

1. Introduction

Approximately 30% of Canadians (Canadian Ground Water Association, 2010) and 50% of Americans (Glennon, 2002) rely on groundwater for their domestic water supplies. Aquifers have been typically considered to be protected from the microbial and chemical contamination that is characteristic of surface water. However, increases in population densities, industrialization and development have led to a rise in withdrawals from aquifers, and together, these factors may compromise the quality of groundwater supplies. Multiple disease outbreaks have been attributed to microbial contamination of groundwater sources; in the U.S alone, 46 disease outbreaks caused by groundwater contamination were reported between 1999 and 2002, accounting for approximately 80% of all microbial illnesses related to drinking water (John and Rose, 2005).

The primary pathogenic microorganisms compromising potable water supplies can be grouped into three categories: enteric viruses, bacteria, and protozoa. Waterborne viruses include, but are not limited to, enteroviruses, coxsackievirus, echovirus, rotavirus, norovirus, and hepatitis A and E. Bacteria of concern include, but are not limited to, enteropathogenic *E. coli*, *Salmonella* and *Shigella* spp., *Campylobacter jejuni*, and *Aeromonas hydrophila*. The main protozoa found in groundwater systems are *Cryptosporidium parvum* and *Giardia lamblia* (MacIer and Merkle, 2000).

The increasing awareness of the threats posed by microbial contaminants to groundwater supplies has lead to a spark of interest in areas of public health and safety (John and Rose, 2005),

as well as a surge in research devoted to the sources, fate and transport of waterborne microorganisms (Brown and Hussain, 2003; John and Rose, 2005). More importantly, it has become clear that uncertainties and risks associated with the contamination of fractured bedrock aquifers are significantly higher than those paired with unconsolidated porous aquifers. It is estimated that 70% of regional aquifers in Canada are situated in fractured media (Natural Resources Canada, 2005). However, in comparison to unconsolidated porous media aquifers, relatively little is understood about particle transport in fractures. Despite the notable differences between porous and fractured media, the well-developed techniques used for characterizing porous aquifers are still being applied to fractured aquifers (Natural Resources Canada, 2005). These techniques, although suitable for the assessment of porous media, are generally inadequate for evaluating fractured aquifers (Natural Resources Canada, 2005). The lack of knowledge related to fractured aquifers (Neuman, 2005), along with tragic events similar to those that occurred at Walkerton, Ontario in the early 21st century, has spurred on much research devoted to bettering the understanding of fractured aquifers as well as bacterial and viral transport through fractured media (Brown & Hussain, 2003).

In response to the Walkerton tragedy, which brought to light the dangers associated with poorly understood and scantily monitored community water supplies, Ontario's provincial government passed the Clean Water Act (2006). This Act demands that each community within the province submit a Source Water Protection Plan that aims to protect both the quantity and quality of water supplies. This Plan is to relay the existing and potential threats to drinking water quality, prioritize each of these threats, and then develop and implement risk management

programs to minimize the effects of these threats. Within this context, therefore, it is important to develop a mechanistic understanding of the transport and retention of biocolloids in fractures, as doing so will aid in determining the risk of biocolloid contamination to fractured groundwater sources.

Flow and transport in fractured media is dictated by the hydrodynamics within complex networks of highly heterogeneous conduits, as well as the surface interactions that occur between the constituent being transported and the media (Berkowitz, 2002; Neuman, 2005). Solute tracer tests applied to fractured aquifers have reported significantly higher groundwater velocities than those typically found in unconsolidated porous media. Malard, Reygrobellet, and Soulie (1994), reported that an injected dye travelled up to 140 feet in 2 hours in a field-scale fracture, while Becker, Reimus and Vilks (1998) found that water in a fractured bedrock region moved 118 feet in 30 minutes. The characteristically high flow rates found in fractured aquifers are known to enhance the risk of contamination by waterborne pathogens (Berger, 2008), as (1) pathogens sorbed onto the matrix are easily dislodged by shear forces and reintroduced to the environment and (2) pathogens in suspension are being transported too quickly to succumb to the attractive forces between the pathogen and the matrix.

Pathogens can be introduced to aquifers through a variety of sources and mechanisms, including seepage from septic systems, leaky sewer lines, direct injection of wastewater effluent into wells, natural percolation through the vadose zone, and sinkholes (John and Rose, 2003). Many factors contribute to the migration of waterborne pathogens, including the size and

isoelectric point of the pathogen (Dowd *et al.*, 1998), saturated versus unsaturated groundwater flow (Powelson, *et al.*, 1991; Jin *et al.*, 2000), and the ionic strength and pH of the groundwater (Fontes *et al.*, 1991). Hydrodynamics, which are highly influenced by flow velocity and the heterogeneity of the flow regime, are also very important (Brush and Thomson, 2003).

1.1 Objectives of Research

The research detailed in this thesis focuses on evaluating the influence of hydrodynamics, which is a conglomeration of individual forces that act upon a particle in motion, on biocolloid transport in saturated fractured media. These forces may include, but are not limited to, pressure forces, inertial forces and frictional forces. It has been well documented in the interfacial science literature that micro-scale hydrodynamics play a significant role in the transport of particles greater than approximately one micron in diameter (Torkzaban *et al.*, 2007; Ahmadi, 2009), but do not significantly affect the transport of smaller particles (Ahmadi, 2009). This phenomenon, however, has never been investigated in fractures, where the larger-scale hydrodynamics are complex, and must also be considered. To bridge this knowledge gap, this research was conducted to elucidate the effects of hydrodynamics on the transport and retention of *E. coli* RS2GFP (2.5 μm x 0.8 μm) and polystyrene microspheres (0.05 μm in diameter) in single, saturated, fractures at the laboratory scale.

More specifically, this research was designed to identify distinct relationships between particle size, specific discharge, and the retention of particles in a single, saturated dolostone

fracture. The use of both *E. coli* RS2GFP and polystyrene microspheres will facilitate the delineation of the aforementioned correlations.

1.2 Scope of Thesis

This thesis contains five chapters in addition to this introductory chapter. Chapter 2 provides a review of particle transport in fractured media, in which the basic principles of particle transport are discussed. Additionally, the research pertaining to microbial transport in subsurface environments is detailed in chronological order. Chapter 3 provides a description of the experimental set up as well as the methods employed in this work. Chapter 4 lists the materials, equipment and analytical techniques employed in this work. Chapter 5 provides detailed results derived from this work along with a discussion pertaining to the results. Chapter 6 contains a list of concluding remarks along with a list of future recommendations.

2. Literature Review

2.1 Characterization of Single Fractures

2.1.1 Equivalent Apertures

Single fractures are typically characterized by bulk aperture measurements, in which one “equivalent” aperture value derived from a series of hydraulic or tracer tests is applied to the entire fracture plane. These measurements are assumed to be comparable to the effective porosity terms associated with homogenous porous media. Most commonly applied are the hydraulic (b_h), mass balance (b_m), and frictional loss (b_f) apertures.

The hydraulic aperture, b_h , of a rough-walled fracture is defined as an equivalent set of smooth parallel plates suitable to satisfy the bulk cubic law, assuming that the flow rate, hydraulic gradient, and fracture dimensions are quantifiable. The hydraulic aperture, b_h , can be calculated as follows:

$$b_h = \left[\frac{12\mu QL}{\rho g W (H_i - H_0)} \right]^{\frac{1}{3}} \quad (1)$$

where, Q [L^3/T] is the flow rate, ρ [M/L^3] is fluid density, μ [M/LT] is fluid viscosity, t [T] is time, and g [L/T^2] is gravitational acceleration, L [L] is the length of the fracture plane parallel to the direction of flow, W [L] is the width of the fracture plane perpendicular to the direction of flow, and $H_i - H_0$ is the difference in hydraulic head between the effluent and influent boundaries of the fracture plane respectively.

There are two large assumptions associated with this approach: (1) the validity of the bulk cubic law; and (2) matrix flow is relatively insignificant. In a very broad sense, researchers (Raven and Gale, 1985; Zimmerman and Yeo, 2000; Brush and Thomson, 2003) have investigated and verified the validity of the bulk cubic law, finding that it generates a viable approximation of the arithmetic mean aperture in relatively smooth fractures that host small mean apertures, at low Reynolds numbers (Dickson and Thomson, 2003). However, measurements of the hydraulic aperture have the potential to under-predict the mean aperture by up to 50% when applied to systems in which fracture roughness, mean aperture, and Reynolds number are increased (Dickson and Thomson, 2003). It is important to note, therefore, that measures of hydraulic aperture are merely an estimation of equivalent aperture, and are in no way a direct approximation of the mean aperture.

The frictional loss aperture, according to Tsang (1992), is measured by calculating the average velocity of the flow field based on the mean residence time, t_m , of a solute tracer, assuming that the definition of specific discharge for flow through smooth parallel plates is applicable to rough-walled fractures. Based on Tsang (1992), it can be calculated as follows:

$$b_f = L \left[\frac{12\mu}{\rho g (H_i - H_0) t_m} \right]^{\frac{1}{2}} \quad (2)$$

where, t_m [T] is the mean residence time of the solute mass.

The mass balance aperture, b_m , is a measure of the aperture necessary to balance a given volume of fluid over the areal extent of a tracer assumed to be migrating in a plug flow manner.

According to Tsang (1992), its application incorporates the assumptions that mass transport must extend over the entire areal extent of the fracture, and that the flow path is linear across the fracture plane. The mass balance aperture, b_m , can be drawn from advective tracer data as defined by Tsang (1992):

$$b_m = \frac{Qt_m}{LW} \quad (3)$$

In order to clarify any inconsistencies within literature, Tsang (1992) studied the definition and applicability of these terms, finding that although equivalent apertures derived from tracer tests were being used interchangeably, they actually produced significantly different results. Tsang (1992) found the following order to be true:

$$b_m \geq b_h \geq b_f$$

Work done in previous studies (Novakowski, 1992; Piggot and Elsworth, 1993; Brush and Thomson, 2003; Dickson and Thomson, 2003) has verified the legitimacy of this ordering. The hydraulic and frictional loss apertures primarily depend on the hydraulic gradient across the fracture plane, and are therefore most sensitive to the regions of high head loss. Such localities are often associated with smaller aperture regions and/or regions of high velocity. Measurements of these apertures are therefore governed by local asperities, and are biased towards the smaller aperture regions and/or regions of high velocity encountered along the flow path. On the other hand, measurements of the mass balance aperture do not depend on the hydraulic gradient across the flow field. Rather, they are controlled by the storage of mass in void space, which takes place in the larger aperture regions. Therefore, according to Tsang (1992), the mass balance aperture is

rendered the most applicable equivalent aperture, as it represents an average aperture along the flow path of tracer transport, and generates the most viable estimate of the arithmetic mean aperture. Zheng *et al.* (2008) demonstrated that the mass balance aperture is also the most appropriate equivalent aperture for modeling solute transport.

Knowing that the frictional loss aperture is sensitive to the smaller aperture regions while the larger aperture regions govern the mass balance aperture, it is possible to evaluate the range of extreme aperture regions along the flow pathway, by applying the tracer aperture ratio, δ , as follows (Dickson and Thomson, 2003):

$$\delta = \frac{b_f}{b_m} \quad (4)$$

In essence, extreme aperture regions govern both the flow regime of the fracture plane as well as the transport of solutes. Larger tracer aperture ratios suggest a smaller range of extreme aperture regions while smaller tracer aperture ratios suggest the opposite. A parallel plate fracture, which in theory has no extreme aperture regions, would have a tracer aperture ratio of one, in which both the frictional loss and mass balance apertures are equivalent. The tracer aperture ratio, although it provides a description of the variation of the aperture field, does not detail the roughness of the aperture field.

Recently, techniques have been developed to measure the actual distribution of apertures in the fracture plane, thus giving information on roughness. These techniques are able to provide measures of the arithmetic mean aperture, $\langle b \rangle$, the standard deviation, σ_b [L], the correlation

length, λ_b [L], and fraction of contact points, c [-], in the aperture field. Additional information on the roughness of the fracture plane can be gathered from the coefficient of variation for the aperture field, which can be defined as the ratio of the standard deviation to the arithmetic mean aperture ($\sigma_b/\langle b \rangle$) (Zimmerman and Bodvarsson, 1996). Realistically speaking, it is possible that a smooth fracture has several extreme aperture regions, in which case it would possess a high tracer aperture ratio, and a low coefficient of variation. Therefore, although they are not mutually exclusive, it is safe to conclude that the tracer aperture ratio and the coefficient of variation are not indicative of each other. It is important to note, however, that the aforementioned techniques provide limited information, as they are highly intrusive, and can only be applied ex-situ.

2.2 Hydrodynamics and Fluid Flow in Single, Saturated Fractures

The hydrodynamics involved in particle transport through fractured media are not well understood, and minor changes in hydrodynamics can implement a significant change in colloid transport behaviour (Becker, 1998). The most general manner of describing the hydrodynamic processes taking place within a rock fracture is given by the Navier-Stokes and the continuity equations, which describe both momentum and mass conservation over the fracture plane. The equations are as follows:

$$\rho(\mathbf{u} \cdot \nabla)\mathbf{u} + \rho \frac{\partial \mathbf{u}}{\partial t} = \mu \nabla^2 \mathbf{u} - \nabla p - \rho \mathbf{g} \quad (5)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (6)$$

where, ρ [M/L³] is fluid density, $\mathbf{u}$ is a velocity vector, p [M/LT²] is fluid pressure, μ [M/ LT] is fluid viscosity, t [T] is time, and $\mathbf{g}$ [T/L²] is gravitational acceleration vector. The left-hand term of Equation 5 describes inertia, and leads to nonlinearity, making the Navier-Stokes equation difficult to solve, especially within domains of rough-walled fractures. However, it can be assumed that inertial forces are negligible in relation to viscous forces, and the Navier-Stokes equation can then be reduced to the Stokes equation by removing the inertial term (Batchelor, 1967). The Stokes equation, also known as the creeping flow equation is as follows:

$$\mu \nabla^2 \mathbf{u} = \nabla P, \text{ where } P = p + \rho g z \quad (7)$$

The linearity of the Stokes equation makes it far simpler to solve than Navier-Stokes; however, it must first be verified that inertial forces are truly negligible. Typically, studies have applied the Reynolds number as a measure of the relative strength of inertial forces to that of viscous forces in flowing fluids. The Reynolds number can be described as follows (Briggs *et al.*, 2009):

$$\text{Re} = \frac{2Wu}{\nu} \quad (8)$$

where, ν [L²/ T] is the kinematic viscosity, in a fracture of width W [L] with physical velocity u [L/T].

Some have solved the Stokes equation numerically for rough walled fractures, and its validity has been described by Brown *et al.*, (1995), Mourzenko *et al.*, (1995), and Brush and

Thomson (2003). However, the application of the Stokes equation to fluid flow in rough-walled fractures is not common, primarily due to mathematical complexities (Koyama *et al.*, 2008).

To further simplify the governing equations of fluid flow, several geometric and kinematic assumptions need to be made. Generally, this is done by assuming viscous flow between two parallel plates under a uniform pressure gradient across the rock fracture. Doing so renders only one of the flow velocity components to be nonzero, leading to Poiseuille flow. Poiseuille flow describes the velocity profile across the fracture aperture, b , as a parabola bound by two parallel plates without roughness (Koyama *et al.*, 2008). The velocity profiles can be explained as follows:

$$u_x = -\frac{1}{2\mu} \frac{\partial p}{\partial x} \left[\left(\frac{b}{2} \right)^2 - z^2 \right], \quad u_y = u_z = 0 \quad (9)$$

Provided that flow is laminar, and is under a steady and uniform hydraulic gradient, total volumetric flow in a rock fracture can be expressed as:

$$Q_x = -\frac{\rho g b^3 W}{12\mu} \nabla p \quad (10)$$

in which b [L] is the aperture of the superficially smooth parallel fracture. Additionally,

$p = \frac{H_i - H_0}{L}$, in which L is the length of the rock fracture parallel to flow and H [L] is a measure

of hydraulic head. In the case of equation 10, volumetric flow is proportional to the cube of the aperture of two parallel plates, thus giving the equation its name: the cubic law (Brush and Thomson, 2003). Studies have verified the behaviour of the cubic law using smooth parallel

plates with apertures ranging from 1 cm to 1 μ m, given that Re was below the critical value of ~ 1200 (Brush and Thomson, 2003).

Research done on single, saturated fractures at both the laboratory (Raven and Gale, 1985; Pyrak-Nolte *et al.*, 1987; Keller *et al.*, 1995) and field scale (Rasmuson and Neretnieks, 1986) have made known the fact that the classical view of a rock fracture as two smooth parallel plates is not adequate to describe flow, thus explaining why mass balance apertures derived from tracer tests are typically greater than those gathered from hydraulic tests. However, although more complex conceptual models have been developed, an alternative has yet to be hailed as justifiable (Berkowitz, 2002).

2.2.1 Channelization

When evaluating flow through a fracture in the x-y plane, strong localization is likely to occur. In other words, a large percentage of flow is concentrated over a small percentage of the fracture area, leading to preferential flow paths. Rasmuson and Neretnieks (1986) have shown that up to 90% of fluid flow can occur within 5-20% of the fracture exit plane. In another case, Brown *et al.* (1998) performed steady-state flow experiments using a transparent epoxy replica of a natural rock, in which the authors applied video imaging and nuclear magnetic resonance imaging (NMRI) to examine the distribution of flow velocity within the fracture. Results from the experiments showed that flow velocities within the fracture plane ranged over several orders of magnitude.

Preferential flow paths can arise from two different phenomenon: the first being strong variations in local hydraulic properties, which is far more prominent at a large scale. The second mechanism comes from structural and geometric heterogeneities within the fracture, in which fluid flow is concentrated along the tortuous paths with the least hydraulic resistance (Bodin *et al.*, 2003; Auradou, 2009), that is, large aperture regions.

Channelization has some very important implications. It suggests that solutes and particles being transported through the fractured medium have far less exposure to the rock matrix than they typically would in a smooth-walled parallel fracture plane (Bodin, *et al.*, 2003). This would essentially limit the potential for reaction, adsorption, and diffusion into the matrix. Applying this ideology to groundwater contamination suggests that fractured media does little to retard and contain the transport of contaminants.

2.3 Solute Transport in Single, Saturated Fractures

Because flow and transport are two phenomena that are highly intertwined, the concepts, ideas and issues discussed in Section 2.2 are a pivotal foundation for understanding the processes involved in solute transport through fractured media. In short, transport is the consequence of: (1) advection of the solute in the fracture plane, (2) hydrodynamic dispersion derived from the local velocity variations with respect to the average velocity, (3) diffusion of the solute within the fracture plane and into the rock matrix and (4) physio-chemical interactions between the solute

and the rock matrix (Bodin *et al.*, 2003). In general, transport can be divided into two primary components: conservative and reactive transport.

2.3.1 Dispersion

Dispersion describes both the spatial and temporal spread of a mass introduced to a flow field. Dispersion is highly influenced by three mechanisms: (1) molecular diffusion, including matrix diffusion, (2) hydrodynamic dispersion, and (3) retention and/or sorption (Bodin *et al.*, 2003), but is also affected by the heterogeneities in velocities caused by complex fracture geometries and channeling. The spreading of mass in a flow field has been well described in unconsolidated porous media because one mechanism usually dominates over the others. For example, at the pore scale, molecular diffusion is dominant, while at a local scale (1-10m), hydrodynamic dispersion caused by advection is most significant. Still yet, at the macroscale (>10 m), it is typical that local variations in hydraulic conductivity due to heterogeneities are responsible for the dispersion of mass.

However, dispersion within fractured media is far more complex. Shapiro and Hsieh (1998), for example, ran slug tests in a single fracture formation and found transmissivity to vary over 7 orders of magnitude. The importance of this lies in the fact that transmissivity can vary over the course of millimeters within the flow regime. Such variations are synonymous to changes found in hydraulic conductivity within a fractured system, suggesting that flow velocities are also highly variable (Becker, 2004). It has been found that within fractured media, dispersion mechanisms important at one location could be negligible in the close surrounding

areas. Furthermore, studies have shown that coupling of two or more dispersion mechanisms can lead to unpredictable transport behaviours (Becker, 2004). Wood *et al.* (2004) discovered that diffusion coupled with advection in a fracture network led to radon transport far beyond what would have been expected if only one mechanism dominated.

The spreading of mass in a flow field is typically described by the advection-dispersion equation (ADE), which is comprised of terms describing both molecular diffusion and hydrodynamic advection (Freeze and Cherry, 1979):

$$D_L = D^* + \alpha_L v \text{ and } D_T = D^* + \alpha_T v \quad (11)$$

where, D^* is the molecular diffusion coefficient, v is the average linear velocity, D_L , and D_T are the coefficients of longitudinal and transverse dispersion, and α_L and α_T are the longitudinal and transverse hydrodynamic dispersivities. Applying equation (11) requires the assumption of Fickian transport. A review provided by Berkowitz (2002), however, indicated that the temporal migration of a plume in fractured media cannot be characterized by an invariable center of mass and constant dispersion coefficients. On the contrary, dispersive transport has been shown to change as a function of time and/or distance traveled by the mass. In essence, such scale-dependant transport can be referred to as “non-Fickian”, thus rendering the application of the ADE to fractured media as precarious.

The complex and non-Fickian behaviour of fractured media has been well documented in literature. Unlike the typical S-shaped breakthrough curves (of concentration versus time) generated by homogenous porous media, conservative solute transport experiments in fractured media have been known to produce breakthrough curves that exhibit rapid initial arrival times,

multiple peaks in relative concentration, and long tails (Becker and Shapiro, 2000). Caution must therefore be taken when applying the ADE to fractured media.

2.3.1.1. Molecular Diffusion

Diffusion is the most straightforward dispersion mechanism to analyze and quantify, as it is entirely independent of groundwater velocity. Being the result of the random kinetic motion of “jostling” water molecules, it is in every sense isotropic (Becker, 2004), and because of its independence from advective processes, diffusion occurs at a relatively slow rate. Combining a variety of tracers in a single experiment can isolate the diffusivity of a given molecule. Using tracers that are soluble, non-reactive, and are transported identically via advection, allows the assumption that differences in breakthrough curves are attributed to variations in diffusivity. For example, Garnier *et al.* (1985) ran transport experiments in fractured chalk using fluorescein, iodide, and deuterium as tracers, and found distinct differences in their effluent breakthrough curves. Often, such differences can be attributed to diffusive exchanges with the fracture matrix. Matrix diffusion can be described as a process by which mass is exchanged between the relatively mobile phase within the fracture plane and the immobile phase within the rock matrix (Becker and Shapiro, 2000).

On large scales, diffusion has minimal impact on the transport of mass. This is especially the case in fast moving, homogenous flow fields, in which flow lines are relatively uniform from one location to the next. However, there are times where neighbouring flow lines vary

significantly, and the diffusion of mass from one flow line to another can have a momentous impact on the overall transport (Becker, 2004).

2.3.1.2 Hydrodynamic Dispersion

The term hydrodynamic dispersion is generally used in conjunction with the advection-dispersion equation, describing the spread and transport of mass via molecular diffusion and advection. The hydrodynamic dispersion terms, D_L and D_T , included in the ADE pertains to the spread of mass due to advection along the direction of average linear velocity and perpendicular to the direction of average linear velocity respectively (Freeze and Cherry, 1979). Unlike the isotropic behavior of diffusion, advective dispersion is most prominent parallel to flow.

Noting that advective dispersion comes from the differences in the fluid flow velocity fields in a fractured system (channeling), caused primarily by the complex geometry and variation in apertures of the fractures (Zhao *et al.*, 2010), delineating its effect on transport can be done in multiple ways. First, as mentioned previously, multiple conservative tracers can be used during a single experiment, allowing for the separation of the effects of diffusion versus advective dispersion. The second approach acknowledges the fact that advective dispersion relies on flow velocities while diffusion does not (Becker, 2004). Breakthrough curves gathered from tracer experiments run at different velocities can help compare and contrast advective dispersion within different flow regimes.

2.4 Colloid Transport

Colloid transport differs significantly from the transport of molecular-scale solutes. Experimental studies in fractured systems have shown that the average transport velocity of colloids is greater than that of both molecular-scale solute traces and water molecules (Reimus, 1995; McKay *et al.*, 2000), a concept commonly referred to as differential transport (Zheng *et al.*, 2009).

In the case of fractured media, the Taylor-Aris theory helps explain the variations in transport between colloids and solutes. Referring to Poiseuille flow as described in equation 9, it states that conservative solutes, because of their high molecular diffusion, sample the entire flow field within a fracture. Colloids, however, are prevented from migrating towards the regions nearest to the walls of the fracture because their physical size and/or repulsive forces prevent them from doing so (Zheng *et al.*, 2009). Such size and charge exclusion allows colloids to sample larger pore-water velocities, on average, than water molecules or molecular-scale solutes. In addition to this, colloids that are sized similarly to portions of the aperture region of a fracture experience straining, and are excluded from entering these regions, thus augmenting the average transport velocity of a colloid even further (Gvirtzman and Gorelick, 1991). Similarly, larger colloids are excluded from entering low velocity regions accessible to smaller colloids, leading to the idea that larger colloids experience a greater average transport velocity than smaller colloids (Jin *et al.*, 2000).

2.4.1 Retention and Inactivation

There are a variety of mechanisms that contribute to the retention of a colloid in fractured media, including: filtration, adsorption, and sedimentation (McDowell-Boyer *et al.*, 1986). Filtration, or straining occurs when the size of a colloid exceeds the diameter of an encountered aperture region, disallowing it to enter that portion of the fractured system. McDowell-Boyer, Hunt and Sitar (1986) came to the conclusion that filtration, although prominent in bacterial transport, is negligible in viral transport. Adsorption, or attachment, describes the processes by which particles are attracted to and adhere to a solid surface (Fetter, 1999). Once sorbed onto a fracture wall, a colloid can be released due to changes in several factors, including: changes in the flow regime, temperature, pH, and concentration of surrounding ions. Sedimentation involves the settling of a particle due to lack of buoyancy, and can be described as follows (Happel and Brenner, 1965):

$$L_s = \left(\frac{1}{18\mu} \right) (\rho_p - \rho_f) g d^2 t \quad (11)$$

where, L_s [L] is the length scale of settling, d [L] is the diameter of the particle, ρ_p [M/L³] is the particle density, ρ_f [M/L³] is the fluid density, μ [M/LT] is the fluid dynamic viscosity, g [L/T] is the acceleration due to gravity, and t [T] is a given time interval. When applying equation 11, sedimentation is only significant when either the particle diameter or density is large enough to allow the particle to reach the lower surface of the fracture within the time interval, t . Moreover, sedimentation becomes significant when particles aggregate, forming a much larger pseudo-particle (Becker *et al.*, 1998).

2.5 *E. Coli* Characteristics

E. coli is a gram-negative, facultatively anerobic, straight, rod- shaped bacterium with dimensions of 2-6 μm x 1.1–1.5 μm , and resides singly or in pairs (Bergey *et al.*, 1984). Although most strains are relatively harmless to humans, some have been found to cause severe illnesses (Foppen and Schijven, 2006). Perhaps the most well-known pathogenic form of *E. coli* is the serotype O157:H7.

E. coli attaches to surfaces based on properties such as surface charge, size, hydrophobicity, and the presence of individual surface characteristics such as flagella, fimbriae, and extracellular lipopolysaccharides (LPS) (Gilbert *et al.*, 1991). With respect to retention via attachment, the distribution of LPS is a very important characteristic due to their physical location on the outside of the cell (Foppen and Schijven, 2006). The existence and/or distribution of LPS contributes to the electrophoretic mobility, or zeta-potential of the cell, which according to Gilbert *et al.* (1991) is the primary contributor to the cell's ability to adhere to other surfaces. It is important to note, however, that zeta potential is a poor representation of the interaction between *E. coli* and surrounding surfaces, as the theory behind zeta potential pertains strictly to hard, spherical particles. *E. coli*, on the contrary, is rod-shaped, has a soft cell wall, and contains local variations in surface charge due to the random distribution of LPS (de Kerchove and Elimelech, 2005). The application of zeta-potential to characterize surface interactions between *E.coli* and collector surfaces should therefore be considered suspect.

When introduced to a subsurface water environment, *E. coli* is resilient, and experiences a relatively slow die-off. Factors that influence die-off include: temperature, predation, antagonism,

light, soil type, pH, toxic substances, and dissolved oxygen (Foppen and Schijven, 2006). Multiple experiments reviewed by Foppen and Schijven (2006) revealed that, from one experiment to the next, most of these factors have similar impacts on die-off, despite the differences in experimental setup and aquifer medium.

2.6 Review of Previous Work Conducted on Saturated, Fractured Media

This section provides the foundation upon which the research presented in this thesis was built. Information presented here is primarily based on empirical studies done at both the field and laboratory scale within the field of groundwater and contaminant hydrogeology.

Early studies applied the concepts of pipe flow to fracture flow in order to examine the relationship between the friction factor and Reynolds number in rough-walled fractures (Lomize, 1951; Louis, 1969). Measures of wall roughness were made using a roughness parameter, ϵ , defined as the absolute height of roughness, and dividing it by the fracture diameter, D , which was equivalent to twice the fracture aperture, b . During laminar flow, it was found that when $\epsilon/D \leq 0.033$, total flow obeyed the cubic law. On the contrary, flow rates deviated from the cubic law once $\epsilon/D > 0.033$. In short, these studies concluded that rough-walled fractures with $\epsilon/D < 0.033$ were considered to be relatively smooth.

Several years later, researchers evaluated fluid flow through rough-walled fractures under various degrees of normal stress (Iwai, 1976; Gale *et al.*, 1990). As opposed to using roughened smooth plates, however, experiments were run through natural or induced rough-walled fractures, in which highly variable aperture fields and contact points (asperities) were present. As fracture

samples deviated from the idealized smooth, parallel plates, it was found that flow patterns were wandering further and further from cubic law. Some claimed that the fallacies of the cubic law can be accredited to tortuous and channellized paths in the flow field (Raven and Gale, 1985).

Once the foundation for fluid flow was laid, researchers began to examine the processes involved in particle transport. Through a series of laboratory and field experiments, Harvey *et al.* (1989) found that microbes travelled faster than molecular scale solutes. Expanding on this, Becker *et al.* (1998) used a series of both field and laboratory tracer tests, all of which employed the same colloid tracer. Running experiments at scales that ranged over three orders of magnitude, the mean transport time of the colloid tracers within each experiment was shorter than that of the solute tracers. It was hypothesized that differences in transport time could be attributed to volume exclusion, aggregation, and or settling. Similarly, Cumby and McKay (1999) experimented with fluorescent carboxylate-coated latex microspheres in fractured shale saprolite, finding that particle size plays a major role in controlling transport. They found the optimal particle diameter to be 0.5 μm . Particles larger than the optimum diameter experienced greater retention due to sedimentation and/or physical straining. Particles smaller than 0.5 μm also experienced increased retention, primarily due to their characteristically high rates of diffusion. Faster diffusion can spurn on more frequent collisions with the fracture wall, potentially leading to increased attachment. Furthermore, smaller particles have the potential to diffuse into zones of relatively immobile pore water at which point they become less recoverable.

To evaluate the legitimacy of microspheres, Passmore *et al* (2010) compared and contrasted the transport of polystyrene microspheres with that of *Escherichia coli* RS2g (1.2 μm

in diameter). Results suggested that microspheres with size and surface properties similar to that of *E. coli* RS2g are viable surrogates to delineate potential pathways of transport in the subsurface. However, difficulty lies in the fact that it is not easy to match both the size and zeta potential of a given microorganism, and therefore, compromises must be made when applying microspheres to transport experiments.

Recently, much research has been devoted to furthering the understanding of particle transport in fractured bedrock. Borchardt *et al.* (2007) inspected deep, confined bedrock aquifers for the existence of human enteric viruses. Often, hydrogeologists assume that such aquifers are protected from microbial contamination, as it is commonly believed that the time it would take for a microbe to transport through a series of aquitards is too long, and that microbial survival time is too short for the contaminants to reach the confined aquifer. However, it was discovered that viruses were able to penetrate and/or bypass overlying aquitards and consequently contaminate deep, confined aquifers. Undoubtedly, the understanding of hydrogeology and contaminant transport in fractured media remains limited, as even the most robust microbial transport models based on colloid filtration theory cannot reliably predict the transport of viruses (Borchardt *et al.*, 2007).

In addition to this, Becker *et al.* (2003) evaluated the transport properties of various bacteria and microspheres in fractured bedrock aquifers. Compared to solutes, both the bacteria and microspheres were highly retained within the fractured system, and exhibited earlier arrival times. Additionally, no two breakthrough curves were the same. With a focus on bacterial transport, Becker *et al.* (2003) imply that differences in cell properties, such as size, morphology,

Gram type and motility account for drastic variations in transport efficiency. For example, motile bacteria experienced greater filtration, as it is likely that they had greater opportunities to interact with the fracture wall, and to migrate into stagnant flow regimes. Furthermore, for reasons unexplained by the authors, Gram-negative rod-shaped bacteria were transported more efficiently than Gram-positive bacteria. The authors conclude that it is undeniable that cell properties dictate transport efficiency, and therefore the transport of one bacteria species would be an unreliable predictor of the transport of another. For the very same reasons, Becker *et al.* (2003) conclude that microspheres are also a limited predictor of bacterial transport.

Taylor *et al.* (2004) discussed and evaluated current strategies for protecting the quality of groundwater sources, finding that most regulations are based on the natural attenuation of microorganisms that would occur in areas defined by bulk, macroscopic groundwater flow velocities. However, this approach ignores the rapid influx of pathogenic microorganisms to a wellhead induced by statistically extreme groundwater velocities. It is important to recognize, therefore, that there is limited protection given by source protection measures that ignore the aforementioned phenomenon, especially in regions relying on untreated groundwater supplies.

3. Experimental Design

The hydrodynamics of a given aperture field are highly governed by fracture wall roughness. Roughness, and ultimately aperture field variability, defines the existence of preferential flow paths (Rasmuson and Neretnieks, 1986), the velocity distribution across the aperture (Dijk *et al.*, 1999; Brush and Thomson, 2003), and the mobile and stagnant regions within the aperture field (Dijk *et al.*, 1999). Combined, these hydrodynamic properties govern the transport of both solutes and colloids through fractures. Furthermore, aperture variability may contribute to the development of hydrodynamic shear (Degueldre *et al.*, 1989; Sharma *et al.*, 1992), and thus the formation of eddies and localized turbulent flow near the fracture walls (Dijk *et al.*, 1999; Brush and Thomson, 2003), which may contribute to attachment and detachment.

The experimental portion of this research is aimed at developing a mechanistic understanding of the influence of hydrodynamics on the transport and retention of colloids in fractures. This will be achieved by injecting a known number of *E. coli* RS2GFP or polystyrene microspheres into dolomitic fracture samples under a range of specific discharges (30, 10 and 5 m/day), and analyzing, both quantitatively and qualitatively, the resulting effluent concentration profiles. Comparing each concentration profile will help isolate the effects of hydrodynamics on particle transport. This chapter explains the methodology used to set up and characterize the fracture planes for these experiments, as well as the techniques used to quantify the effluent concentration profiles derived from each test.

3.1 Fracture Sample Preparation

Laboratory-scale experiments were performed using two unique dolostone fracture samples, each of which were acquired from the DoLime Quarry in Guelph, Ontario and were returned to a laboratory at McMaster University. Selection of the rock samples was based on the prominence of stylolites and or bedding planes, as these features represent planes of weakness, and therefore facilitate the induction of fractures. Once returned to the laboratory, the rock samples were cut into rectangular prisms using a diamond blade on a STIHL Cutquik® saw, in order to define the plane of weakness and enhance the maneuverability of the samples. Hydrostone cement was then poured onto the portions of the rock not smoothed by the Cutquik® saw to form a smooth surface, and facilitate the attachment of a fiberglass reinforced polymer (FRP). FRP was used to reinforce the sample in all directions other than that of the plane of weakness, so that when the fracture was induced, it followed the plane of weakness. Similar to method used in Reitsma and Keuper (1994), a uniaxial force was applied to each sample, inducing a fracture along the plane of weakness. As shown in Figure 3-1, triangular metal bars extending the length of the rock samples were fabricated to channel the uniaxial force along the plane of weakness, and metal bands were wrapped around the samples to ensure they would not open during the fracturing process. The fracturing technique applied in the laboratory mimics the in situ stress relief fractures that bedrock experiences due to excessive tension or compression. Two rock samples were successfully fractured using the aforementioned techniques and their dimensions are included in Table 3-1.

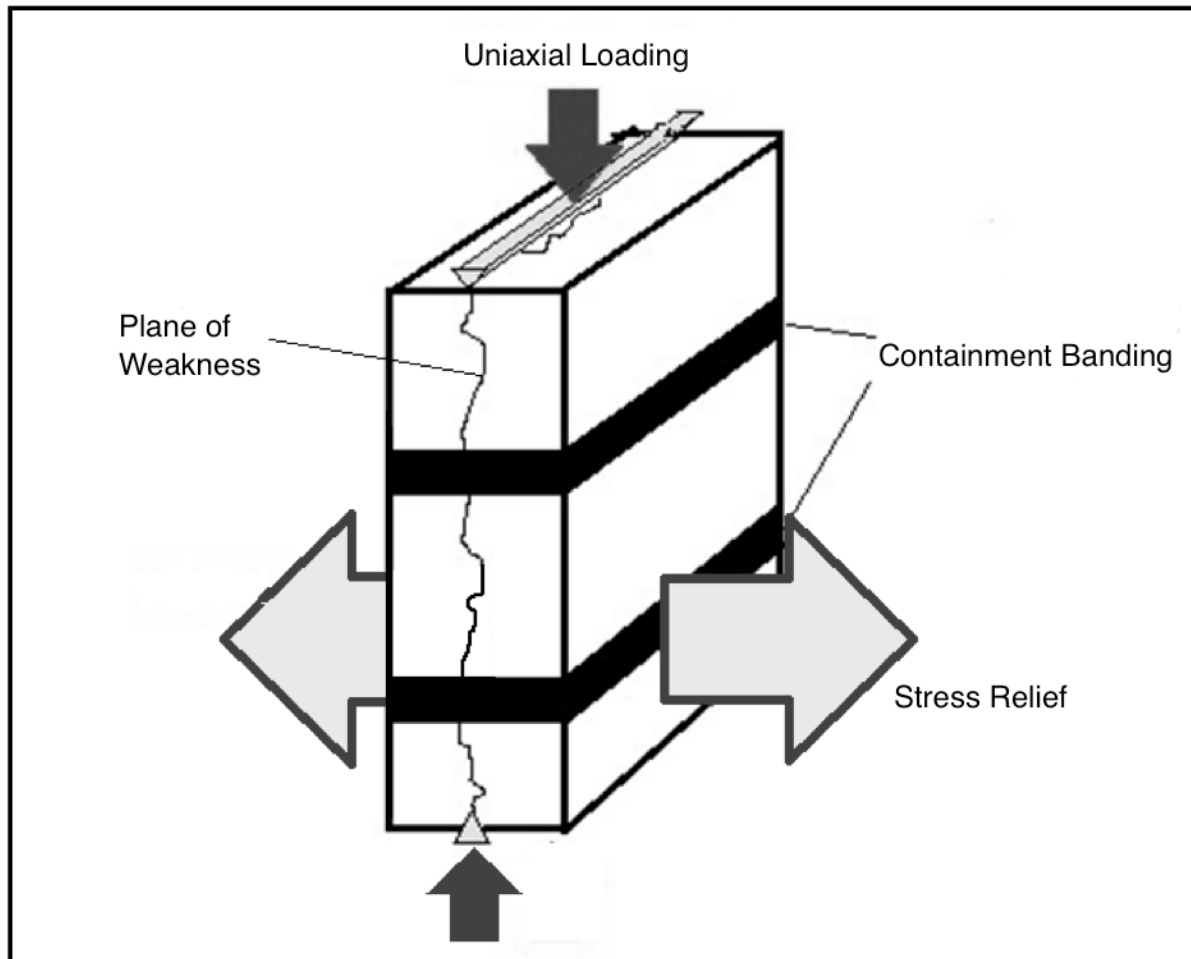


Figure 3-1: Induction of fractures

Table 3-1: Fracture Sample Properties

Property	Fracture 1 (F1)	Fracture 2 (F2)
Length (cm)	41	49
Width (cm)	31	30
Volume of influent recirculation system (mL)	224.26	211.61
Volume of effluent recirculation system (mL)	224.05	175.16

3.2 Experimental Setup

3.2.1 Hydraulic Tests

The fractured samples were setup in such a way that flow ran parallel to the two longer sides. Each of these longer sides was sealed with 100% silicone (GE Silicone II 100% Silicone Rubber Caulk) to create no-flow boundaries. It was imperative that the two fracture walls were retained in their original position throughout all the experiments as studies have shown that even a displacement of 0.5 mm of one wall relative to the other can alter the hydraulic conductivity by up to five orders of magnitude (Durham and Bonner, 1994). Plexiglass end caps, which served as constant pressure boundaries, were placed on the two shorter ends of the sample, sealing the remainder of the fracture plane. Six ports were drilled into each end cap. Each port was equipped

with a Swagelock fitting, which allowed various forms of tubing to feed water to and from the end cap without any leakage. Any unused ports were fitted with PVC tubing (Nalgene 380 PVC) and were clamped shut.

Each hydraulic test involved the injection of water through the fracture plane using a peristaltic pump (Masterflex, L/S 7523-70), and the exit of water through a constant-head outlet port located at the downstream portion of the fracture. A tipping bucket rain gauge (Davis, Rain Collector II) was used to collect water from the constant-head outlet and measure the volumetric flow rate. The tipping bucket was wired to a data logger (Lakewood Systems Ltd., UL16 GC), which recorded the number of tips within a given time interval, and thus ensured and verified that there were no changes to the system throughout the duration of an experiment. The rain gauge was calibrated regularly throughout the course of these experiments. Inclined piezometers, as shown in Figure 3-2, were installed at both the upstream and downstream ends of the fracture, allowing the measurements of head loss throughout the duration of the experiments.

Prior to the injection of water, the fracture plane was flushed with carbon dioxide to remove any gas of atmospheric composition, after which prepared water was immediately injected. Carbon dioxide, because it is highly soluble in water, was completely dissolved once water was injected into the fracture plane, thus minimizing the development of a gas phase. Milli Q water was used for all experiments. Milli Q water was stored in 20L carboys and was left to equilibrate to room temperature. The prepared water was frequently degassed using nitrogen, which, along with the carbon dioxide flushing of the fracture plane, helped minimize the

development of a gaseous phase. Once prepared, water was injected into the fracture plane at a very slow flow rate to prevent an excessive build up of pressure.

After saturation was complete, prepared Milli Q water was pumped through the fracture plane at a constant flow rate, and the resulting hydraulic gradient across the fracture plane was measured. These tests were executed under a range of flow rates, enabling the delineation of the relationship between flow rate and head loss. It is important to note that hydraulic tests were regularly performed throughout the duration of the experimental portion of this research to ensure that all properties of the fracture plane remained unchanged.

3.2.2 Solute Tracer Tests

The experimental setup for the solute tracer tests involved two modifications made to the setup used for the hydraulic tests described in Section 3.2.1. First, once it was known that equilibrium with respect to the flow rate was achieved, access to the piezometers was cut off to minimize the loss of solute mass. Second, a recirculation system was added to each end cap, ensuring that all contents entering and exiting the fracture were perfectly mixed. Additionally, a vial facilitating the injection and withdrawal of samples was installed on each circulation system.

The recirculation system is comprised of perforated nylon tubing that was passed through two ports on opposite ends of each end cap. The nylon tubing was perforated every 4 mm, and the end of each tube was attached to pump tubing (MasterFlex, L/S 16) to form a closed-loop system, allowing contents injected in the sampling vial to mix with the contents held in the end cap. With the feed pump turned off, Rhodamine dye was injected into the influent recirculation

system to determine the amount of time required to attain a perfectly mixed solution in the end cap. Figure 3-2 displays the experimental setup for the solute tracer tests.

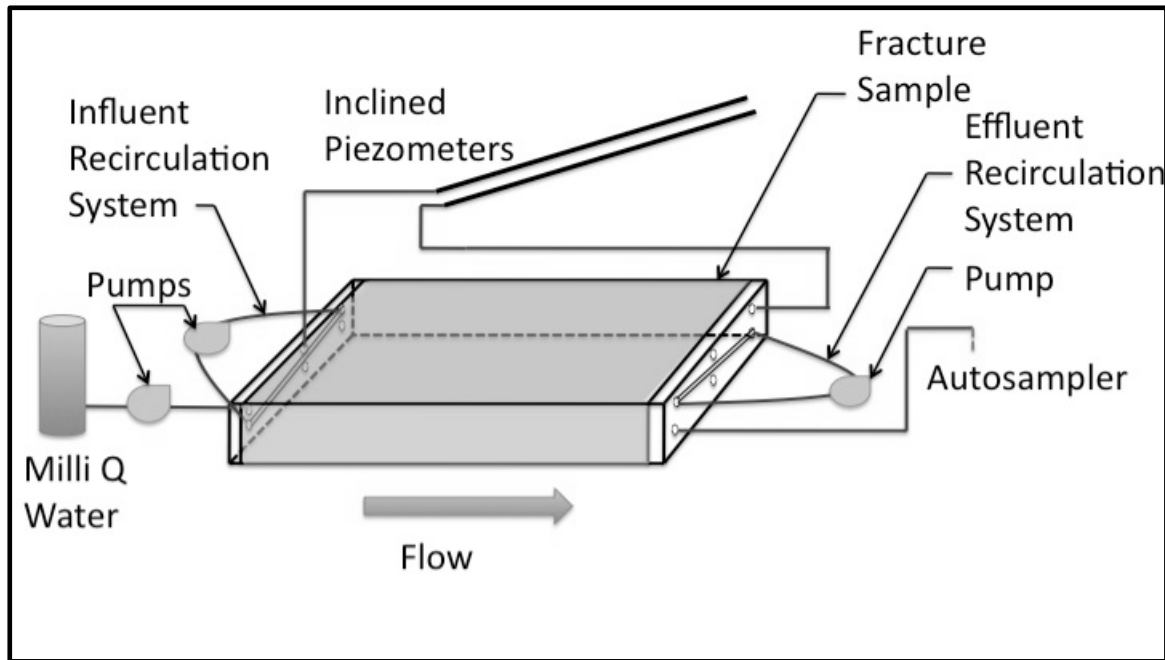


Figure 3-2: Experimental Setup

Prior to the commencement of a solute tracer test, prepared Milli Q water, as described in Section 3.2.1, was pumped through the fracture plane for 12-15 hours at a relatively high flow rate. This ensured that the fracture plane was both saturated and flushed of all constituents from previous experiments. Following this, the feed pump was turned off and a 3 mL syringe (BD 3 mL syringe) was used to inject 3 mL of a 7.29 mg/L Br^- solution into the influent sampling vial. Once the amount of time required to perfectly mix the end cap was attained, a sample was taken from the influent sampling vial to measure the initial concentration. Additionally, a sample was

taken from the effluent end cap to measure the possible background concentration of Br^- . The feed pump was then turned on, initiating a pulse input of mass, and marking the beginning of the solute tracer test.

Samples were strategically collected from the effluent end cap using a Foxy® 200 fractionator, such that small sampling intervals at the beginning of the test captured the characteristics of the peak of the breakthrough curve, while larger sampling intervals towards the end of the test described the tail of the curve. To account for the lag time between the effluent end cap and the fractionator, Rhodamine dye was injected into the effluent end cap, and once perfectly mixed, the time required for the dye to be transported from the end cap to the fractionator was measured. This was done for both fractures at each of the three flow rates. Bromide concentrations were analyzed using high-pressure liquid chromatography (HPLC), as described in Section 4.1.

The purpose of the solute tracer tests was to measure the mass balance and frictional loss apertures. Bromide was selected as an appropriate tracer due to its conservative nature, as well as the ease by which it is quantified. To verify the validity of the tracer tests, mass balance calculations were executed, ensuring that $m\text{Br}^-_{\text{in}} \approx m\text{Br}^-_{\text{out}}$.

3.2.3 *E. coli* RS2GFP Tracer Tests

E. coli RS2GFP, a nonpathogenic strain of *E. coli*, was used in the biocolloid tracer experiments. This strain of *E. coli*, derived from the RS1 strain, possesses an attached green fluorescent protein (GFP) that allows the bacteria to fluoresce when activated by ultraviolet light.

This is ideal for enumerating the bacteria, as well as the visualization experiments conducted in transparent epoxy fractures. *E. coli* RS2GFP is also resistant to two antibiotics, rifampicin and kanamycin, thus reducing the possibility of contamination in a laboratory setting. The *E. coli* RS2GFP used in the biocolloid tracer tests was acquired from the Emelko Laboratory in the Department of Civil Engineering at the University of Waterloo. The culture used at the University of Waterloo was initially grown by Dr. Larry Halverson from the Department of Agriculture and Biosystems Engineering at Iowa State University, Ames, IA, USA.

The setup for the *E. coli* tracer test was identical to that employed for the solute tracer tests, and is illustrated in Figure 3-2. Prior to the commencement of the *E. coli* tracer tests, the fracture plane was saturated and flushed at a relatively high flow rate with a degassed 1% phosphate buffer saline (PBS) for 12-15 hours. Flushing, as mentioned in Section 3.2.2, ensures that all constituents from previous experiments are removed from the fracture plane. The feed pump was then turned off, and 1 mL of an approximately 10^7 CFU/mL *E. coli* RS2GFP suspension was injected into the influent recirculation system. The suspension was left to mix according to the time derived from the rhodamine dye tests required for perfect mixing. Once the *E. coli* suspension was fully mixed in the influent end cap, the feed pump was turned on, marking the commencement of the *E. coli* tracer test. A sample was then taken from the effluent sampling vial to determine if any background concentration of *E. coli* was present. Consequent samples were gathered in a similar manner to that described in section 3.2.2. Once collected, the samples were serially diluted in a 1% PBS solution to ensure a countable concentration, and then plated on an agar gel. The enumeration of *E. coli* RS2GFP is described in Sections 4.2 and 4.3.

3.2.4 Microsphere Tracer Tests

Carboxylate modified yellow-green polystyrene (CMP) microspheres, with an excitation/emission maxima of 441/486 nm, were used as a colloid tracer. These microspheres are a desirable colloid tracer as they are nearly spherical in shape, are monodisperse in diameter, resistant to biodegradation, and are stable at any temperature below 100 °C (Becker *et al.*, 1998). The surface of these microspheres are coated with a hydrophilic polymer that contains a variety of carboxylic acids, thus giving them a negative charge when placed in a solution with pH<5. The CMP microspheres used in these experiments were acquired from Polysciences Inc., Warrington, PA, and were 0.05 µm in diameter, thus mimicking the size of a typical virus. This is of great importance, as particles this size are not subject to micro scale hydrodynamic forces, and are therefore transported differently.

The experimental setup for the microsphere tracer tests was virtually identical to that of the solute tracer tests, as illustrated in Figure 3-2. To ensure the microspheres maintained their fluorescence for the duration of the experiment, a dark shelter was constructed over the experimental setup, minimizing the intrusion of light. Prior to injection, the stock of microspheres was sonicated for two minutes, to ensure a monodisperse state. A known number of microspheres were then injected into the influent recirculation system, and were left to mix. Once a perfectly mixed solution was obtained within the influent end cap, the feed pump was turned on, marking the commencement of the tracer test. Samples were collected using the Foxy® 200 fractionator, and because of the bright fluorescent dye contained within each microspheres, they were conveniently analyzed using a Fluorescence Spectrophotometer (Cary Eclipse, Varian).

Samples were analyzed within 24 hours of being collected to ensure the microspheres did not lose any fluorescence.

4. Analytical Techniques

4.1 List of Equipment

Fractionator

A Foxy® 200 fractionator was used to automatically collect samples from the tracer tests. Time intervals were input manually to suit the sampling required for each test.

High Pressure Liquid Chromatography (HPLC)

High-pressure liquid chromatography (HPLC) (Varian ProStar 330) was applied to quantify the bromide samples derived from the solute tracer tests. The HPLC equipment was comprised of an auto sampler (Varian, 410), a solvent delivery module (Varian, 230), and a conductivity detector (Dionex, CD25). A guard column (Dionex AGI2A) and a 4 x 200 mm column (Dionex, ASI2A) with an anion suppressor (Dionex, AMMSIII 4mm) were used to separate the bromide solution.

Fluorescence Spectrophotometer

A fluorescence spectrophotometer (Cary Eclipse, Varian) was employed to analyze the concentration of carboxylate-modified microspheres in samples collected from the colloid tracer tests.

Spectrophotometer

A spectrophotometer (Beckman Coulter DU 530) was used to measure the optical density of the *E. coli* RS2GFP suspension. Both the blank and bacterial suspensions were prepared using the same broth, and were evaluated at a wavelength of 520 nm.

Autoclave

The autoclave used was a Hirayama Hiclave HV-50. The sterilization of both solids and liquids was achieved at 121°C and at a pressure of 20 kPa for approximately 60 min.

Centrifuge

A Beckman Coulter Allegra 25R centrifuge was used to isolate a pellet of *E. coli* from suspension. Optimal results involved running the centrifuge for ten minutes at 8500 rpm (5000g).

Incubator

A VWR 1575R incubator, set to a temperature of 37°C, and a rotational speed of 180 RPM, was used to culture the *E. coli* RS2GFP.

Colony Counters

A Stuart SC6 colony counter enhanced the precision by which colonies of *E. coli* RS2GFP were counted.

4.2 List of Reagents

Eluent for HPLC

The eluent solution for the HPLC was made up of 0.3 mM NaHCO_3 and 2.7mM Na_2CO_3 .

Stocks of each component comprised of the following:

NaHCO_3 stock:

- 21 g Sodium Bicarbonate (NaHCO_3),
- 500 mL Milli-Q water

Na_2CO_3 stock:

- 53g Sodium Carbonate (Na_2CO_3)
- 500 mL Milli-Q water

The eluent was then prepared by mixing 2.4 mL of the NaHCO_3 stock and 21.6 mL Na_2CO_3 stock with 4 L of Milli-Q water.

Regenerant for HPLC

The regenerant used for the chemical suppressor was a 12.5 mM H_2SO_4 solution which was comprised of the following:

- 2.8 mL Sulfuric Acid (H_2SO_4)
- 4 L Milli -Q water

PBS Stock Solution

A 1% PBS solution was used as the liquid medium in the bacteria tracer tests. Additionally, *E. coli* RS2GFP samples were serially diluted using a 1% PBS solution. The 1% PBS solution was derived from a large stock of 10% PBS solution that was made prior to running the bacteria tracer tests.

The recipe for the 10% PBS solution is as follows:

- 80.0g Sodium Chloride (NaCl)
- 2.0g Potassium Chloride (KCl)
- 14.4g Sodium Phosphate (Na_2HPO_4)
- 2.4g Potassium Dihydrogen Phosphate (KH_2PO_4)
- 1 L Milli-Q water

The pH of this solution was adjusted to approximately 7.4 using NaOH.

Antibiotics

Preparation of the antibiotics involved the following:

- Rifampicin: 0.1 g of Rifampicin was added to 100 mL of methanol, and stored at 4°C in an amber bottle.
- Kanamycin: 1 g of kanamycin was added to 100 mL of Milli Q water, filter sterilized using a 0.22 μm filter, dispensed into 5 mL bottles, and stored at -80°C.

Broth

Using an Erlenmeyer flask, the broth was prepared as follows:

- 10 g HiVeg Hydrolysate (HIMEDIA RM030v-500G)
- 5 g Yeast Extract (Bacto BD 212750)
- 10 g Sodium Chloride (NaCl)
- 1L Milli-Q water

The mixture was autoclaved, and after cooling, 10 mL of both Rifampicin and Kanamycin were added to the broth. This helped minimize the development of competitors of *E. coli* RS2GFP within the broth.

Luria-Bertani (LB) Agar

Preparation of the agar was identical to that of the broth, other than the fact that 15 g of Agar (Bioshop AGR003.500) was also added to the broth mixture.

4.3 Bromide Quantification

Using high-pressure liquid chromatography, bromide was quantified based on methods detailed by Motter and Jones (2008). In short, the process is as follows: samples derived from the solute tracer tests are injected into a stream of carbonate/bicarbonate eluent, are brought through a selection of ion exchange columns, and are then sent to a conductivity detector. The two

columns involved in this process are a guard column and an analytical column. The guard column is responsible for protecting the analytical column from the invasion of particulate and organic matter. The analytical column is set up in a way that it separates anions based on their relative affinities for a low-capacity, strongly basic anion exchanger (Motter and Jones, 2008). A suppressor, located along the stream from the analytical column to the conductivity detector, ensures a continuous suppression of background conductivity of the eluent, and thus, by means of acidification, enhances the response of the analytes that are being targeted. Once separated, the conductivity detector measures anions in their acidified form. They are identified based on their retention times within the column, and are quantified based on their response relative to the standard response of the eluent (Motter and Jones, 2008). The conductivity detector is connected to the Varian Star Chromatography Workstation Version 6.20 software, which is capable of plotting a chromatogram that details the exact response of the targeted analyte.

In order to accurately quantify bromide concentrations in the solute tracer test samples, it is imperative to generate a standard curve. This was achieved by diluting a stock of 100 mg/L Br₂ down to 50 mg/L, 25 mg/L, 10 mg/L, 5 mg/L and 1 mg/L, and measuring each of their responses. Standard curves were deemed to be acceptable when R^2 was greater than 0.99. A sample standard curve is included in Appendix A.

4.4 Enumeration of *E. coli* RS2GFP

4.4.1 Inoculation

The first step in the inoculation process involved the preparation of *E. coli* RS2GFP for long-term storage. First, 1 mL of the stock *E. coli* RS2GFP (from the University of Waterloo) was added to 100 mL of broth in a 250 mL Erlenmeyer flask. To encourage bacterial growth, the inoculated broth was placed in an incubator at 37°C while being stirred at 180 rpm. After approximately 3 hours, the optical density was measured on an hourly basis using a spectrophotometer at a wavelength of 520 nm. Broth not inoculated by bacteria was used as a blank. The desired culture was to contain roughly 10^8 coliform forming units (CFU)/mL in the log growth stage, which corresponds to an absorbency of 0.5 units on the spectrophotometer. Once a reading of 0.5 units was acquired, the culture was left to cool, after which 10% (v/v) sterilized glycerol was added and thoroughly mixed. The culture was then distributed into 2 mL cryogenic vials, and stored at -80°C until required for an experiment.

Preparing *E. coli* RS2GFP for a tracer experiment followed similar steps to preparing it for long-term storage. First, a 2 mL cryogenic vial of *E. coli* RS2GFP was thawed, and added to 125 mL of broth in an Erlenmeyer flask. The culture was then placed in an incubator at 37°C while being stirred at 180 rpm, and was left for 9-12 hours. After 8 hours, the optical density was measured at a wavelength of 520 nm, ensuring that it fell somewhere between 0.5 and 0.9 units. Once the ideal optical density was achieved, the culture was centrifuged at 5000 g, leaving a pellet of *E. coli* RS2GFP. The broth was decanted, and the pellet was re-suspended in an

autoclaved 1% PBS solution. The *E. coli* RS2GFP was “washed” another two times with a 1% PBS solution in the centrifuge. After the final washing, the bacteria were suspended in approximately 25 mL of PBS, and stored at 4°C. The survival of *E. coli* RS2GFP in a 1% PBS solution was evaluated and it was found that no significant die off occurred within the first week of it being made.

4.4.2 Enumeration Techniques

Immediately after the washing process, the *E. coli* RS2GFP suspension was serially diluted using a sterile 1% PBS solution. The dilutions from 10^{-6} – 10^{-8} were plated in triplicate on agar in a petri dish, and were placed in the incubator at 37°C. After 24 hours, the plates were taken out of the incubator, and CFUs were counted. Once averaged, the three plates revealed the number of CFU/mL in the *E. coli* RS2GFP suspension. Details on plating techniques are discussed below.

Samples gathered from the *E. coli* tracer tests were serially diluted to concentrations of 10^{-2} , 10^{-3} , and 10^{-4} in a 1% PBS solution. Accounting for three orders of magnitude ensured that each sample was countable. Each dilution was done in triplicate to satisfy statistical significance. Once the dilutions were prepared, 0.1 mL from each was spread, using sterile techniques, on a petri dish containing agar. Samples were left to dry, and were then incubated at 37°C. After 24 hours, the plates were removed from the incubator, and the number of CFUs on each plate was counted. This revealed the concentration of *E. coli* RS2GFP within each of the samples gathered from the tracer test. Typically, the range of countable colonies on a plate is 30-300 (Standard

Method 9215A, APHA *et al.* 2006). However, Emelko *et al.* (2008) evaluated organism counts ranging from 1 to 1000, and found that counts as low as 10 do not increase the uncertainty. Therefore, counts ranging from 10-300 were deemed acceptable.

It is important to note that there are significant limitations to the aforementioned plating technique. Random errors in sample collection, sample processing and sample counting all contribute to biased results. For example, random losses (or gains) of bacteria during the serial dilution processes were virtually inevitable due to imperfectly mixed dilutions. Another source of error includes under or over counting colonies, and/or misidentifying a CFU, causing repeated counts of a given dilution to be inconsistent (Schmidt *et al.*, 2010).

4.5 Enumeration of Carboxylate Modified Polystyrene Microspheres

The stock of microspheres acquired from Polysciences Incorporated contained 3.64×10^{14} microspheres/mL. Knowing this concentration exceeded the detection limit of the fluorescence spectrophotometer, the stock was sonicated for two minutes, and serially diluted down to 3.64×10^{11} microspheres/mL. Various dilutions were then extracted from the original series of serial dilutions, facilitating the development of a standard curve, which can be found in Figure 4-1. This curve portrays a range of concentrations detectable by the fluorescence spectrophotometer, and thus influenced the number of microspheres injected into the fracture plane during the colloid tracer tests.

Samples collected from the colloid tracer tests were measured in triplicate using the fluorescence spectrophotometer. The response of each sample was converted to a concentration using the standard curve portrayed in Figure 4-1.

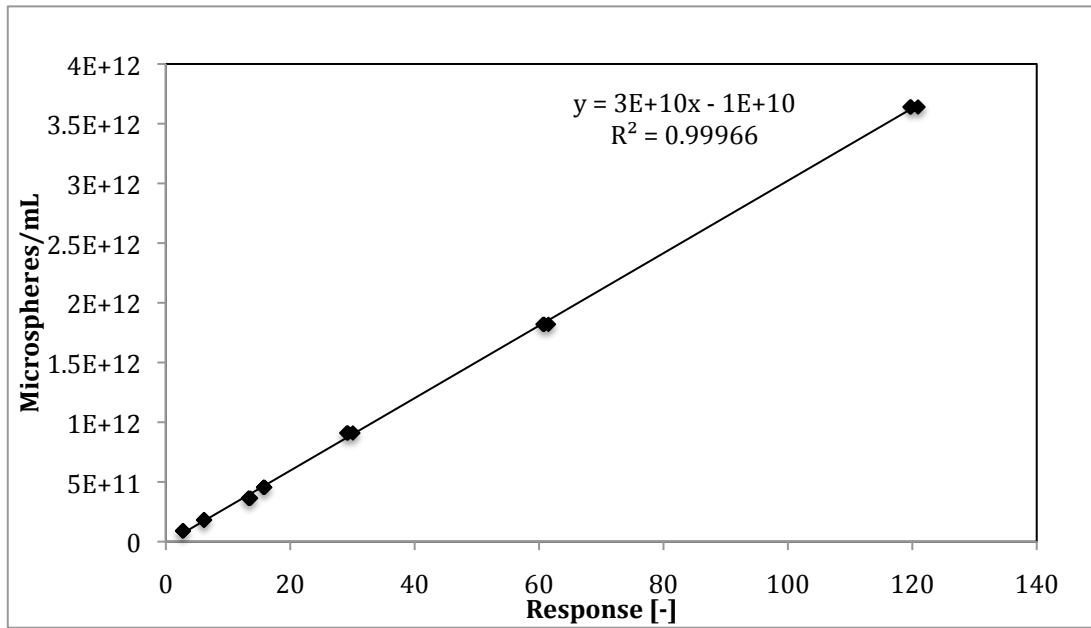


Figure 4-1: Microspheres standard curve.

5. Results and Discussion

This chapter presents and discusses the results of the experiments used to evaluate hydrodynamics within saturated fractured media. Included within this chapter are results from hydraulic tests, and solute, and particulate tracer experiments. These experiments, when analyzed in tandem, draw out the effects of hydrodynamics on particulate transport in saturated fractures. Two dolomitic limestone fracture samples were employed in these experiments.

5.1 Characterization of the Aperture Field

Hydraulic and solute tracer tests were employed to evaluate the characteristics of each aperture field. Three different equivalent apertures for each fracture plane were derived from a combination of these tests, each providing unique information about the aperture field, and thus information regarding the variability of the aperture field. The equivalent apertures used to characterize the aperture field are the hydraulic (b_h), mass balance (b_m), and frictional loss (b_f) apertures.

5.1.1 Hydraulic Tests

Hydraulic tests were used to determine the hydraulic aperture of each fracture plane, and helped ensure, based on a linear relationship between head loss and specific discharge, a laminar flow regime was maintained throughout all experiments. The flow rates applied in the hydraulic tests ranged over an order of magnitude to ensure that the specific discharges selected for the solute and bacteria tracer tests were well within the laminar range, and not on the border of

turbulent flow. The cubic law is not valid in the turbulent flow regime. The hydraulic aperture was calculated using the cubic law, as described by Equation 6 in Section 2.1.1. The measured hydraulic aperture of each fracture sample is included in Table 5-2.

Figure 5-1 shows the results of the hydraulic tests, and displays the inherent linear relationship between specific discharge, as defined by the hydraulic aperture, and head loss. Linearity of this relationship indicates a laminar flow regime, and thus validates the application of the cubic law for estimating the hydraulic aperture. To verify the existence of laminar flow, Reynolds number [-] was calculated as follows:

$$Re = \frac{\rho q b_h}{\mu} \quad (12)$$

Re fell well below the critical value of 1 at all specific discharges for both fracture samples, reinforcing the conclusion that a laminar flow regime truly does exist.

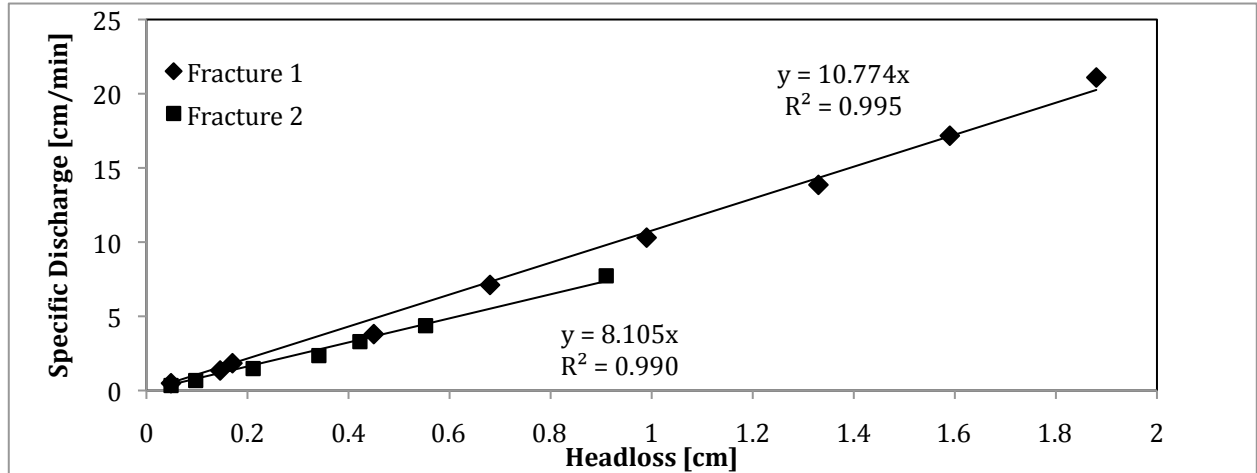


Figure 5-1 Head loss versus Specific Discharge

5.1.2 Solute Tracer Tests

Solute tracer tests were carried out on each fracture sample at three different specific discharges: 30 m/day, 10 m/day, and 5 m/day. The specific discharges selected for this research attempted to represent the typical velocities of natural fractured systems. Bromide, in the form of sodium bromide, was the conservative solute tracer used to determine both the mass balance, b_m , and frictional loss, b_f , equivalent apertures. The way in which each of these equivalent apertures was calculated is described in detail in Section 2.1.1. The calculation of e_f requires the determination of the mean residence time of the tracer within the fracture plane. According to Fahim and Wakao (1982), mean residence time is defined by the following:

$$t_m = \frac{\int_0^t C(t)_{out} \cdot t dt}{\int_0^t C(t)_{out} \cdot dt} - \frac{\int_0^t C(t)_{in} \cdot t dt}{\int_0^t C(t)_{in} \cdot dt} \quad (13)$$

Equation (14) was approximated as follows:

$$t_m = \sum t \cdot E(t) \cdot \Delta t \quad (14a)$$

where,

$$E(t) = \frac{C_{out}(t)}{\sum C_{out}(t) \cdot \Delta t} - \frac{C_{in}(t)}{\sum C_{in}(t) \cdot \Delta t} \quad (14b)$$

where, C_{out} [M/L³] is the concentration of tracer exiting the fracture plane at time, t [T], C_{in} [M/L³] is the concentration of tracer entering the fracture plane at time t , and Δt [T] defines the time interval between sampling events. Research conducted by Zheng (2008) shows that the concentration of tracer within the influent circulation system decreases exponentially, and can be modeled as follows:

$$C_{in}(t) = \frac{M_{Br}}{V_{recirc}} \cdot EXP\left(\frac{-Qt}{V_{recirc}}\right) \quad (15)$$

where M_{Br} [M] is the initial mass of bromide tracer injected into the influent recirculation system, and V_{recirc} [L³] is the volume of the influent recirculation system.

Because the tracer exiting the fracture plane was instantly diluted upon entering the effluent recirculation system, samples collected by the fractionator were not representative of the actual concentration of bromide exiting the fracture. To account for this dilution, a mass balance

was conducted on the effluent recirculation system, which was modeled as a continuous flow mixed reactor (CFMR). This was done as follows:

$$C_{eff-frac}(t) = \frac{V_{recirc}}{Q} \cdot \frac{\Delta C_{meas}(t)}{\Delta t} + C_{meas}(t) \quad (16a)$$

and can be approximated as

$$C_{eff-frac}(t) = \frac{V_{recirc}}{Q} \cdot \frac{C_{meas}^t - C_{meas}^{t-\Delta t}}{\Delta t} + C_{meas}^t \quad (16b)$$

in which $C_{eff-frac}(t)$ [M/L³] represents the concentration of tracer exiting the fracture plane at time t , V_{recirc} [L³] is the volume of the effluent recirculation system, and C_{meas} [L³] is the measured concentration of tracer in the effluent recirculation system.

Figure 5-2 compares the breakthrough curve of the measured concentration of bromide to that of the back-calculated concentration for F2 at a specific discharge of 30 m/d. It is evident that the peaks of the back-calculated breakthrough curves are larger, and appear sooner than those of the actual measured breakthrough curve. This is simply due to the fact that there is both a dilution and lag factor introduced by the recirculation system. The error introduced through back-calculating can be seen in the increased noise in the back-calculated breakthrough curves relative to the measured breakthrough curves. The remaining breakthrough curves resulting from the bromide tracer tests can be found in Appendix A.

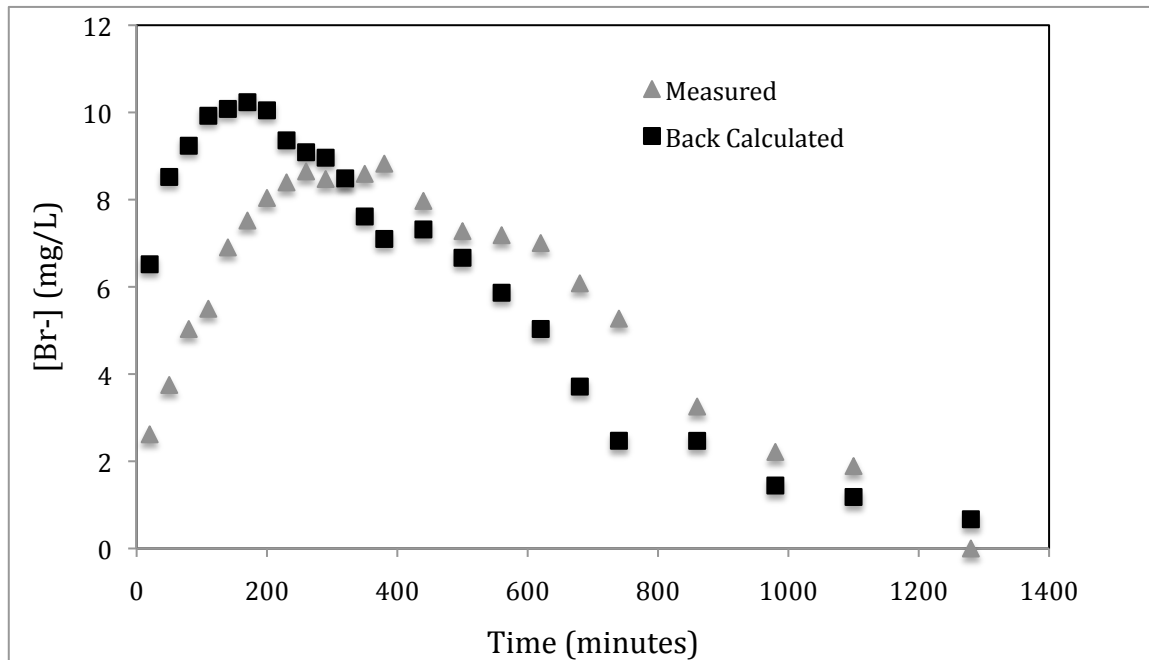


Figure 5-2: Measured and back-calculated effluent concentration profiles for F2 ($q = 30$ m/day)

Limitations to the aforementioned back-calculating methods also manifest themselves in measures of percent recovery of mass. Being a conservative tracer, and operating within a closed system, approximately 100% of bromide should be recovered once injected into the flow field. Statistics displayed in Table 5-1, however, show that percent recovery ranged from 76.35% to 103.02%. The deviation from 100% recovery can be explained in several ways. Reasons for percent recovery to exceed 100% include: (1) background concentrations of Br⁻ from previous tests contribute to an addition of mass not accounted for during the initial injection; (2) quantification errors, including error in HPLC quantification method as well as error in the integration of breakthrough curves using the trapezoid method. It is important to note, however,

Table 5-1: Recovery and Residence of Bromide from Tracer Tests

Fracture Id	Specific Discharge	Percent Recovery	Mean Residence
	[m/d]	[%]	[min]
F1	30	81.02	139.80
	30	91.41	145.00
	10	86.63	726.46
	5	101.44	1136.39
F2	30	99.28	160.07
	30	97.11	134.38
	10	103.02	290.83
	5	76.35	761.34

that in relation to the trapezoid method of integration, the error due to HPLC quantification methods is miniscule. In addition to the potential quantification errors mentioned above, reasons for percent recovery to fall below 100% include: (1) the diffusion of mass into stagnant portions of the flow field and/or matrix and (2) the sampling period did not extend long enough to account for the entirety of the characteristically long tail of solute to breakthrough the fracture.

Table 5-1 also shows the mean residence time of bromide for each tracer test. In F2 there appears to be a linear relationship between specific discharge and mean residence time. However, in F1 this relationship deviates from linearity. This may be due to an increased presence of

stagnant water accessible to the tracer both within the fracture plane and in the matrix. Diffusion into immobile regions, which is far more dominant at lower flow rates, would have induced a more tortuous flow path during the tests run at low specific discharges, thus resulting in significantly higher residence times.

It is noticeable from table 5-2 that the various methods used for estimating the equivalent aperture of a fracture plane are sensitive to different portions of the aperture field. In all cases $b_m > b_h > b_f$ which is consistent with the findings of others (e.g. Tsang, 1992; Dickson and Thomson, 2003).

5.1.3 *E. coli* RS2GFP Tracer Tests

In order to facilitate the delineation of hydrodynamic effects on particle transport in saturated fractures, the specific discharges applied to solute tracer tests were also applied to the *E. coli* RS2GFP tracer tests. Additionally, the methods used to calculate the concentration of tracer exiting the fracture plane during solute tracer tests were applied to the *E. coli* RS2GFP tracer tests, such that measures of breakthrough, retention and residence times were translatable and comparable between the different types of tracer tests.