/11**

		mob.	zeta	cond (uS)		mob.	zeta	cond (uS)
1 mM								
trial 1				485	trial 2			481
	1	-1.31	-17.39		1	-1.65	-22	
	2	-2.09	-27.78		2	-2.18	-28.91	
	3	-1.17	-15.6		3	-1.28	-16.96	
	4	-1.41	-18.74		4	-2.4	-31.94	
	5	-2.14	-28.42		5	-1.2	-15.95	
mean		-1.62	-21.59		mean	-1.74	-23.15	
trial 3					trial 4			488
	1	2.54	33.82		1	-1.47	-19.54	
	2	-1.11	-14.76		2	-1.97	-26.18	
	3	-2.31	-30.7		3	-1.96	-26.08	
	4	-2.13	-28.28		4	-1.65	-21.99	
	5	-1.43	-19.04		5	-2.17	-28.81	
mean		-0.89	-11.79		mean	-1.84	-24.52	
5 mM								
trial 1				1963	trial 2			1985
	1	-1.65	-16.67		1	-1.5	-19.89	
	2	-1.58	-21.04		2	-1.74	-23.08	
	3	-1.71	-22.7		3	-2.03	-26.92	
	4	-1.21	-16.12		4	-0.82	-10.94	
	5	-1.16	-15.44		5	-1.94	-25.84	
mean		-1.38	-18.39		mean	-1.6	-21.33	
trial 3				1966				
	1	-2.17	-28.87					
	2	-1.62	-21.52					
	3	-1.13	-15.07					
	4	-1.18	-15.71					
	5	-0.95	-12.65					
mean		-1.41	-18.764					

Table C4 (continued) *B. fragilis* Zeta Potential Measurements, 3/31/11								
	mob.	zeta	cond (uS)			mob.	zeta	cond (uS)
20 mM								
trial 1			4383		trial 2			4477
1	-1.35	-17.95			1	-1.18	-15.71	
2	-1.54	-20.46			2	-0.87	-11.59	
3	-2.69	-35.7			3	-1.32	-17.5	
4	-1.52	-20.19			4	-1.3	-17.31	
5	-1.04	-13.83			5	-1.94	-25.77	
mean	-1.63	-21.62			mean	-1.32	-17.58	
trial 3			4449		trial 3	(redo)		4493
1	-1.07	-14.21			1	-1.13	-15.05	
2	-0.8	-10.62			2	0.39	5.17	
3	-1.82	-24.19			3	-1.22	-16.18	
4	-1.56	-20.71			4	-1.05	-14.02	
5	1.49	19.76			5	-0.89	-11.84	
mean	-0.75	-9.99			mean	-0.78	-10.38	
trial 3	(again)							
1	-1.27	-16.91						
2	-2.31	-30.74						
3	-0.34	-4.56						
4	-2.33	-31.01						
5	-0.58	-7.69						
mean	-1.37	-18.18						
50 mM								
trial 1			10188		trial 2			
1	-0.36	-4.74			1	-2.45	-32.52	
2	-0.33	-4.4			2	-0.77	-10.21	
3	-2.29	-30.48			3	-1.5	-19.99	
4	-0.66	-8.76			4	-1.6	-21.4	
5	-1.38	-18.32			5	-1.53	-20.4	
mean	-1	-13.34			mean	-1.57	-20.88	
trial 3			10136					
1	-1.21	-16.06						
2	-1.55	-0.62						
3	-0.89	-11.89						
4	-1.23	-16.41						
5	-1.93	-25.71						
mean	-1.36	-18.14						

Appendix D. Contact Angle Measurements

Contact angle measurements between liquid drop and bacterial lawn

***E. faecium* E1162 bacterial lawn**

trial #	water θ	glycerol θ	diiodomethane θ
1	18.3	22.9	47.4
2	16.9	22.9	45.4
3	24.5	25.7	48.8
4	24.9	25.6	51.3
ave θ	21.2	24.3	48.2
stdev	4.1	1.6	2.5

***E. faecium* E1162Δesp bacterial lawn**

trial #	water θ	glycerol θ	diiodomethane θ
1	17.2	32.9	44.0
2	17.7	32.8	44.1
3	14.7	26.0	44.5
4	14.7	27.0	44.3
5		41.0	
6		38.6	
7		34.6	
8		33.2	
9		35.1	
10		33.0	
11		28.3	
12		28.0	
ave θ	16.1	32.5	44.2
stdev	1.6	4.6	0.2

***E. coli* K-12 bacterial lawn**

trial #	water θ	glycerol θ	diiodomethane θ
1	20.7	19.2	57.0
2	18.1	19.6	62.1
3	22.4		56.9
4	20.0		60.7
5	17.2		50.9
6	15.4		50.9
7	13.6		48.1
8	12.5		51.1
9	11.6		
10	11.5		
11	12.6		
ave θ	16.0	19.4	54.7
stdev	3.9	0.3	5.2

***B. fragilis* bacterial lawn**

trial #	water θ	glycerol θ	diiodomethane θ
1	28.7	40.5	55.3
2	25.4	40.7	57.5
3	30.4	30.5	64.7
4	25.2	24.9	58.0
5	27.3		57.7
6	24.3		63.6
7	24.1		
8	23.6		
9	36.6		
10	30.8		
ave θ	27.6	34.2	59.5
stdev	4.1	7.8	3.8

Curriculum Vitae

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Associate Professor, Department of Physical Sciences, Division of Natural Sciences, Mathematics and Technology, Alverno College, Milwaukee, WI.
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Education

PhD. Geosciences, University of Wisconsin – Milwaukee, 2016. Dissertation titled *Transport of potential microbial source tracking markers in sandy materials*

M.S. Geosciences, University of Wisconsin - Milwaukee, 1990. Master's thesis titled *The hydrologic interaction between the Milwaukee River and the ground water system at the Blue Hole abandoned landfill, Milwaukee, Wisconsin*.

Teacher Certification, Post Baccalaureate Program, University of Wisconsin-Milwaukee, 2002. Received Wisconsin teaching certification in Earth and Space Science, Chemistry, Physics, and Broadfield Science

B. S. Geology, University of Minnesota - Duluth, 1985

Teaching Experience

- **Associate Professor**, Alverno College, 2003-present. Undergraduate courses taught include: Environmental Geology; Foundations of Earth Science; Earth Science; Global Effective Citizen (Power of Water); Foundations of Chemistry; Integrated Science; Chemistry Survey/Biochemistry Lab
- **Science Teacher**, Catholic Memorial High School, 2000-2003, courses taught included: Integrated Science; Chemistry, Honors Chemistry.
- **Interpretive Ranger**, GeoCorps Internship, Glacier National Park, Montana, summer 2010 (12 weeks); interpreted park geology, glaciers, and water resources.
- **Instructor**, Natural Science, Lakeland College Life-long Learning Program, 1992, 1994; natural science course covering chemistry, physics, earth science, and biology.

Areas of Specialization

- Geology, Hydrogeology, Environmental Geology, Earth Science, Science Education, Hydrology, Introductory Chemistry

Areas of Competence

- Interdisciplinary studies of environmental science and humanities, sustainability studies.

Other Professional Experience

- **Senior Hydrogeologist**, Tetra Tech, Inc., 1987-2000. Senior hydrogeologist in environmental consulting firm. Responsible for investigating and evaluating complex environmental problems in specific geologic settings; preparing and presenting proposals to clients; writing, reviewing, and editing technical scientific reports; project management including personnel oversight, training field staff, troubleshooting during project activities; tracking project costs and timelines, scheduling and communications between personnel, subcontractors, and regulators to successfully complete the project on time and on budget; preparation of formal and informal reports and presentations to clients, regulatory agency personnel, and peers.

- **Director, Corporate Health and Safety**, HSI GeoTrans, Inc., 1998-2000. Responsible for overseeing the corporate health and safety program for 13 offices across the country, as well as health and safety planning for the Milwaukee office staff.

College Committees and Department Responsibilities

- Associate Professor, Physical Sciences Department (2003 - present)
- Program Director, Environmental Science (2003 – present)
- Effective Citizenship Committee (2013-present)
- Communications Ability Department (2004-2013)
    - Quantitative Analysis Subcommittee (2004-2013)
- ACTS Environment Committee (2008-present)
- Institutional Representative for Wisconsin Space Grant Consortium (WSGC) (2004-2009)

Curriculum Development

- Alverno summer fellowship supported work on problem solving and analysis in the science curriculum, 2005-present.
- Developed or significantly adapted four courses:
    - Foundations of Earth Science
    - Earth Science for Educators
    - Environmental Geology
    - The Power of Water – Global Effective Citizen

    - Sustainability and Technology
- Developed or significantly adapted curriculum to include use of geographic information system (GIS)
    - GEC – The Power of Water
    - Environmental Geology
    - Sustainability and Technology
- AC 309 External development committee
- Coastal Cities course: Baltic Cities, consultant

Fellowships Grants and Awards

2016 STEM to LEAF curriculum development fellowship, Alverno College

2016 West Campus Stormwater Study grant, Fund For Lake Michigan

2012, 2014 Virtual Field Experience development grant participant; Baraboo WI, and Badlands of SD, Paleontological Research Institution/NSF

2012, 2014 Best Graduate Student Research Presentation award' University of Wisconsin-Milwaukee Geosciences Department

2011 Ghana partnership delegate grant, Presbytery of Milwaukee Mission Partnerships

2010 GeoCorps Internship, Glacier National Park; Geological Society of America

2009-2013 Steering Committee; Alverno Scholars Grant; National Science Foundation

2008-2016 Doctoral Incentive Program recipient, Alverno College

2008 Developing problem solving and analysis in the science curriculum, Alverno summer faculty fellowship

2007-2009 Principle Investigator; Expanding GIS Across the Curriculum grant; Wisconsin Space Grant Consortium

2007 International travel faculty photos database; Alverno faculty fellowship

2006-2007 Principal Investigator, Alverno College Rain Garden Demonstration Project grant; Wisconsin Department of Natural Resources and United States Environmental Protection Agency

2005-2007 Principle Investigator; Geospatial Science & Technology Across the Curriculum grant; Wisconsin Space Grant Consortium

2005 Alverno Asian Studies Initiative fellowship; Freeman Foundation

2004-2006 Participant; Adapting Diagnostic Digital Portfolio Technology to Track Assessments of Advanced Student Learning Outcomes in Analysis and Problem Solving Abilities in STEM Courses; National Science Foundation grant

1985-1987 Graduate Student GPOP Fellowship; University of Wisconsin- Milwaukee

Presentations, Workshops, Panels

American Water Resources Association, Wisconsin Section Annual Meeting, March 2014, "Comparison of *Escherichia coli* and *Bacteriodes fragilis* transport within saturated quartz sands"

Geological Society of America Annual Meeting, October 2011, "Effects of Enterococcal Surface Protein (Esp) on the Transport of *Enterococcus faecium* Within Saturated Aquifer Sands"

Wisconsin Space Grant Consortium Annual Meeting, Milwaukee School of Engineering, Milwaukee, Wisconsin, August, 2009. "Expanding GIS Across the Curriculum"

Alverno College, Distilling Essence general conference, 2008.: "Distilling the Essence of Water"

University of Wisconsin-Milwaukee, May 2008. Women In Science Workshop

Wisconsin Space Grant Consortium Annual Meeting, Superior, Wisconsin, August, 2007. "GIS Across the Curriculum"

Alverno College Institute, June, 2007, Panel member "Teaching for Student Engagement".

American Water Resource Association, Wisconsin Section Annual Meeting, 1986, "Electrical Resistivity at the Blue Hole abandoned landfill, Milwaukee, WI".

Publications and Reviews

Doctoral Dissertation: "Transport of potential microbial source tracking markers in sandy material". University of Wisconsin-Milwaukee, May 2016

Colloids and Surfaces B: Biointerfaces, 2014 Nov 1;123:439-45. Epub 2014 Oct 5, "Comparison of the transport of Bacteroides fragilis and Escherichia coli within saturated sand packs"

Environmental Science and Technology, 2012 Feb 7;46(3):1511-8. Epub 2012 Jan 26, "Influence of enterococcal surface protein (esp) on the transport of *Enterococcus faecium* within saturated quartz sands"

Proceedings of the Geological Society of America Annual Meeting, October 2011, "Effects of Enterococcal Surface Protein (ESP) on the Transport of *Enterococcus Faecium* Within Saturated Aquifer Sands"

Proceedings of the 19th Annual Wisconsin Space Conference, August 2009. "Expanding GIS Across the Curriculum"

Proceedings of the 17th Annual Wisconsin Space Conference, August 2007, "Integrating GIS Across the Curriculum"

Master's thesis: "The hydrologic interaction between the Milwaukee River and the ground water system at the Blue Hole abandoned landfill, Milwaukee, Wisconsin". University of Wisconsin-Milwaukee, August 1990.

Book Review; Review of chapter 14, The Atmosphere, in The Good Earth: Introduction to Earth Science, McConnell et al, 2008.

Professional Organization Memberships and Certifications

- Wisconsin Women Environmental Professionals (member)
- National Science Teacher Association (member #2136105)
- American Institute of Professional Geologists, Certified Professional Geologist #9196
- State of Wisconsin Registered Professional Geologist #67-113
- State of Wisconsin Registered Professional Hydrologist #65-111
- Wisconsin Society of Science Teachers
- Wisconsin Space Grant Consortium (WSGC)
- Geological Society of America (GSA)
- UWM GIS Council