ASSESSMENT OF LABORATORY METHODS FOR QUANTIFYING AQUEOUS BACTERIAL DIFFUSION

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by
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ABSTRACT

Laboratory studies of bacterial transport and attenuation in a porous media were conducted under low velocity and near diffusion-dominated conditions. This thesis reviews and summarizes velocity data from a great deal of pertinent bacterial transport and attenuation investigations. Peclet numbers calculated from the literature data and data from this study correlated well with the Peclet plot. Three independent methods were investigated for determining bacterial diffusion rates: dynamic light scattering measurements, the diffusing sphere technique, and the double reservoir diffusion technique. One motile and four non-motile bacteria were used in these mono-culture experiments: *Pseudomonas fluorescens* 840406-E, *Pseudomonas fluorescens* m18, *Klebsiella oxytoca*, *Burkholderia cepacia* G4PR1, and a *Pseudomonas* isolate. Dynamic light scattering measurements were used to measure the Brownian movement of the bacteria. These measurements yielded free-water bacterial diffusion coefficients of $\sim 1\times10^{-13}$ $m^2 \cdot sec^{-1}$ for all five bacteria. Estimates of bacterial diffusion coefficients using the Einstein-Stokes and the volume-fraction modified Einstein-Stokes equation agreed with measurements made using dynamic light scattering. The diffusing sphere model and technique was re-designed to measure free-water bacterial diffusion. The diffusion coefficient for fluorescein (4.4×10^{-10} $m^2 \cdot sec^{-1}$), found by simulating diffusing fluorescein results, corresponded

well with published values for the free-water diffusion of fluorescein. Many attempts were made to adapt this technique to measure free-water bacterial diffusion coefficients. Rapid sinking of the diffusing sphere prevented measurement of the bacterial diffusion and the technique could not be used to measure free-water bacterial diffusion. The double reservoir diffusion cell model was designed and constructed to study the diffusion-dominated transport and attenuation of bacteria through saturated quartz sand. While the double reservoir diffusion technique was successful for determining an effective diffusion coefficient of chloride (6.1×10^{-10} $m^2 \cdot sec^{-1}$), the technique could not be adapted for measurement of bacterial diffusion in this study. The inability of the double reservoir diffusion test to quantify bacterial diffusion through saturated quartz sand was attributed to the difficulty in the enumeration of bacterial concentrations without disturbing experimental conditions. As well, the low values for bacterial diffusion ($\sim1\times10^{-13}$ $m^2 \cdot sec^{-1}$) provided a bacterial diffusive flux low enough that experimental errors may have influenced the results. Achieving and maintaining adequate experimental conditions for the laboratory study of diffusion-dominated bacterial transport and attenuation remains one of the most daunting challenges facing researchers in this field today.

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DEDICATION

To my father, who taught me how to run and never give up.

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CHAPTER 1

INTRODUCTION

Transport and attenuation of bacteria in the subsurface has been a concern for a number of years. By 1897 it was known that proper sewage disposal is vital to community health and general sewage guidelines were being proposed (Rafter, 1897). Yet, the present understanding of bacterial transport and attenuation in the subsurface is limited and based upon extrapolation from knowledge of conservative species transport (Bitton and Harvey, 1992; Harvey, 1991). However, in the past decade interest in bacterial transport in the subsurface has increased for several reasons. Some of these include bacterial facilitated transport of contaminants (Lindqvist and Enfield, 1992; Saiers and Hornberger, 1996), spread of pathogenic microorganisms in the subsurface (Bales et al., 1995; Craun et al., 1997), and bioremediation of contaminated sites (Cookson Jr., 1995; King et al., 1992; Riser-Roberts, 1992).

1.1 Previous work

Early work focused on practical public health and engineering concerns (i.e., proper sewage disposal and identifying safe water sources). One of the first bacterial transport field experiments investigated how close a well may be located to a broken

sewer line without being contaminated (Ditthorn and Luerssen, 1909). Since 1909, the study of bacterial transport has defined factors controlling bacterial transport in the subsurface. Still, the many factors influencing bacterial transport and attenuation in the subsurface make bacterial transport experiments very complex. There are many published reviews of literature concerning bacterial transport in the subsurface (Abu-Ashour et al., 1994; Bitton and Harvey, 1992; Corapcioglu and Haridas, 1984; Crane and Moore, 1984; Gerba et al., 1991; Harvey, 1996; Keswick et al., 1982; Lawrence and Hendry, 1996; McDowell-Boyer et al., 1986; Peterson and Ward, 1987; Peterson and Ward, 1989; Viraraghavan, 1978; Yates and Yates, 1988). Table 1.1.1 presents factors that have been identified as important in controlling bacterial transport and attenuation in the subsurface. These include three major areas: microbial conditions, environmental conditions, and media properties.

Table 1.1.1. Mechanisms controlling bacterial transport and attenuation in the subsurface. Adapted from Bitton and Harvey (1992), Crane and Moore (1984), and Yates and Yates (1988).

Microbial conditions	Sorption; origin and type of microorganism; and its physiological state.
Environmental conditions	Temperature, presence of soluble organics, ionic strength, pH, and groundwater chemistry
Media properties	Groundwater velocity, water content, average pore/grain size, pore/grain size distribution, tortuosity, porosity, surface characteristics, and organic content.

Attenuation of bacteria in porous media is usually assumed to be a sorption or filtration controlled mechanism. Most bacterial transport experiments are modelled assuming either filtration (Abu-Ashour et al., 1994; Gross et al., 1995; Harvey and Garabedian, 1991; Harvey et al., 1995; Hornberger et al., 1992; Johnson et al., 1995; McCaulou and Bales, 1994) or sorption (Corapcioglu and Kim, 1995; Harvey and Garabedian, 1991; Hendry et al., 1997; Hendry et al., 1999; Lawrence and Hendry, 1998; McCaulou and Bales, 1994; Tan et al., 1994) of bacteria in the porous media. Sharma and McInerney (1994) showed that the size of bacterial cells and the pore-size ratios may influence bacterial transport and attenuation. However, in most sand-packed column experiments the average pore throat is sufficiently large so filtration is not a concern (Matthess et al., 1988). Further, McDowell-Boyer et al. (1986) indicated that filtering would not be a consideration for cell/ pore ratios <0.05. However, sorption of bacteria is one of the most important mechanisms governing bacterial transport and attenuation in the subsurface (Bales et al., 1997; Harvey, 1996; Lindqvist and Bengtsson, 1991). Most laboratory bacterial transport experiments relate sorption of bacteria in porous media to other factors (e.g., origin of bacteria, bacterial cell size, organic content of the porous media, bacterial motility, and fluid chemistry).

Harvey et al. (1995) showed through field and laboratory tests that bacteria cultured in the lab and later placed in a subsurface environment exhibited different transport and attenuation characteristics when compared to indigenous subsurface bacteria. Several researchers showed that bacterial transport is strongly influenced by the organic

content of the porous media and the media surface characteristics (Gannon et al., 1991a; Gross and Logan, 1995; Johnson et al., 1996; Krone et al., 1958; Rijnaarts et al., 1996b; Scholl and Harvey, 1992).

Some bacteria are motile and capable of moving through liquids. Motile bacteria move by intermittent winding and unwinding of their corkscrew-shaped flagella (Purcell, 1977). Chemotaxis, bacterial migration in response to a chemical gradient, has been studied extensively in the laboratory (Barton and Ford, 1995; Ford et al., 1991; Harwood et al., 1989; Rivero et al., 1989). Numerous models predict chemotactic transport rates as a function of an attractant gradient and motility of the bacteria (Brosilow et al., 1996; Rivero et al., 1989; Segel, 1977). Chemical or nutrient sources can provide a significant stimulant for bacterial movement and growth (Brock and Madigan, 1991).

Experiments by McCaulou et al. (1995) showed that a temperature increase from 4 to 18°C caused a decrease in irreversible bacterial sorption in sand packed columns. The initial sorption between bacteria and solid surfaces is sometimes described as a surface charge dependant process (Gross and Logan, 1995; Johnson et al., 1996). A change in the fluid ionic strength can influence the charge potential between the bacteria and the solid surface. In column experiments by Johnson et al. (1996), decreased ionic strength of the fluid media (0.07 to 10^{-5} M) caused a two orders of magnitude decrease in the fraction of bacteria irreversibly sorbed in the columns. This reduction is in agreement with other investigations of groundwater chemistry and ionic strength

effects on bacterial transport and attenuation (Gannon et al., 1991b; Gross and Logan, 1995; Hornberger et al., 1992; Scholl and Harvey, 1992; Scholl et al., 1990; Yates and Yates, 1988). Changes in the pH affect the attenuation of bacteria (Scholl and Harvey, 1992). Studies have not been conclusive enough to establish a general relationship between pH and sorption of bacteria (Kinoshita et al., 1993; Scholl et al., 1990).

Column experiments conducted under controlled laboratory conditions have been used to define some of the dominant mechanisms controlling bacterial transport (Harvey, 1996). Sorption, one of the key controlling mechanisms, has been examined in column and field studies (Bales et al., 1995; Barton and Ford, 1995; Camper et al., 1993; Gannon et al., 1991b; Harvey, 1996; Hendricks et al., 1979; Johnson et al., 1996; Lindqvist and Bengtsson, 1991; McCaulou and Bales, 1994; Mills et al., 1994; Saiers and Hornberger, 1996; Weiss et al., 1995). Unfortunately, much of the work focuses on experiments conducted with high fluid velocities relative to those found in the subsurface (Table 1.1.2).

Table 1.1.2. Average fluid velocities from field and laboratory bacterial transport investigations.

Velocity ($m \cdot day^{-1}$)	References
1.9 to 11.7	Bales et al. (1989)
0.09	Bales et al. (1997)
0.5, 2.4, and 10.8	Camper et al. (1993)
8.6	Gannon et al. (1991b)
0.5	Harvey et al. (1995)
0.09, 0.17, and 0.19	Hendry et al. (1997)
0.12 to 3.2	Hendry et al. (1999)
10.5	Hornberger et al. (1992)
4.4	Lindqvist and Bengtsson (1995)
5.8	McCaulou et al. (1995)
5.2	Rijnaarts et al. (1996b)
17.3	Rijnaarts et al. (1996a)
2.7	Smith et al. (1985)
1	Toran and Palumbo (1992)
17.4	Wollum II and Cassel (1978)

Typical average groundwater velocities are <0.1 $m \cdot day^{-1}$ (API, 1989). The average fluid velocity used in column experiments plays an important role in the sorption of microorganisms in porous media (Gannon et al., 1991b; Hendry et al., 1997; Lindqvist and Bengtsson, 1995). Studies have found an inverse relationship between fluid velocity and sorption in sand-packed column experiments (Bales et al., 1989; Gannon et al., 1991b; Hendry et al., 1997; Hendry et al., 1999; Smith et al., 1985; Tan et al., 1994; Wollum II and Cassel, 1978). Likely, as the fluid velocity decreases, the contact time between the bacteria and the solid surfaces increases. This likely increases the potential for binding between the bacteria and the solid surface. As the

potential for binding between bacteria and the solid surfaces increases, then bacterial sorption increases as the fluid velocity decreases. Using pulsed and/ or finite bacterial concentrations, laboratory studies have determined two relationships: as the fluid velocity decreases, the peak output concentration of bacteria decreases (Bales et al., 1989; Gannon et al., 1991b; Hendry et al., 1997; Hendry et al., 1999; Smith et al., 1985; Tan et al., 1994; Wollum II and Cassel, 1978) and the arrival time (corrected for the velocity difference) decreases as well (Bales et al., 1989; Gannon et al., 1991b; Wollum II and Cassel, 1978).

1.2 Diffusion in the subsurface

Most bacterial transport experiments have used extremely high groundwater velocities (Table 1.1.2). As well, very little work has been conducted under low velocity or diffusion-dominated transport conditions (Ford et al., 1991; Reynolds et al., 1989; Rijnaarts et al., 1993; Sharma and McInerney, 1994). Consequently, our understanding of bacterial transport and attenuation in porous media under low velocity conditions is lacking. A full understanding of bacterial transport in the subsurface requires a close examination of the effects of sorption under the diffusion-dominated or low velocity conditions commonly found in the subsurface.

Most of the previous diffusion-dominated experiments in the literature either have not quantified bacterial diffusion coefficients (Ford et al., 1991; Lovely and Dahlquist, 1975), have incorrectly represented the physical problem in the mathematical model and solution (Ford and Lauffenburger, 1991), or have not properly addressed some of

the controlling mechanisms of bacterial transport in the experiments (Jenneman et al., 1985; Sharma et al., 1993). Using a stopped-flow diffusion chamber, Ford et al. (1991) determined bacterial motility and chemotaxis transport rates (1.1×10^{-9} and 8×10^{-9} $m^2 \cdot sec^{-1}$, respectively), with no measurement of bacterial diffusion. In a companion paper, Ford and Lauffenburger (1991) mathematically modelled bacterial motility and chemotaxis experiments; however, the mathematical solution they used did not fully represent their physical problem. The authors assumed a finite source diffusing into an infinite volume (as defined by Crank, 1956), instead of the finite volume found in their experiments.

The static flow column is a simple method for studying bacteria movement in porous media, but not without complications. Using static flow conditions, Reynolds et al. (1989) examined the transport of anaerobic bacteria in sand packed nutrient-saturated tubes. The authors found bacterial growth penetration rates >0.01 $m \cdot day^{-1}$ through the tubes. In similar experiments, Sharma et al. (1993) found faster growth penetration rates of bacteria (~0.1 $m \cdot day^{-1}$) through nutrient-saturated sand packed columns. Sharma and McInerney (1994) filled columns with nutrient-saturated porous media and monitored bacterial movement by sampling the distal column end using a syringe technique similar to Reynolds et al. (1989). The growth penetration rates found by these authors demonstrate that growth-induced movement of bacteria may overwhelm diffusion-dominated conditions within the columns.

A non-dimensional coefficient, the Peclet number (P_e) can be used to describe flow through a porous media system by comparing the advective-dispersive and diffusive fluxes (Fig. 1.2.1). The P_e number indicates whether a flowing system is under advective flow dominated or diffusion-dominated conditions (Fetter, 1992; Perkins and Johnston, 1963). The P_e number is defined as the average fluid velocity*soil pore diameter/effective diffusion coefficient, (Perkins and Johnston, 1963). Diffusion-dominated flow systems are characterized by P_e values $<1\times10^{-1}$ (Perkins and Johnston, 1963). In the example of Sharma et al. (1993) where the authors determined growth-induced movement of bacteria of ~0.1 $m\cdot day^{-1}$, the equivalent P_e number for their physical model was $>1\times10^{5}$. Results from Sharma et al. (1993) indicate that growth-induced movement of bacteria can be greater than diffusion alone. Though no reference has been found that used the P_e number for analyzing bacterial transport in porous media, it seems well suited for gauging diffusion-dominated conditions in bacterial transport studies.

In this thesis, P_e numbers were calculated for the studies shown in Table 1.1.2. An assumed value for the effective diffusion coefficient (1×10^{-14} $m^2\cdot sec^{-1}$) was used. The stated soil pore diameter found each paper was also used. In some cases, a soil type was stated, but no particle diameter. In these cases, an estimate of the soil particle diameter was used (Table 2.2, Freeze and Cherry, 1979). If no value for pore diameter was found, an estimate was made based upon square packing of the soil particles (pore size ~ 0.7*particle size, Krone et al., 1958). Unless information was provided, the coefficient of hydrodynamic dispersion (D_h) was estimated using an assumed

dispersivity of 1×10^{-3} m (Gelhar, 1986; Gillham and Cherry, 1982). The P_e number estimates are presented in Table 1.2.1 and ratio of D_h and P_e number versus P_e number are plotted on Figure 1.2.1 and Figure 1.2.2. The P_e plot shown in Figures 1.2.1 and 1.2.2 is based upon a linear extrapolation (beyond $P_e = 1\times10^4$) of the work by Perkins and Johnston (1963).

Table 1.2.1. Peclet number calculations for the results of some field and laboratory bacterial transport investigations.

Velocity ($m\cdot day^{-1}$)	References	P_e
1.9 to 11.7	Bales et al. (1989)	2×10^6 to 1×10^7
0.09	Bales et al. (1997)	2×10^5
0.5, 2.4, and 10.8	Camper et al. (1993)	6×10^5, 3×10^6, and 1×10^7
8.6	Gannon et al. (1991b)	1×10^7
0.5	Harvey et al. (1995)	3×10^5
8.6, 18.3, and 20.2	Hendry et al. (1997)	3×10^6, 6×10^6, and 7×10^6
0.11 to 3.2	Hendry et al. (1999)	4×10^4 to 1×10^6
10.5	Hornberger et al. (1992)	9×10^6
4.4	Lindqvist and Bengtsson (1995)	2×10^6
5.8	McCaulou et al. (1995)	2×10^6
5.2	Rijnaarts et al. (1996b)	3×10^6
17.3	Rijnaarts et al. (1996a)	4×10^6
2.7	Smith et al. (1985)	6×10^5
1	Toran and Palumbo (1992)	1×10^6
17.4	Wollum II and Cassel (1978)	1×10^6

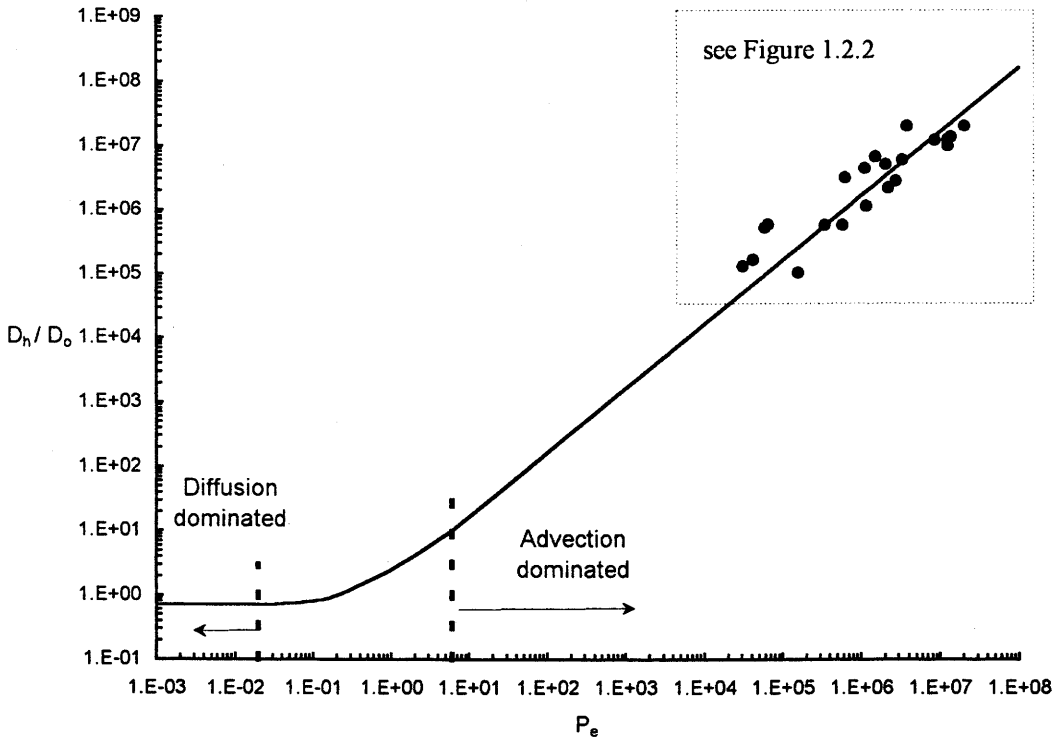


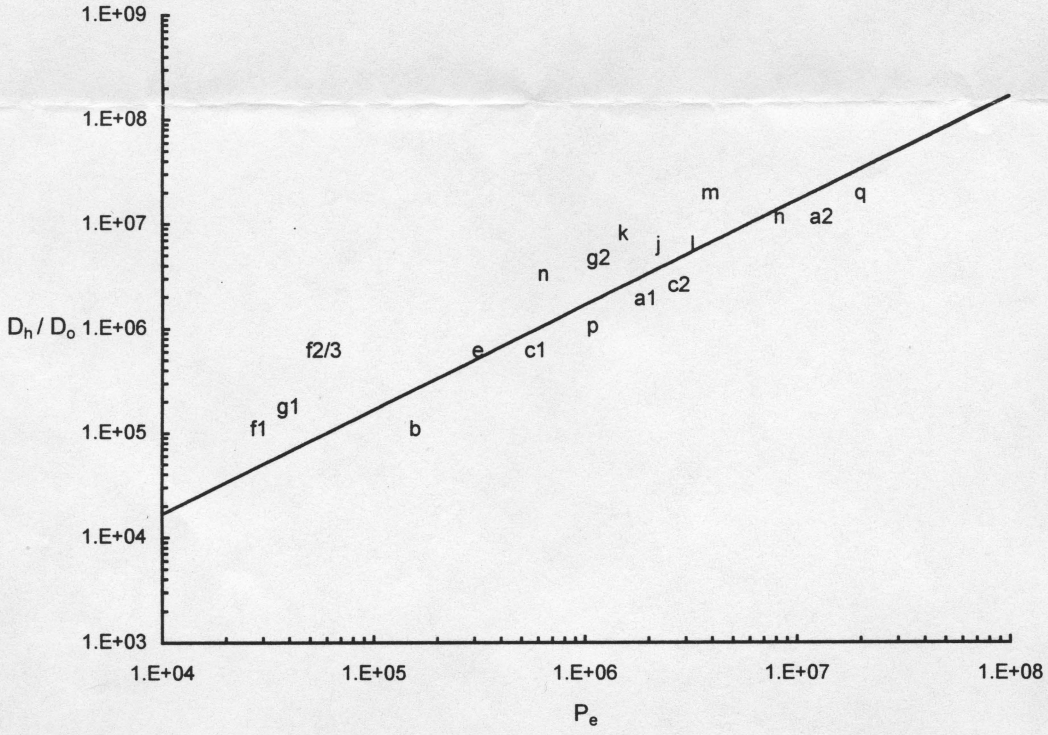
FIGURE 1.2.1. The Peclet plot. The relationship between the Peclet number (vd/D_e) and the ratio of the coefficient of hydrodynamic dispersion (D_h) and the coefficient of effective diffusion (D_e) for fluid flowing through a porous media (after Perkins and Johnston, 1963).

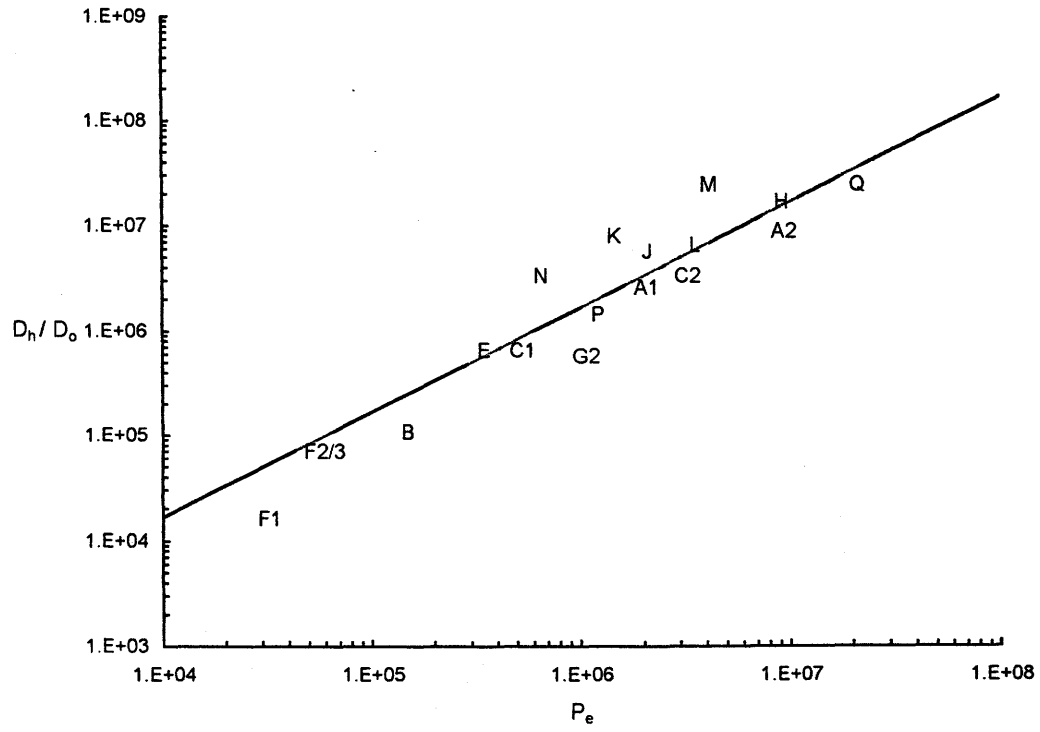
ERRATUM

Assessment of laboratory methods for quantifying aqueous bacterial diffusion, Reiter (1999)

The following correction is required to page 12, Figure 1.2.2.

The correct labels of the scaled area of the Peclet plot area are,





References	Plot label
Bales et al. (1989)	A1 and A2
Bales et al. (1997)	B
Camper et al. (1993)	C1, C2, and A2
Gannon et al. (1991b)	A2
Harvey et al. (1995)	E
Hendry et al. (1997)	F1, F2, and F3
Hendry et al. (1999)	F1 and G2
Hornberger et al. (1992)	H
Lindqvist and Bengtsson (1995)	J
McCaulou et al. (1995)	K
Rijnaarts et al. (1996b)	L
Rijnaarts et al. (1996a)	M
Smith et al. (1985)	N
Toran and Palumbo (1992)	P
Wollum II and Cassel (1978)	Q

FIGURE 1.2.2. Scaled area of the Peclet plot shown on Figure 1.2.1 with accompanying legend text.

The P_e plot provides a clear representation of the bacterial transport data and illustrates the lack of bacterial transport data under diffusion-dominated conditions or even near diffusion-dominated conditions. The bacterial transport data found in the literature correlate well with the extrapolation of Perkins and Johnston's earlier work (1963), the P_e plot. The close relationship between the bacterial transport data and the P_e plot indicates that the P_e number may be a useful tool for evaluating physical conditions of bacterial transport investigations. Additionally, the P_e number would simplify modelling of bacterial transport data, modelled using a modified advective-dispersive equation. A modified advective-dispersive equation has been used in previous bacterial transport investigations (Corapcioglu and Haridas, 1984; Corapcioglu and Haridas, 1985; Grondin and Gerba, 1986; Hendry et al., 1997) and follows the theory commonly used in modelling transport of dissolved species (Anderson, 1979; Ogata and Banks, 1961).

Free-water diffusion coefficients (D_o) represent the Brownian movement of a diffusing species in water. The diffusion of isolated particles (diameter <1 μm) in a suspension can be estimated using the Einstein-Stokes equation (Smoluchowski, 1917). In the same manner, estimates of free-water diffusion of bacteria (D_B) can be found using the Einstein-Stokes equation (1926)

$$D_B = \frac{kT}{6\pi\mu P} \qquad \text{Eq. 1}$$

where k is the Stefan-Boltzmann constant (1.38×10^{-23} kg·cm^2·sec^{-2}·Kelvin^{-1}), T is the temperature (Kelvin), μ is the absolute viscosity of the surrounding fluid media (kg·cm^{-1}·sec^{-1}), and P is the radius of the bacteria (cm). Yao et al (1971) and Rijnaarts et al. (1993) applied Eq. 1 to bacterial experiments and showed a good correlation between data from experiments and estimates found with the Einstein-Stokes equation.

Rijnaarts et al. (1993) measured D_o values using dynamic light scattering (Pusey and Tough, 1985) for Coryneform bacteria and Pseudomonads. Estimates of bacterial radii ($0.5\cdot[l\cdot w]^{0.5}$) were found using light microscopy measurements (Rijnaarts et al., 1993). D_B values calculated with estimates of bacterial radii and the Einstein-Stokes equation (Eq. 1) correlated very well with measured D_o values. The similarity between bacterial D_o values and D_B estimates indicates that the Einstein-Stokes equation is a close estimate of D_o values for bacteria (Rijnaarts et al., 1993). The D_B estimates are presented in Table 1.2.2 along with measured D_o values (Rijnaarts et al., 1993).

Table 1.2.2. Estimates of bacterial diffusion coefficients (D_B) using the Einstein-Stokes equation and measured free-water diffusion coefficients of bacteria (D_o) (from Rijnaarts et al., 1993).

Strain	D_B ($m^2 \cdot sec^{-1}$)	D_o ($m^2 \cdot sec^{-1}$)
Coryneform strain DSM 6685	5.4×10^{-13}	5.90×10^{-13}
Rhodococcus sp. strain C125	1.9×10^{-13}	1.74×10^{-13}
Rhodococcus erythropolis A177	2.3×10^{-13}	1.95×10^{-13}
Corynebacterium sp. strain DSM 44016	4.7×10^{-13}	3.78×10^{-13}
Gordona sp. strain DSM 44015	3.4×10^{-13}	2.61×10^{-13}
Pseudomonas fluorescens p62	3.8×10^{-13}	3.75×10^{-13}
Pseudomonas putida mt2	3.8×10^{-13}	3.65×10^{-13}

In addition to growth-induced migration, advection, and diffusion; bacterial motility can influence bacterial transport (Jenneman et al., 1985) and attenuation (Korber et al., 1989). Bacterial motility is sometimes described as a series of run and tumble events (Berg and Brown, 1972) or a drunkard's walk (Taylor, 1921). Some authors have measured the velocity of motile bacteria to quantify motility (Ford and Lauffenburger, 1991; Korber et al., 1989). However, bacterial motility is better understood as a dispersing mechanism, like diffusion (Weiss, 1983). Taylor (1921) studied a similar drunkard's walk problem and found that as time elapsed the dispersal of an irregularly motile particle was best approximated by Fick's laws of diffusion. In a good example of this, Slater et al. (1981) used dynamic light scattering measurements to determine D_o values for motile and non-motile bacteria. The D_o values determined by these authors were three orders of magnitude greater for motile than for non-motile bacteria

(i.e., 3.6×10^{-10} and 4.6×10^{-13} $m^2\cdot sec^{-1}$). Therefore in laboratory studies of bacterial diffusion, bacterial growth and motility must both be controlled.

1.3 Thesis Goal

There are many important reasons for understanding bacterial transport and attenuation using diffusion-dominated conditions: the prevalence of low velocity conditions in the subsurface and the lack of understanding of bacterial sorption under these low velocity conditions. The goal of this thesis is to better understand bacterial transport and attenuation using low velocity and diffusion-dominated conditions. The first objective of this thesis is to determine D_o values for selected non-motile bacteria: *Pseudomonas fluorescens* m18, *Klebsiella oxytoca*, *Burkholderia cepacia* G4PR1, and a *Pseudomonas* isolate; and a motile bacterium *Pseudomonas fluorescens* 840406-E. D_o values are found using dynamic light scattering measurements. The second objective is to assess the applicability of the diffusing sphere technique (de Beer et al., 1997) for measurement of free-water bacterial diffusion. The diffusing sphere technique was adapted and evaluated for its applicability for measuring bacterial D_o values. The final objective is to study bacterial-porous media interactions under low velocity and diffusion-dominated conditions using the double reservoir diffusion technique (Kemper and van Shaik, 1966).

CHAPTER 2

THEORY AND CONCEPTS

The concentration dependent diffusion of a species in a homogeneous media can be represented by Fick's first law

$$F = -D\frac{\partial C}{\partial x} \qquad \text{Eq. 2}$$

where F is the mass flux of the diffusing species through a unit of area per time [$M \cdot L^{-2} \cdot T^{-1}$], D is the diffusion coefficient of the species through the media [$L^2 \cdot T^{-1}$], and $\partial C/\partial x$ is the concentration gradient of the species in the direction of the measured mass flux [$M \cdot L^{-4}$]. Using Fick's first law and assuming continuity of mass, Fick's second law for a one-dimensional case is

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \text{Eq. 3}$$

In 1902, Einstein (1926) developed an estimation for the diffusion coefficient of bacteria (D_B) in the case of a discrete particle in a suspension of smaller particles, the Einstein-Stokes equation (Eq. 1). Further development of the Einstein-Stokes equation by Batchelor (1976) showed that the Einstein-Stokes equation is applicable to

cases where the concentration of the suspended particles is high enough that the particles can not be considered discrete. The volume-fraction modified Einstein-Stokes equation, is

$$D_B(\phi) = (1 + 1.45\phi)\frac{kT}{6\pi\mu P} \qquad \text{Eq. 4}$$

The unitless volume fraction of particles (bacteria) in a suspension ϕ is defined by

$$\phi = \frac{Nv}{(N_O v_O + Nv)} \qquad \text{Eq. 5}$$

where N and ν are the number and volume of each particle (bacteria) in the suspension and N_O and ν_O are the number and volume of molecules in the surrounding fluid. The remainder of the terms in Eq. 4 are defined the same as in the Einstein-Stokes equation (Eq. 1).

Because of increased travel path, diffusion through porous media is reduced by a factor called the apparent tortuosity (τ). More complex definitions of diffusion in porous media systems further define tortuosity to include terms accounting for porosity, volumetric water content, pore-size distribution, electrostatic interactions, and viscosity (Kemper and van Shaik, 1966). Though surface charges are important in clayey materials, it was assumed that these surface charge effects had a negligible impact on the diffusion of bacteria through the clean quartz sand. Although several mathematical definitions exist for τ (Fetter, 1992) but the relationship between the

effective diffusion through porous media D_e and D_o is usually defined as (Shackelford and Daniel, 1991)

$$D_e = D_o \tau \qquad \text{Eq. 6}$$

CHAPTER 3

MATERIALS AND METHODS

3.1 Aqueous solutions and suspensions

In this study, artificial groundwater (AGW) was used to simulate natural groundwater conditions. AGW was also used as a solute to rinse and re-suspend the bacteria throughout all of the experiments. The AGW was prepared by leaching deionized water through local lacustrine sand, filtering with a 0.45 μm membrane filter (Gelman Sciences Inc., Ann Arbor, MI), and autoclaving (Hendry et al., 1997). Results of an analysis of the AGW are presented in Table 3.1.1.

Table 3.1.1. Results of the chemical analysis of the autoclaved artificial groundwater ($mg \cdot L^{-1}$) (from Hendry et al, 1997).

Ca^{2+}	Mg^{2+}	K^{+}	Cl^{-}	PO_4^{2+}	Alkalinity*	pH	NO_3^{2-}	SO_4^{2-}
22.2	3.43	1.41	0.1	0.1	66.65	7.3	<1.0	<1.0

* Alkalinity is represented as $mg \cdot L^{-1}$ of $CaCO_3$.

3.2 Aqueous tracers

Both the diffusing sphere tests and the double reservoir diffusion tests (both presented below) were conducted using dissolved tracers with known D_o values. In the diffusing sphere tests fluorescein (100 $mg \cdot L^{-1}$, formula mass = 376.28 $g \cdot M^{-1}$) was used (Berg

and Brown, 1972; Lawrence et al., 1994) and in the double reservoir diffusion tests sodium chloride was used (100 $mg \cdot L^{-1}$ as Cl^-, from Hendry et al., 1997). D_o values for fluorescein of 5.5×10^{-10} and 3.3×10^{-10} $m^2 \cdot sec^{-1}$ were found in Berg and Brown (1972) and Lawrence et al. (1994), respectively. D_o values for chloride at 25°C (2.03×10^{-9} $m^2 \cdot sec^{-1}$) were found in Weast (1987).

3.3 Bacteria

D_o values were determined for five bacterial strains: four native subsurface bacteria and a genetic variant in mono-culture experiments. Experiments were conducted using a motile bacterium *Pseudomonas fluorescens* 840406-E, its non-motile Tn5 mutant *P. fl.* m18 (Korber et al., 1989), and the non-motile bacteria: *Klebsiella oxytoca*, *Burkholderia cepacia* G4PR1, and a *Pseudomonas* isolate.

P. fl. 840406-E and *P. fl.* m18 were isolated in earlier investigations for their successful surface colonization during slide culture experiments in a biofilm study (Korber et al., 1989). Cultures of *K. oxytoca*, G4PR1, and a *Pseudomonas* isolate were used in previous experiments studying advection-dominated bacterial transport in sand-packed columns (Hendry et al., 1997; Hendry et al., 1999). The *Pseudomonas* isolate (*P.* 5) was isolated from cores recovered at a research site near Birsay, Saskatchewan, Canada (Shaw and Hendry, 1999) at a depth of 90 m in the 71 Ma to 72 Ma year old clay-rich Snakebite Formation (Caldwell, 1968). Shields and Reagin (1992) isolated G4PR1 in a laboratory study for its ability to degrade trichloroethylene.

Bacteria were cultured in 50% tryptic soy broth (TSB, Difco, Sigma-Aldrich Inc., Oakville, ON) and harvested after two days. Harvested cells were rinsed by centrifuging the culture, pouring off the supernatant, resuspending in sterilized AGW, and agitating with a vortex mixer (Hendry et al., 1997). The bacteria were rinsed three times before preparation of each stock suspension. After harvesting and rinsing, the concentration of viable bacteria in the stock suspensions varied from 1×10^{8} to 1×10^{10} colony forming units per ml ($CFU\cdot mL^{-1}$) of AGW, as determined by spread plating on 50% TSB plates. Concurrent direct filter counts of DAPI (Sigma-Aldrich Inc., Oakville, ON) stained bacteria in suspension (Hobbie et al., 1977; Porter and Feig, 1980) showed little difference over the course of the experiment between the total number of bacteria and the number of viable bacteria in the suspensions. Stock suspensions kept for <31 days had minimal change in concentration of viable bacteria in the stock suspension (1×10^{7} to 5×10^{5} $CFU\cdot mL^{-1}$).

Working suspensions of the bacteria were made immediately prior to all bacterial experiments by preparing a 1/10 to 1/100 AGW dilution of the bacterial stock suspension. To reduce clumping of the bacteria in the suspension and better approximate the diffusion of discrete particles; a non-ionic surfactant (2-6 $\mu L\cdot 100\ mL^{-1}$ of Tween 80™, Sigma-Aldrich, Oakville, ON) was admixed into the working suspension.

3.4 Characterization of the bacterial strains

The length and width of *K. oxytoca*, G4PR1, and *P.* 5 were previously determined by examining wet mount slide preparations of bacterial suspensions (Lawrence and Hendry, 1998). Approximately 100 measurements of each bacterial strain were made by wet mount examination of bacteria using phase-contrast light microscopy (Lawrence and Hendry, 1998).

3.4.1 Bacterial motility

Korber et al. (1989) quantified motility rates of *P. fl.* 840406-E and compared them to the non-motile strain *P. fl.* m18. No attempt was made to quantify the motility rate of *P. fl.* 840406-E. However, the motility and non-motility of each strain was verified prior to each experiment by phase-contrast microscopy examination of a wet mount slide preparation.

3.4.2 Viscosity of the bacterial suspensions

The bacterial working suspension viscosity (25°C, 1×10^8 CFU·mL^{-1}) for all five bacteria were compared to deionized water using an Ostwald viscometer (Daniels et al., 1962).

3.4.3 Liquid bulk densities of the bacterial suspensions

In the diffusion tests, maintaining diffusion-dominated conditions was very important for determining bacterial D_o values. Density differences between an AGW solution and a suspension of bacteria may provide a density gradient - which would adversely

influence the diffusion tests. The liquid bulk densities [$M \cdot L^{-3}$] (25°C, 1×10^8 CFU·mL^{-1}) of all five bacterial working suspensions were compared to the density of AGW using a gravimetric method with a 50 mL measurement volume.

3.4.4 Settling of stained and unstained bacterial suspensions

Duplicate working suspensions of all five bacteria were prepared and added into test tubes (total volume per tube, 10 mL). Tween 80™ (2-6 μL·100 mL^{-1}, Sigma-Aldrich, Oakville, ON) was admixed with each working suspension. Bacteria in one set of the five working suspensions were stained using SYTO9 (Molecular Probes Inc., Eugene, OR) at a concentration of 5 μM. Both the stained and unstained bacterial suspensions were uniformly and simultaneously mixed for 30 seconds.

As an indicator of bacterial suspension settling, changes in fluid turbidity were monitored. Measurements of the changes in turbidity of all suspensions were made using a Klett Colorimeter (Klett Manufacturing Co, NY) after 1, 10, and 60 minutes. During the 60 minute measurement period, the test tubes were neither moved nor disturbed.

3.4.5 Bacterial cell density

The mass or gravimetric densities of the individual bacterial cells for all five bacteria were determined by density gradient centrifugation using Percoll® (Sigma-Aldrich, Oakville, ON) and coloured density marker beads (Sigma Aldrich, 1997). Percoll® was diluted using 1.5 M NaCl to form a density gradient liquid as detailed by

Pharmacia Biotech, Inc. (1997). The density marker beads and a 1 mL aliquot of each bacterial suspension were admixed to separate centrifuge tubes containing the formed Percoll® and NaCl density gradient as described by Pharmacia Biotech, Inc. (1997). The gross masses of the tubes were equalized with deionized water (added deionized water was <7% of the total volume) and centrifuged at 30,000×g for 15 minutes at a fixed rotor angle of 44°. Gravimetric densities of bacteria were determined by interpolating the vertical position of the bacterial layer against the position of the coloured density marker bead layers.

3.4.6 Bacterial cell hydrophobicity

The cell surface hydrophobicities were determined for all five bacteria using the n-hexadecane method or BATH (bacterial adherence to hydrocarbons) method described by Rosenberg (1984). Serial dilutions of the stock bacterial suspensions were made and the (%) light absorbance at a wavelength of 650 ηm was recorded. A small volume (40 μL) of n-hexadecane was added to each dilution, agitated for two minutes, and the absorbance (650 ηm) measured again after five minutes and thirty minutes.

3.5 Diffusion test techniques

3.5.1 Dynamic light scattering technique

In this thesis D_o values were determined with the dynamic light scattering (DLS) technique using photon correlation spectroscopy (Pusey et al., 1974). The five bacterial working suspensions were prepared (concentrations for all suspensions were

~1×10^8 CFU·mL^{-1}) and delivered via courier in <24 hours to Protein Solutions Inc., Charlottesville, VA for analysis. During shipping, the samples were stored, along with a non-contacting ice-pack, in a lower than ambient temperature container (4 to 10°C). All samples were diluted 1/10 with deionized water immediately prior to DLS measurements (final sample concentration ~1×10^7 CFU·mL^{-1}). It was unclear whether significant clumping might occur during shipping. Clumping would increase the average bacterial cell size and therefore decrease the D_o determined by DLS measurements. Though precautions were taken to reduce clumping (i.e., triple rinsing and resuspension of bacteria in autoclaved AGW), it was unknown whether bacterial clumping would be a problem.

DLS measurements were made using an SEM F60 (Protein Solutions Inc., Charlottesville, VA) with an He-Ne laser at a wavelength of 632.8 ηm. The laser was directed through the sample and the scattered light was measured with a pinhole/ photomultiplier at a scattering angle of 60°. Brownian motion of the bacteria in suspension causes intensity fluctuations in the scattered light (Camerini-Otero et al., 1974; Pusey et al., 1974). The measured intensity autocorrelation function; wavelength; and the suspension temperature, viscosity, and refractive index were used to convert the intensity fluctuation to a D_o value (Russel et al., 1989; Vold and Vold, 1983). In this experimental set-up, the Brownian motion was measured. There was no accounting for bacterial motility or bacterial diffusion incorporating bacterial motility and Brownian motion (Nobbmann, 1998). The DLS measured D_o value was used with the Einstein-Stokes equation to calculate a representative bacterial radius.

3.5.2 Diffusing sphere technique

D_o values were also measured using a new method designed to investigate two-dimensional bacterial diffusion, the diffusing sphere technique. In this technique, a small pulse of known tracer concentration C_o is injected into a nearly identical fluid volume (e.g., temperature, pressure, density, viscosity, and bacterial concentration). Assuming an instantaneous, finite tracer volume uniformly distributed in a sphere of radius a', under diffusion-dominated conditions there will be a net diffusive flux of the tracer outwards and non-tracer volume inwards (Li and Gregory, 1974; Russel et al., 1989; Weiss, 1983).

The diffusing sphere technique was based upon a problem described by Crank (1956), where a small concentrated of tracer volume of concentration C_o and known radius diffuses into an identical volume, except for the tracer. In this experiment, a small volume of stained bacteria was injected into a suspension of identical bacteria, except for the stain. The change in concentration of the stained bacteria at a measured distance from the sphere was quantified as the stained bacteria diffused outwards. Using a microinjection technique suggested by de Beer et al. (1997) the diffusion of fluorescein and fluorescent bacteria were measured using confocal laser microscopy and the diffusing sphere technique. The data was modelled using a one-dimensional axisymetric analytical solution (Eq. 7) after Crank (1956). At some distance r from the centre of the concentrated sphere the analytical solution (modified from Crank, 1956) for the concentration C at some time t is (Maloszewski, 1997)

$$\frac{C}{C_O} = \frac{1}{2} erf\left(\frac{r+a'}{\sqrt{4D_o t}}\right) - \frac{1}{2} erf\left(\frac{r-a'}{\sqrt{4D_o t}}\right) - \frac{\sqrt{D_o t}}{r\sqrt{\pi}}\left\{\exp\left[-\left(\frac{r-a'}{\sqrt{4D_o t}}\right)^2\right] - \exp\left[-\left(\frac{r+a'}{\sqrt{4D_o t}}\right)^2\right]\right\} \quad \text{Eq. 7}$$

A schematic diagram of the diffusing sphere model is shown in Fig. 3.5.2.1. The physical model consisted of a flow cell with a central, vertically mounted stainless steel tube (pulsing line) and inflow and outflow flushing lines at each end. The flow cell was constructed by laminating microscope slides and fixing a glass microscope coverslip on the top with silicone. An aquarium-standard silicone sealant (GE Silicones, Mississauga, ON) was used in construction of both the diffusing sphere model and the double reservoir diffusion model.

Initial diffusing sphere experiments studying fluorescein diffusion were performed as a confirmation of the physical model and technique. The physical model was saturated with AGW and the pulsing line was filled with fluorescein (100 $mg \cdot L^{-1}$ of AGW). The physical model was mounted on a Nikon Microphot-SA photomicroscope stage, the focal plane adjusted, and the physical model left undisturbed for ~2 minutes.

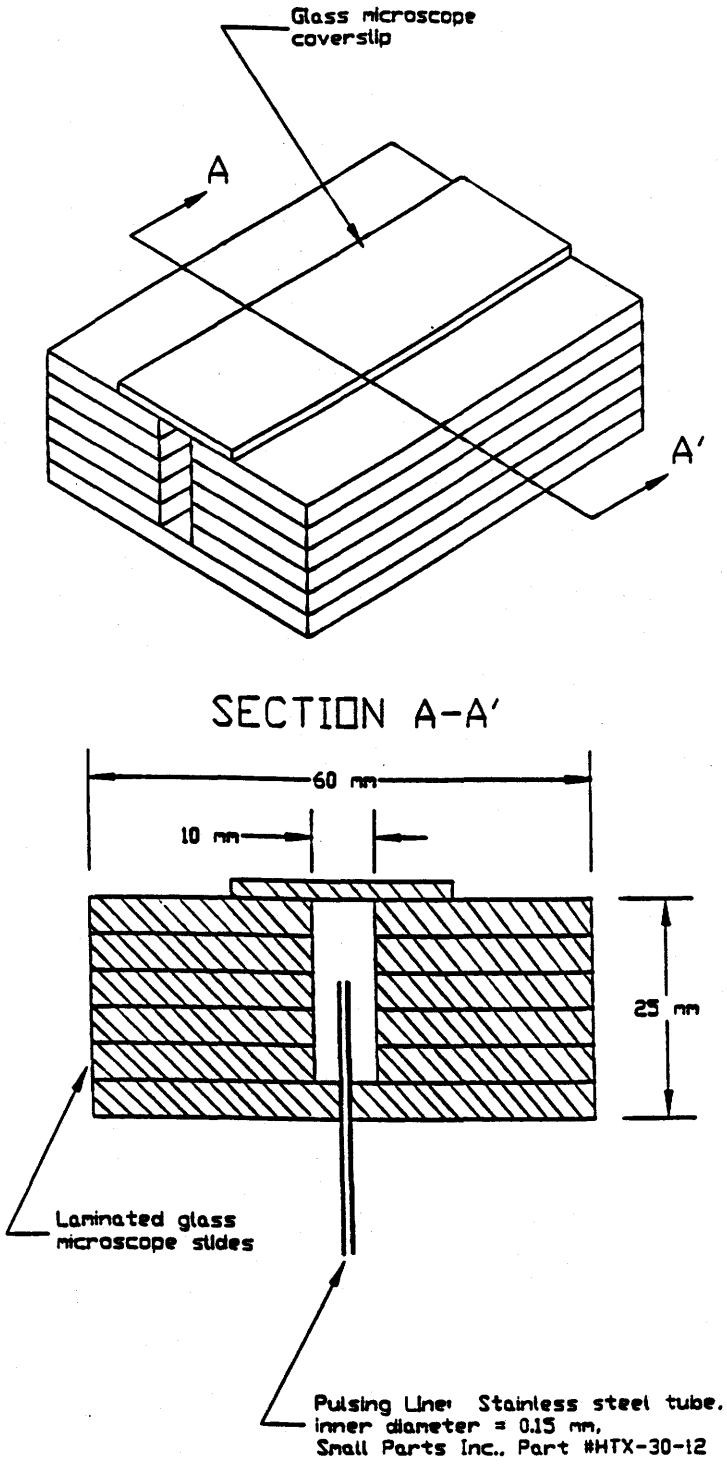


FIGURE 3.5.2.1. Diffusing sphere model. Glass microscope slides were laminated with silicone sealant to form a flow cell. A stainless steel tube (0.15 mm ID) was used to vertically inject a small pulse of fluorescein or a sphere of stained bacteria.

A small volume of fluorescein (~10 ηL) was pumped into the diffusing sphere model through the stainless steel pulsing line in <0.25 seconds using a syringe pump (SP230i, World Precision Instruments, Sarasota, FL). A BioRad MRC1000 scanning confocal laser microscope, mounted on the photomicroscope was used to take non-disruptive images of the diffusing fluorescein. The concentrated volume of fluorescein, the diffusing sphere, was observed in plan view with the scanning confocal laser microscope. The excitation and emission wavelengths were 488 and 522-532 ηm, respectively. After each diffusing sphere experiment (<1 min.), the flow cell volume was purged with AGW and left undisturbed for ~2 minutes prior to the next experiment. Observations indicated that after 1 minute there were no observable fluid currents remaining in the flow cell.

The digital images of the diffusing sphere were taken and stored as a time series. The time series of the diffusing sphere was analyzed using an image analysis program (Scion Image ver1.62, National Institute of Health, U.S.) to quantify concentration as a function of r and t. Images were analyzed by measuring the brightness at six areas equidistant from the centre of the diffusing sphere of a measured radius (Fig. 3.5.2.2). Brightness measurements at each of the six locations were normalized with respect to the maximum brightness at that location, the six values were averaged, and each average was normalized with the maximum average for that time series. The final set of brightness values versus time was modelled with the analytical solution (Eq. 7) visually by a trial and error method. In order to fit the analytical solution to the

normalized data, the analytical solution was normalized with respect to its maximum value C_{MAX} and fitted to the data (i.e., $C/C_o * C_o/C_{MAX}$).

After completion of the fluorescein experiments, the five bacteria were examined using the diffusing sphere technique. However, instead of a fluorescein solution and AGW, stained and unstained bacterial working suspensions were used.

The bacteria were stained in the working suspension immediately prior to each diffusing sphere experiment. To prepare the stained working suspension, bacteria in a 1 mL aliquot of the working suspension were stained using a nucleic acid stain (SYTO9, Molecular Probes Inc., Eugene, OR) at a concentration of 5 μM for 10 minutes. The only difference between the stained aliquot and the unstained working suspension was the presence of the small volume of stain (~4 $\mu L \cdot mL^{-1}$ of SYTO9, or 0.4% of the total volume of the aliquot). Since specific details of the stain are proprietary and are not readily available, a specific density of 1.100 at 20°C referred to water at 4°C (Windholz, 1976) was assumed for the stain bulk density (Molecular Probes, 1998). Assuming complete dissolution of the stain, the bulk liquid density of the stained working suspension aliquot would change <0.04% after staining. The nearly identical properties of the stained and the unstained bacterial working suspensions provided consistency in the fluid phase and avoided gradients other than the stained bacteria/ total bacteria concentration (i.e., density gradients and counter diffusion of the solute).

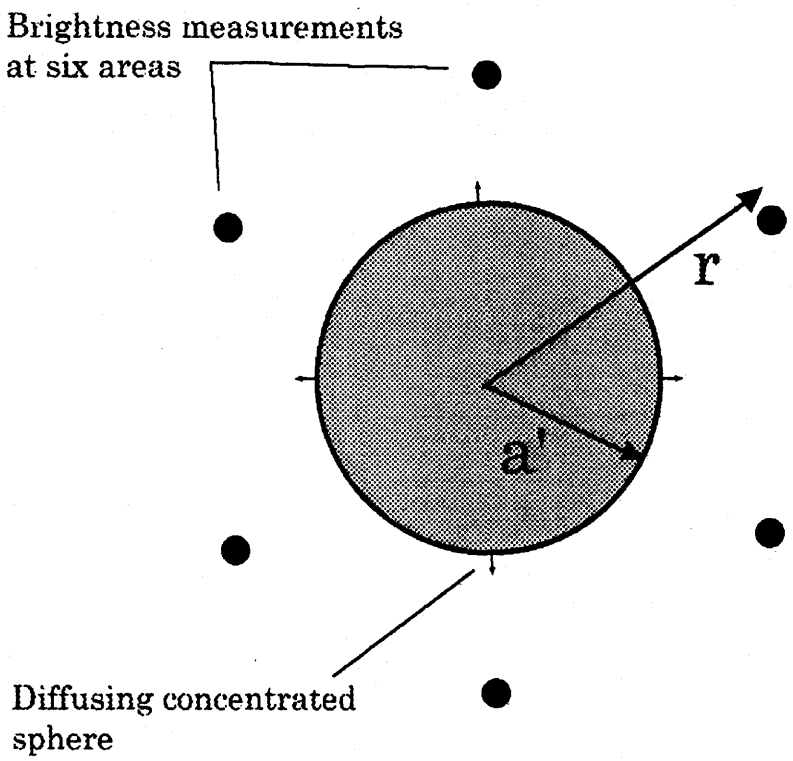


FIGURE 3.5.2.2. A plan view showing the diffusing sphere methodology. Average brightness at six areas equidistant from the centre of the diffusing sphere were measured at every time step and combined. The combined brightness values versus time and the analytical model were used to determine free-water diffusion coefficients.

3.5.3 Double reservoir diffusion technique

Double reservoir diffusion cells and single reservoir diffusion cells have been used to characterize the adsorption and diffusion of complex contaminants in geological materials (Barone et al., 1992a; Barone et al., 1992b; Donahue, 1994; Rowe, 1988). Breakthrough and contaminant profiles from these types of tests using field specimens were consistent with parameters back-calculated from field observations (Quigley et al., 1987).

To gain a better understanding of bacterial transport and attenuation bacterial sorption under diffusion-dominated conditions, bacterial transport was examined in this thesis using the double reservoir diffusion technique and a suspension of *P. fl.* m18 in AGW (Rowe, 1988). The double reservoir diffusion model would provide a better enumeration of bacterial concentrations in the model and allow for repeated measurement of bacteria in the aqueous phase.

A schematic diagram of the physical model used in this investigation is shown in Fig. 3.5.3.1. The physical model consisted of two - 76.2 mm (ID) polyvinyl chloride (PVC) female adapters (SOC × FPT), which are referred to as half-cells. The half-cells were machined to a depth of 5.0 mm on the socket ends (Fig. 3.5.3.1). The machined socket ends of the half-cells were fitted together with a PVC sample ring (Fig. 3.5.3.2) inserted between them. The threaded ends of the half-cells were closed

and sealed with rubber o-rings and end plugs. Four identical physical models were constructed and used in replicate double reservoir diffusion bacterial tests.

The sample ring was filled with uniform medium grained silica sand (Unimin Corp., Le Seur, MN). The average grain size, by sieve analysis, was 1.03 mm and <10%(w/w) of the sand was smaller than 840 μm. The porosity of the completed sample ring was 0.40, by gravimetric mass analysis.

Nylon textile mesh (Tetko Inc., Cleveland OH) (openings 600 μm × 600 μm) was placed on either side of the sand filled sample ring, trimmed, and fixed against the face of the sample ring with silicone. The completed sample ring was inserted into the socket ends of each half-cell and sealed with silicone.

The physical models were designed to allow for measurement of Cl^- and aqueous bacterial concentrations in each reservoir, with minimal disturbance to the experiment. In this study, the term aqueous bacteria refers to planktonic bacteria that are suspended in fluid, thus are not attached to a solid surface. Two stainless steel rods (1.57 mm diameter) were mounted into each half-cell (Fig. 3.5.3.1). Real time Cl^- concentrations were determined by measuring the fluid electrical resistivity between the two rods (Allen and Taylor, 1923). Using a datalogger (21X, Campbell Scientific Inc., Edmonton, AB) and an AC half-bridge measurement, the ratio of output voltage to input voltage ($volts \cdot volts^{-1}$) was measured, timed, and stored. The stored voltage ratios were converted to Cl^- concentrations ($mg \cdot L^{-1}$) using calibration curves.

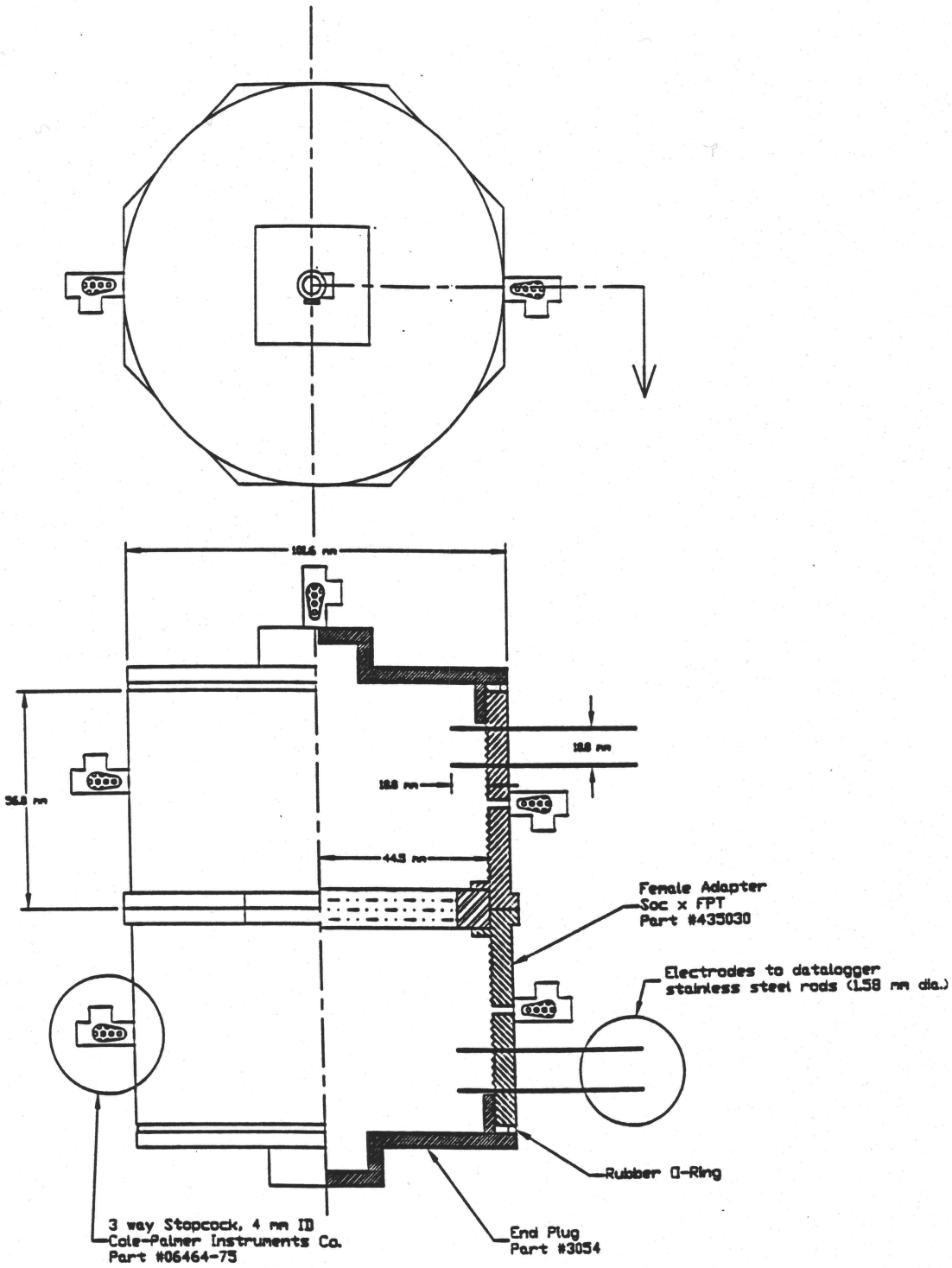


FIGURE 3.5.3.1. Double reservoir diffusion model. The model was constructed with two 3.5 inch (ID) PVC female adapters, two end plugs, and a 3.5 inch (OD) PVC sample ring packed with medium grained silica sand.

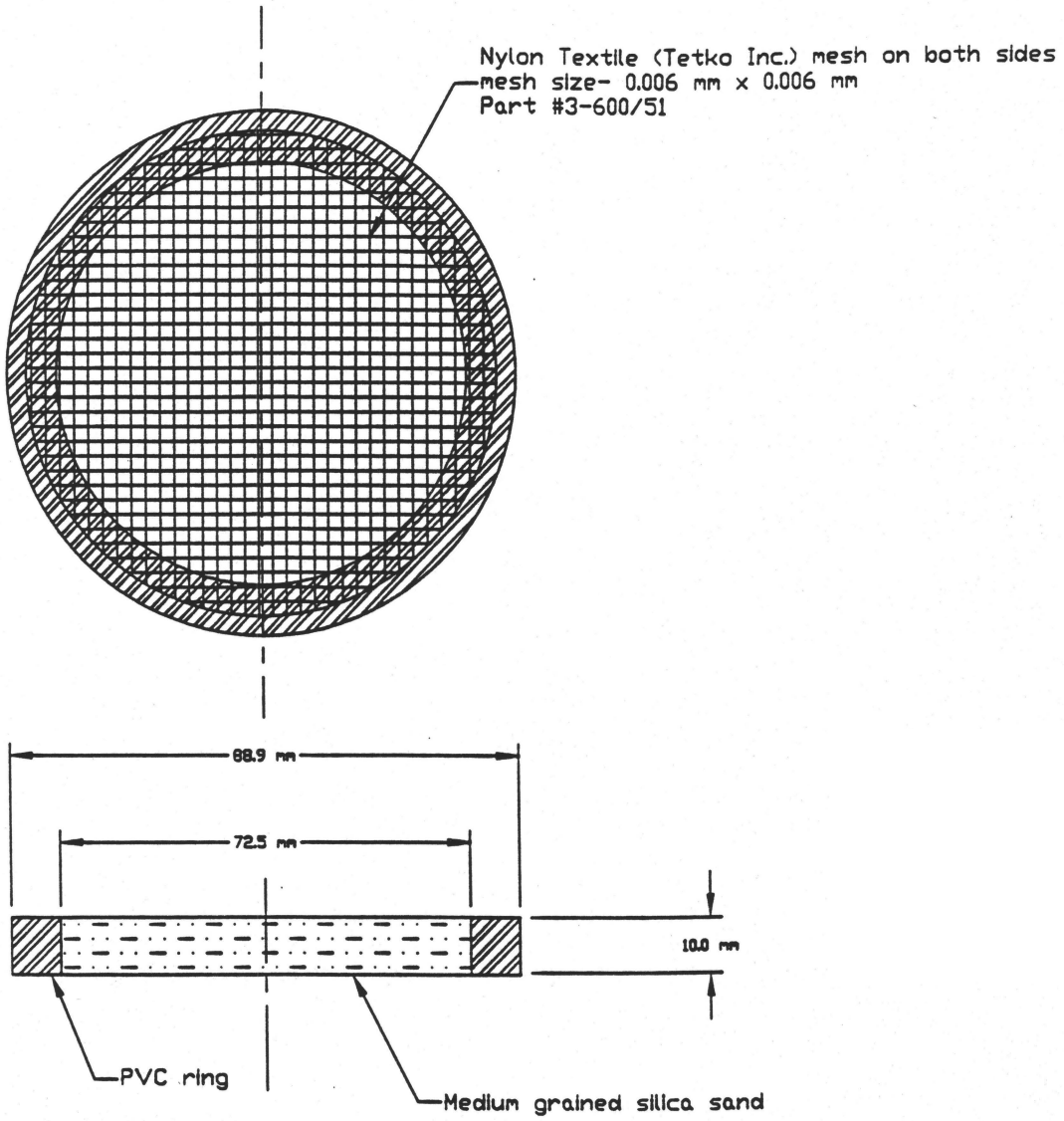


FIGURE 3.5.3.2. Sample ring for the double reservoir diffusion model. A 3.5 inch (OD) PVC ring was filled with media grained silica sand and nylon textile mesh fixed on either side.

Calibration curves unique to each half-cell (for all calibration curves $r^2>0.995$, n=8) were determined prior to the double reservoir diffusion tests by saturating each completed double reservoir diffusion model with a range of AGW and chloride solutions (0 to 100 $mg \cdot L^{-1}$ as Cl^-) and measuring the fluid resistivity.

Two 3-way stopcocks installed on each reservoir (Fig. 3.5.3.1) allowed daily sampling to determine viable aqueous bacterial concentrations. Each double reservoir diffusion model was kept horizontal in wooden cradles to eliminate an elevation gradient across the sand-packed sample ring.

The set-up for the double reservoir diffusion experiments is shown in Fig. 3.5.3.3. Constant aqueous concentrations of bacteria and Cl^- were maintained in the source reservoirs by continuous re-circulation from a source flask. Continuous re-circulation provided close monitoring of source reservoir concentrations and contamination of the diffusion cells. Daily samples were taken to determine the bacterial concentrations in the source flask, recharge flask, and the four collection reservoirs. During the sampling period, the pump was stopped, the valves to the source reservoirs were closed, and each diffusion model was gently rocked for ~30 seconds. While sampling, approximately 3 mL of fluid was removed from each collection reservoir, replaced with an equivalent volume of sterile AGW, and then each diffusion model was again gently rocked for ~30 seconds. After sampling, the valves to the pump were opened and the pump was turned on again. For one double reservoir diffusion test the total

volume of fluid removed from the collection reservoirs over the test period (~800 hr) was <5% of the total fluid volume in each of the reservoirs.

An antibiotic (kanamycin, 50 $mg \cdot L^{-1}$) was added to all fluids used in the double reservoir diffusion tests to minimize contamination of the physical models. The antibiotic had no observed effect on the half-cell chloride calibration curves. Concentration of viable aqueous bacteria in each reservoir, the source flask, and the recharge flask were determined by plate counts on selective growth media plates (50% tryptic soy agar with kanamycin at a concentration of 50 $mg \cdot L^{-1}$).

Results from the double reservoir diffusion tests were normalized (C/C_o) to relative concentrations prior to modelling. Normalized concentrations were modelled using POLLUTE™ (ver6), a commercial one-dimensional transport model (Rowe et al., 1983). The best-fit model was determined visually by a trial and error method.

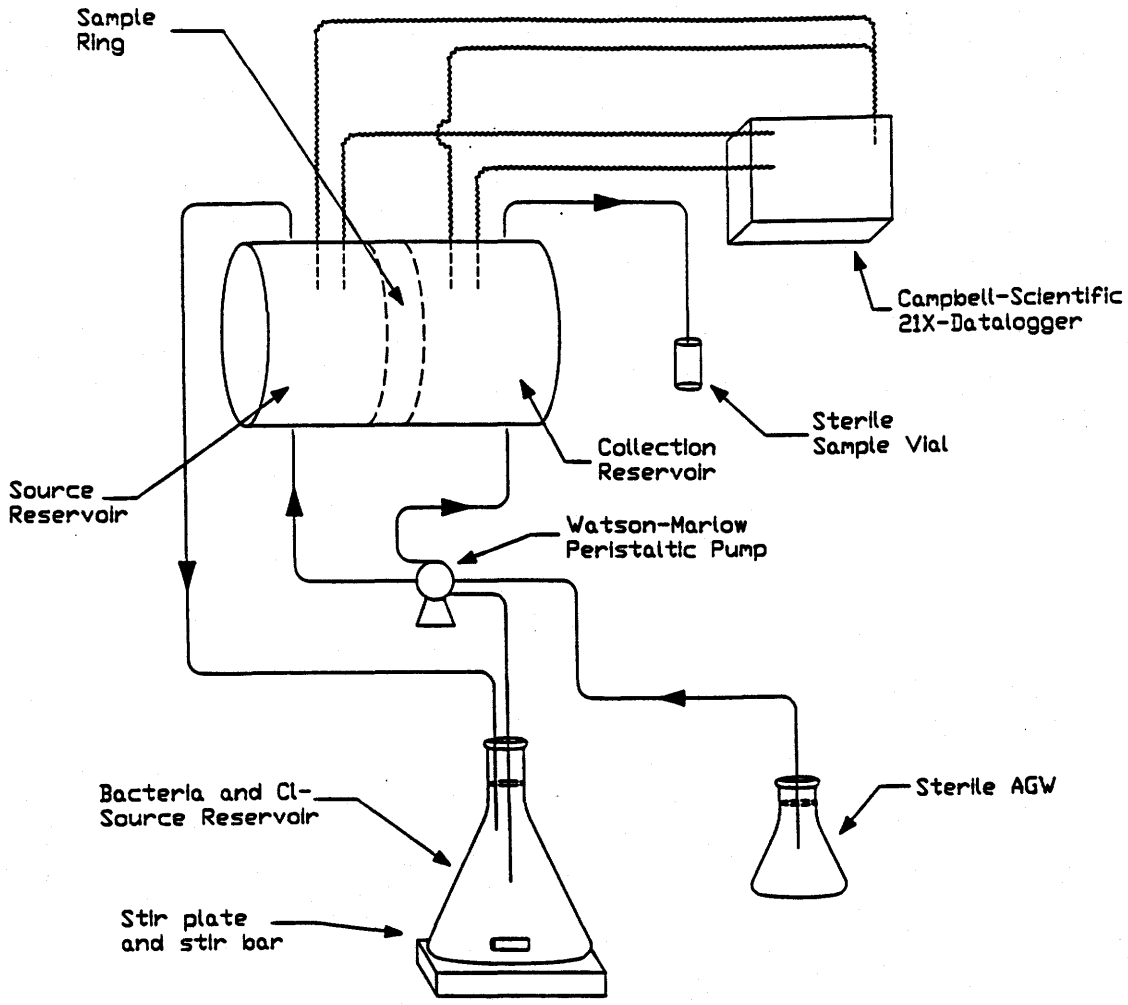


FIGURE 3.5.3.3. Experimental set-up for the double reservoir diffusion tests. This figure shows the set-up for one of the four double reservoir diffusion models. A measured concentration of bacteria and Cl^- was maintained in one reservoir and the change in concentration of both the bacteria and the Cl^- was measured in the other reservoir.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization of the bacteria

Many bacterial properties affect the diffusive transport and attenuation of bacteria, including: bacterial suspension viscosity, fluid bulk density, bacterial cell density, and bacterial cell hydrophobicity. Therefore prior to the bacterial diffusion experiments, the five bacterial strains were characterized.

4.1.1 Viscosity of the bacterial suspensions

The Einstein-Stokes equation provides a good order of magnitude estimate of colloidal diffusion rates. Many authors have used this estimate in bacterial studies (Jang, 1985; McDowell-Boyer et al., 1986; Wilke and Chang, 1955; Yao et al., 1971). The fluid viscosity value used by these authors in the Einstein-Stokes equation either was the value for water at the same temperature or was left undeclared. However, no justification was found in these studies for assuming the viscosity of water in the estimate of bacterial diffusion. Viscosity values for water are well constrained over common groundwater temperatures (4 to 20°C, 1.567 to 1.002 $g \cdot sec^{-1} \cdot m^{-1}$) (Freeze and

Cherry, 1979; Weast, 1987). However, experiments were conducted to determine the viscosity of bacterial suspensions at 25°C.

The viscosity of the bacterial working suspensions (25°C, 1×10^{8} CFU·mL^{-1}) for the five bacteria were compared to deionized water using an Ostwald viscometer (Daniels et al., 1962). There was no discernable difference between the deionized water and the viscosity of the bacterial working suspensions. Thus, the equivalent viscosity of water at 25°C was assumed in estimates of bacterial diffusion values (0.8904 g·sec^{-1}·m^{-1} within an experimental error of ± 0.019 g·sec^{-1}·m^{-1}).

4.1.2 Liquid bulk densities of the bacterial suspensions

The liquid bulk densities (25°C, 1×10^{8} CFU·mL^{-1}) of all five bacterial working suspensions were checked using a gravimetric method. No differences were observed between the density of the bacterial working suspensions and that for AGW. A density of 0.99707 g·mL^{-1} within an experimental error of ± 0.00099 g·mL^{-1} was determined for both fluids (25°C). The close similarity between the density of the bacterial suspensions and the density of AGW indicated that there should be minimal density gradient effects between the source reservoir and the collection reservoir during the double reservoir diffusion tests.

4.1.3 Settling of stained and unstained bacterial suspensions

Prior to conducting the diffusing sphere experiments, measurements were made of the possible settling of stained and unstained bacterial suspensions using a Klett

Colorimeter (Klett Manufacturing Co., NY). Measurements were made after 1, 10, and 60 minutes. No turbidity changes were observed in any of the working suspensions over the 60 minute observation period. The absence of turbidity change in the bacterial suspensions indicated a lack of settling of the bacteria. Thus, over the time period of the diffusing sphere experiments (<5 minutes) settling would not be concern. However, some settling was observed in suspensions left undisturbed for ~24 hr. This posed a concern in the double reservoir diffusion tests. Large amounts of settling in the double reservoir diffusion tests would reduce the quantity of bacteria in suspension and therefore reduce the diffusive flux through the porous sample ring. However, daily agitation of both the source and collection reservoirs may help to mitigate bacteria settling out of suspension.

4.1.4 Bacterial density determinations

Although bacterial suspensions remained well dispersed for >60 minutes (from the settling experiments, Section 4.1.3), there still remained a concern that bacterial densities [$M \cdot L^{-3}$] might adversely influence the bacterial diffusion experiments. In these supplementary experiments, the density of the five bacteria were determined using a density gradient centrifuge method with Percoll® and coloured density marker beads. The results of centrifugation tests for the five bacteria are presented in Table 4.1.4.1.

Table 4.1.4.1. Mean density [$M \cdot L^{-3}$] of bacterial cells determined using a density gradient centrifugation.

Bacterium	Density ($g \cdot cm^{-3}$)			Increase in turbidity
	Low	Mean	High	
P. fl. 840406-E	1.072	1.085	1.133	Fast
P. fl. m18	1.074	1.094	1.135	Fast
P. 5	1.074	1.092	1.137	Fast
G4PR1	0.951	1.051	1.061	Slow
K. oxytoca	0.936	1.052	1.061	Slow

After centrifuging, the density marker beads formed a narrow layer and the bacteria settled over a small range which is linearly defined as: high, mean and low. Although identical culturing, harvesting, and preparation techniques were used for the five bacteria variance, in the turbidity of the growth medium as the bacteria multiplied indicated potentially different growth rates between the strains. Different growth rates would influence bacterial cell properties at the time of harvesting the bacterial cultures (White et al., 1996). The slower growing bacteria would have a younger mean cell population than the older cell populations. This could affect the mean cell density determined with the density gradient centrifugation tests. This explanation agrees with the changes in turbidity observed prior to harvesting the bacteria. The density gradient tests showed that the faster growing or older bacteria were denser than the slower growing or younger bacteria.

Guerrera (1985) used Percoll® density gradients to determine the gravimetric density of 18 different bacterial strains obtained from lake and rock samples, as well as other pure cultures. The author found an average and standard deviation of bacterial gravimetric densities of 1.065 ± 0.036 g·cm^{-3}. Likewise, Harvey et al. (1997) found an average and standard deviation bacterial gravimetric density for laboratory cultured groundwater bacteria of 1.068 ± 0.021 g·cm^{-3}. These values compared well with the average gravimetric density determined in these experiments (average and standard deviation 1.075 ± 0.022 g·cm^{-3}).

4.1.5 Bacterial cell hydrophobicity

Bacterial cell surface hydrophobicity and contact angle play a significant role in determining bacterial sorption (van Loosdrecht et al., 1990). However, because of the many factors controlling bacterial transport and attenuation (e.g., groundwater chemistry and velocity, bacterial growth and death), results from hydrophobicity tests have not correlated well with bacterial sorption to porous media (Gannon et al., 1991a; Lindqvist and Bengtsson, 1991; Lindqvist and Bengtsson, 1995; van Loosdrecht et al., 1990).

A comparative analysis of the results of the double reservoir diffusion tests for each of the five bacteria would require an understanding of the bacterial surface characteristics. Qualitative hydrophobicity tests were used to further the understanding of the bacterial surface characteristics. The results of the hydrophobicity tests for the five bacteria using the n-hexadecane method (Rosenberg,

1984) are presented in Table 4.1.5.1. There was no observed difference between the hydrophobicities determined after the two time intervals (5 and 30 minutes).

Table 4.1.5.1. Hydrophobicities of the bacteria using the n-hexadecane method.

Bacterium	Hydrophobicity
P. fl. 840406-E	Very hydrophilic
P. fl. m18	Very hydrophilic
P. 5	Slightly hydrophobic
G4PR1	Slightly hydrophilic
K. oxytoca	Very hydrophilic

The n-hexadecane tests provide a general indication of bacterial strain dependent attenuation possible during the double reservoir diffusion experiments. In batch sorption tests, van Loosdrecht et al. (1990) found that bacteria that were more hydrophobic, as determined through n-hexadecane tests, showed greater sorption to geological material. The hydrophobicity tests indicated that P. 5 was the most hydrophobic of all the bacteria used in this study. Therefore, P. 5 would likely be attenuated more in the porous media during the double reservoir diffusion tests than the four other bacterial strains.

4.2 Diffusion test techniques

4.2.1 Dynamic light scattering technique

The DLS technique has been successfully used to determine D_o values for bacteria in a suspension (Rijnaarts et al., 1993). Rijnaarts et al. (1993) determined D_o values for bacteria using a photo correlation spectroscopy technique described in detail by Pecora (1985). Calculated values of D_B using measured bacterial radii closely approximate D_o values from DLS measurements (Table 1.2.1, from Rijnaarts et al. 1993).

Both the double reservoir diffusion technique and the diffusing sphere technique are used to quantify bacterial diffusion by observing changes in bacterial concentration occurring because of bacterial diffusion. In contrast, dynamic light scattering (DLS) measurements were made of the five bacteria used in this thesis to directly determine D_o values. DLS has been successfully applied to isolate and determine D_o values of both motile and non-motile bacteria (Pusey et al., 1974). Applying the DLS technique, D_o values for the five bacteria were determined with no incorporation of motility (Table 4.2.1.1).

Table 4.2.1.1. D_o, calculated bacterial radius, volume-fraction, and volume-fraction corrected bacterial radius from dynamic light scattering measurements.

Bacterium	Diffusion coefficient D_o ($m^2 \cdot sec^{-1}$)	Calculated radius (μm)	ϕ (Eq. 5)	Volume-fraction corrected radius (μm) (Eq. 4)
P. fl. 840406-E	4.87×10^{-13}	0.49 ± 0.05	0.002	0.49
P. fl. m18	3.74×10^{-13}	0.64 ± 0.05	0.005	0.64
P. 5	2.57×10^{-13}	0.93 ± 0.07	0.015	0.95
G4PR1	1.95×10^{-13}	1.22 ± 0.17	0.034	1.28
K. oxytoca	1.65×10^{-13}	1.44 ± 0.22	0.054	1.55

Using the measured D_o values from the DLS measurements, the average bacterial radii were determined using the Einstein-Stokes equation (Eq. 1). With the calculated bacterial radii, the volume-fraction (Eq. 4) of each tested sample was determined assuming a water molecule radius of 2.5 Å (Weast, 1987) and a bacterial and molecule concentration of 1×10^7 CFU·mL^{-1} and 3.34×10^{22} molecules·mL^{-1}, respectively. A corrected average bacterial radius was determined using the calculated volume-fraction (Table 4.2.1.1). Since the volume-fractions were relatively low (<0.1) there was only a small change in the representative bacterial radii. Under these conditions, the bacterial radii may be determined directly from D_o without correction for the volume-fraction (Eq. 1). The variance in the calculated volume-fractions, shown in Table 4.2.1.1, is caused by the difference in the bacterial radii calculated with the Einstein-Stokes equation.

The DLS measured D_o values were compared to the D_B and $D_B(\phi)$ estimates from measured bacterial dimensions for *K. oxytoca*, G4PR1, and *P.* 5 (Section 3.4). Though the bacteria used in this study were rod-shaped, an assumption was necessary for calculation of a representative bacterial radii. This was done using measurements of bacterial length (l) and width (w) from phase-contrast microscopy examination of a wet mount slide preparation. The bacterial radii were then estimated using the square-area approximation ($\{0.5\cdot[l\cdot w]^{1/2}\}$, Rijnaarts et al., 1993). D_B was calculated assuming $kT = 4.08\times10^{-17}$ kg·cm^2·sec^{-2} and $\mu = 9.55\times10^{-6}$ kg·cm^{-1}·sec^{-1}. $D_B(\phi)$ was calculated using D_B; an average water molecule radius of 2.5 Å (Weast, 1987); and water molecule and bacterial concentration of 3.34×10^{22} molecules·mL^{-1} and 1×10^{7} CFU·mL^{-1}, respectively. The measured D_o values and the calculated D_B and $D_B(\phi)$ estimates were similar (average % difference, 36%). Thus, the Einstein-Stokes equation and the volume-fraction modified Einstein-Stokes equation provided very good order of magnitude estimates of bacterial diffusion coefficients in comparison to DLS determined D_o values (Table 4.2.1.2).

Table 4.2.1.2. Calculated D_B and $D_B(\phi)$ values based on estimates of bacterial radius. DLS measured D_o values from this thesis are presented for comparison of measured to estimated aqueous bacterial diffusion coefficients.

Bacterium	Estimated radius (μm)	D_B ($m^2 \cdot sec^{-1}$)	$D_B(\phi)$ ($m^2 \cdot sec^{-1}$)	D_o ($m^2 \cdot sec^{-1}$)
P. 5	0.92	2.5×10^{-13}	2.5×10^{-13}	2.6×10^{-13}
G4PR1	0.67	3.4×10^{-13}	3.4×10^{-13}	2.0×10^{-13}
K. oxytoca	0.53	4.3×10^{-13}	4.3×10^{-13}	1.7×10^{-13}

The average *P.* 5 bacterial radius estimated (Eq. 1) from DLS measurements (0.93 μm) was very similar to the radius estimate ($\{0.5 \cdot [l \cdot w]^{1/2}\}$ from phase-contrast microscopy measurements (0.92 μm). However, the DLS determined bacterial radii for *K. oxytoca* and G4PR1 (1.22 and 1.44 μm, respectively) were different than those estimated from phase-contrast microscopy measurements (0.67 and 0.53 μm; percent difference 82 and 172%, respectively). This indicated that bacterial clumping may have occurred. Bacterial size and shape is suggested as playing an important role in bacterial transport and attenuation in porous media (Rijnaarts et al., 1996a). Preferential clumping of bacterial would affect the cell/ pore ratio (McDowell-Boyer et al., 1986) and increase the attenuation of bacteria being transported through porous media.

4.2.2 Diffusing sphere technique

Bacterial diffusion was examined with a physical model and method developed as part of this thesis, the diffusing sphere technique. Initial experiments using the diffusing sphere technique were conducted by injecting a small volume of fluorescein into an AGW solution. Figure 4.2.2.1 is a typical concentration dissipation curve for the diffusion of fluorescein using the diffusing sphere technique. Presented on Figure 4.2.2.1 is the observed fluorescein diffusion data and the best-fit simulation using the analytical solution (Eq. 7) fitted visually by a trial and error method. The average D_o value for fluorescein from modelling the concentration dissipation curves of $4.4\times10^{-10} \pm 1.5\times10^{-10}$ $m^2 \cdot sec^{-1}$ was similar to reported D_o values for fluorescein of 5.5×10^{-10} $m^2 \cdot sec^{-1}$ (Berg and Brown, 1972) and 3.3×10^{-10} $m^2 \cdot sec^{-1}$ (Lawrence et al., 1994). In both the fluorescein and later bacterial diffusion experiments, the concentration rapidly increased to a peak concentration and gradually diminished after that point. The maximum concentration was measured as a peak brightness (C_{MAX}).

Diffusing sphere experiments were conducted with all bacteria using the same injection, analysis, and modelling technique. A suspension of stained bacteria was injected into a suspension of unstained bacteria. All five bacterial strains were used in separate mono-culture experiments. During these experiments, literally, thousands of attempts were made trying to apply the diffusing sphere technique to each of the bacterial suspensions. The key problem in applying the technique to the stained bacteria was that the sphere of stained bacteria kept sinking into the surrounding

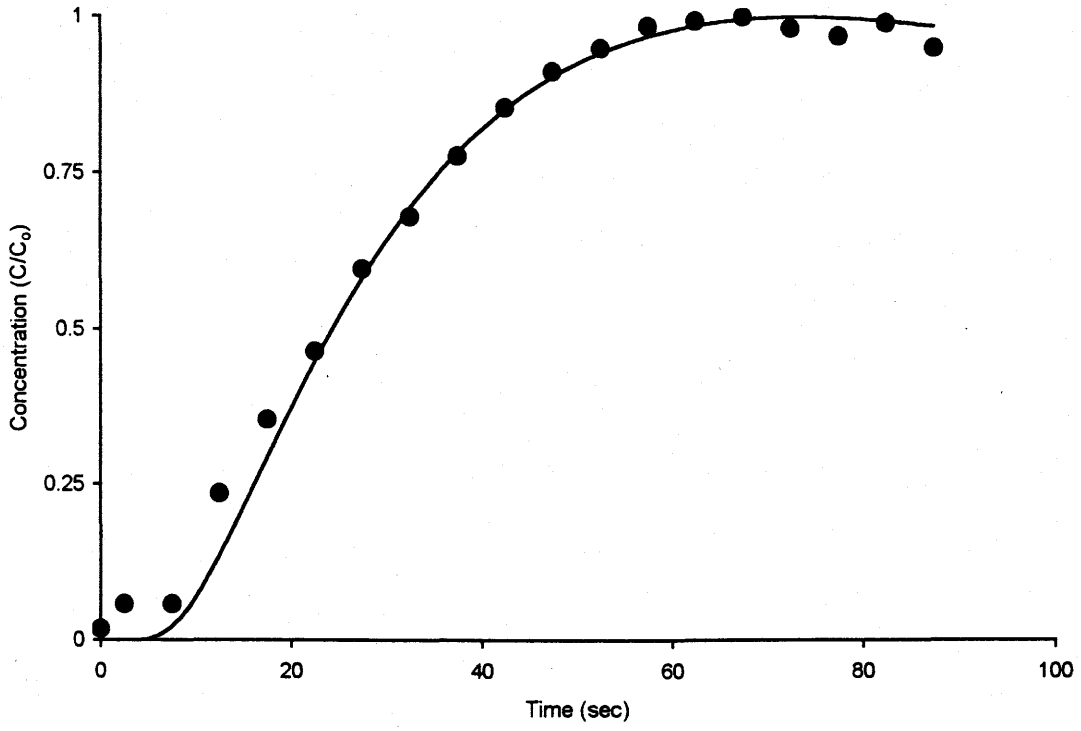


FIGURE 4.2.2.1. Typical dissipation curve found for fluorescein using the diffusing sphere technique. In this example, the diffusion of a sphere (r = 251 μm) of fluorescein was measured at a distance of 477 μm from the centre of the sphere (solid circles). A best-fit analytical model (solid line) of the data was determined with a D_o value of 4.2×10^{-10} $m^2\cdot sec^{-1}$.

suspension shortly after injection (~4 mm in 20 seconds). Little lateral spreading was observed while the sphere of stained bacteria sank into the surrounding unstained bacterial suspension (~20 seconds).

There was no obvious reason for the sinking of the stained bacterial suspension. The concentrations of bacteria in the sphere and the surrounding suspension were nearly identical. After staining, the percent difference between the concentrations of bacteria in the stained and unstained suspensions was <0.4%. The preparation technique of the concentrated sphere and the surrounding fluid were identical except for the final step, the staining. No published physiological effects of the stain on bacteria were found, so staining effects on the bacteria were not known.

Experiments to observe and compare settling rates of the stained and the unstained suspensions in test tubes indicated no observable settling of either the stained or the unstained suspension (Section 4.1.3). Therefore, it was unlikely that the sphere of stained bacteria was sinking because of density differences between the fluid and the stained bacteria. Initial work with fluorescein indicated that the diffusing sphere technique was satisfactory for a dissolved solute and allowed a concentrated sphere of fluorescein to gradually diffuse outwards without interference. Yet, the technique was not applicable to stained and unstained bacterial suspensions. The potential causes of the sinking diffusing sphere problems included: laser heating of the bacteria altering the density, micro-currents in the physical model remaining after rinsing, and stain influence on gravimetric density of bacterial cells. An additional mechanism that may

cause sinking of the sphere of stained bacteria is clumping of the bacteria in the either the stained or the unstained suspension. No testing was done of the difference in aggregation of bacteria in the stained injected bacterial suspension and in the unstained bacterial suspension.

No analysis was done of bacterial cell gravimetric density changes resulting from heating of the bacterial cells with an incident scanning confocal laser. However, it is doubtful that preferential heating of the bacteria by the scanning confocal laser microscope was the cause of the sinking bacteria. Heating of the stained bacteria would have decreased their gravimetric density and caused the sphere to rise, not fall. It was unclear if currents in the flow cell remained after rinsing the last stained sphere out of the flow cell. Small currents in the flow cell could disrupt the required diffusion-dominated conditions. The time between experiments was varied (10 seconds to 15 minutes) to identify how long currents were present in the physical model after rinsing. After ~1 minute delay between rinsing and injection of the next sphere of stained bacteria, there were no observable current effects on the injected sphere of stained bacteria. Except for the stain, both the stained and the unstained bacterial suspensions were identically prepared. If the stain was the cause of stained sphere sinking, the difference in gravimetric density of the bacteria [$M \cdot L^{-3}$] would have been observable during the time period of the settling experiments (60 minutes, Section 4.1.3). Modelling the diffusion of the stained bacteria could not be conducted because the sphere of stained bacteria sank so rapidly. No cause has been established for the sinking of the sphere of stained bacteria.

4.2.3 Double reservoir diffusion technique

Many physical models are available to examine the transport and attenuation of dissolved ionic species in geological materials (e.g., single reservoir diffusion cells, double reservoir diffusion cells, column tests, and batch tests) (Kemper and van Shaik, 1966; Quigley et al., 1987; Shackelford, 1991). Double reservoir and single reservoir diffusion cells have been used to study adsorption and diffusion of volatile organics in a clayey soil (Barone et al., 1992b; Donahue, 1994). The single and double reservoir diffusion cells provide a solids-liquid ratio comparable to that found in the subsurface (Freeze and Cherry, 1979). Thus, they may provide a better approximation of bacterial sorption to geological materials under diffusion-dominated conditions than the batch test method (Voice et al., 1983). In addition, parameters obtained from double reservoir diffusion tests have correlated well with field observations (Quigley et al., 1987). In this thesis, double reservoir diffusion tests were used to define an effective bacterial diffusion coefficient D_e (where, $D_e = \tau \cdot D_o$) through a saturated quartz sand media.

The initial double reservoir test results for Cl^- diffusion and the modelled best-fit curve for the Cl^- breakthrough data are presented in Fig. 4.2.3.1. The Cl^- data presented in Fig. 4.2.3.1 were the average concentrations for all four collection reservoirs at every time interval. Previous results from double reservoir diffusion tests using K^+, Cl^-, and Na^+ have shown a characteristic breakthrough curve shape (i.e., initial lag, exponential increase, and an exponential decrease leveling off to a plateau) (Rowe, 1988). The Cl^-

data followed this expected trend (Fig. 4.2.3.1), thus indicating diffusion was likely the dominant transport mechanism for the Cl^- in these double reservoir diffusion experiments.

The modelled (POLLUTE™) best-fit effective diffusion coefficient for Cl^- was 6.1×10^{-10} $m^2\cdot sec^{-1}$. Using the published D_o value for Cl^- at 25°C (2.03×10^{-9} $m^2\cdot sec^{-1}$, Li and Gregory, 1974) and Eq. 6, a τ value of 0.30 was found. This τ value compares well with the range of τ values for conservative tracers (0.5 to 0.01) diffusing in geological materials (Freeze and Cherry, 1979). Alternatively, the D_e value for Cl^- in quartz sand during the double reservoir diffusion tests (assuming $\tau = 0.3$ and the previously stated D_o value) was 6.1×10^{-10} $m^2\cdot sec^{-1}$. Previously stated D_e values for Cl^- in undisturbed clayey samples in double reservoir diffusion tests range from 5.7×10^{-10} to 6×10^{-10} $m^2\cdot sec^{-1}$ (Rowe, 1988). The D_e value for Cl^- found during this thesis compared well with the previously stated D_e values for Cl^- (a percent difference of 7 and 2%, respectively). Though the D_e comparison was between two different types of geological materials, the validation with both the D_e and the τ values supported the use of this physical model and technique for determination of conservative dissolved species D_e values in the range of the Cl^- value.

Bacterial diffusion and attenuation was examined using the double reservoir diffusion technique and a suspension of *P. fl.* m18 in AGW. Breakthrough results of *P. fl.* m18

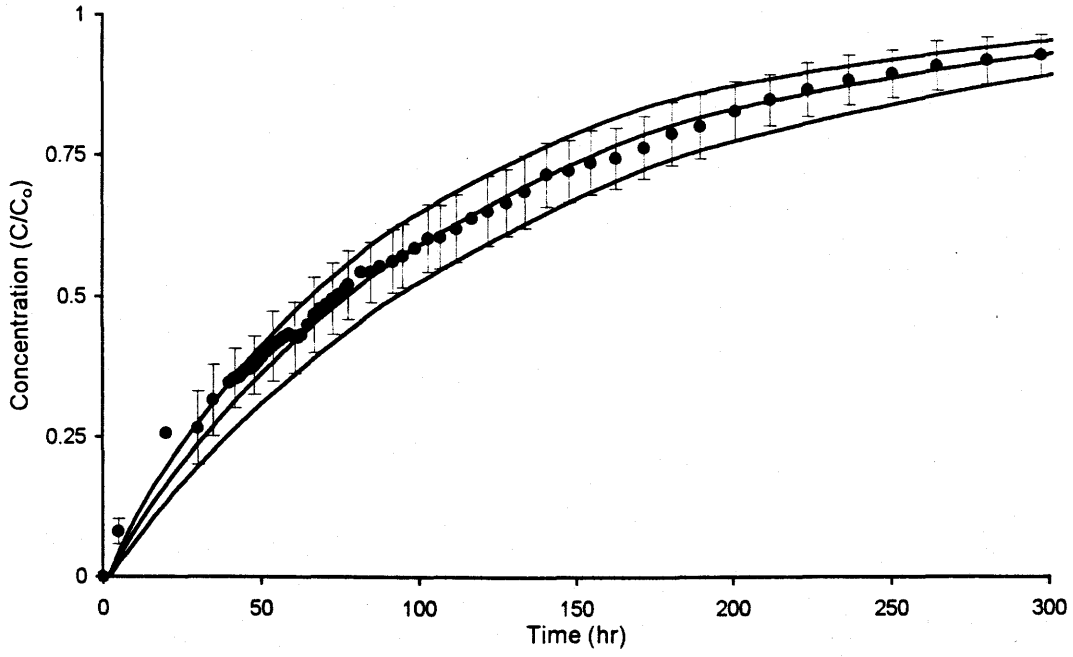


FIGURE 4.2.3.1. Breakthrough curve for Cl^- using the double reservoir diffusion technique. Average data with standard deviations for the four collection reservoirs are plotted as solid circles. The best-fit analytical models are represented by the plotted lines. The D_e values for the best-fit models are, from top to bottom: 7×10^{-10}, 6×10^{-10}, and 5×10^{-10} $m^2 \cdot sec^{-1}$, respectively.

shown as the average concentration of the four collection reservoirs along with the averaged Cl^- breakthrough curve are presented in Fig. 4.2.3.2. The breakthrough of *P. fl.* m18 was retarded compared to Cl^-. The shape of the Cl^- breakthrough data was similar to other double reservoir diffusion test results (Rowe, 1988). However, the shape of the *P. fl.* m18 breakthrough data was unusual. The *P. fl.* m18 breakthrough was rapid and lacked the gradual increase and plateau of most double reservoir diffusion test results.

The diffusion of *P. fl.* m18 in the double reservoir diffusion tests was simulated using an estimated D_e value for *P. fl.* m18. The D_e value for *P. fl.* m18 was estimated using the volume-fraction modified Einstein-Stokes equation (Eq. 4) and the τ determined from the Cl^- modelling. Assuming an average solvent molecule and bacterium radius of 2.5 Å (Moore, 1962; Weast, 1987) and 1.0 μm, respectively and a molecule and

bacterial concentration of 3.34×10^{22} molecules·mL^{-1} and 1×10^{8} CFU·mL^{-1}, respectively: then $\phi = 0.00019$. When $kT = 4.08\times10^{-17}$ kg·cm^2·sec^{-2}, $\mu = 9.55\times10^{-6}$ kg·cm^{-1}·sec^{-1}, and $P = 1\times10^{-4}$ cm: then $D_B(\phi) = 2.4\times10^{-13}$ m^2·sec^{-1}. A D_e for *P. fl.* m18 of 7.3×10^{-14} m^2·sec^{-1} was determined using the $D_B(\phi)$ estimate and the τ from the Cl^- modelling (0.30). Model simulations using the $D_B(\phi)$ derived estimate did not correspond well with the observed *P. fl.* m18 breakthrough data. The $D_B(\phi)$ modelled breakthrough reached a concentration of $C/C_o = 1\times10^{-25}$ after 800 hr, compared to the actual *P. fl.* m18 concentration of $C/C_o \sim 1$ after the same time. A

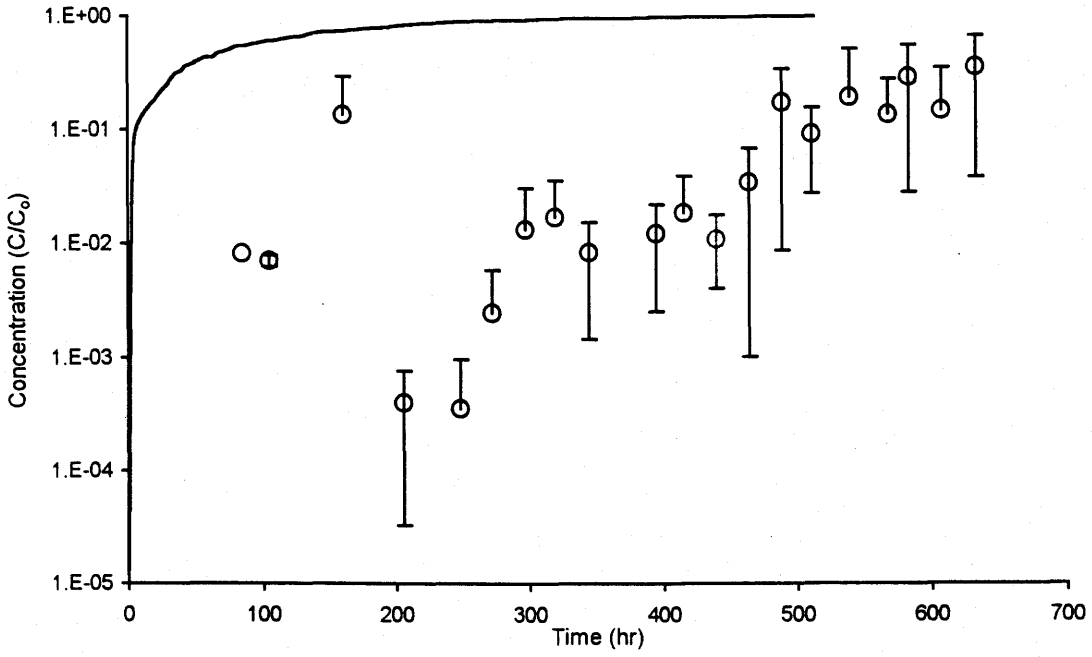


FIGURE 4.2.3.2. Breakthrough results from the double reservoir diffusion experiments for Cl^- and *P.fl.* m18. The average Cl^- breakthrough data is plotted as a solid line. The average and standard deviations of the *P.fl.* m18 breakthrough data for the four collection reservoirs from the double reservoir diffusion tests are represented by the circles.

concentration this low would not have been observable using the microbial analysis technique applied in this study. The minimum detectable concentration of viable bacteria was 1×10^{2} CFU·mL^{-1}, based on a 1 mL sample volume (Brock and Madigan, 1991).

Simulations of the effective diffusion of *P. fl.* m18 through the quartz sand were done with the τ value determined from the Cl^- modelling in this investigation. However, assuming the Cl^- determined τ value in these *P. fl.* m18 simulations was incorrect and introduced some error into the simulation. van der Kamp et al. (1996) showed with laboratory experiments that the effective porosity of a porous media was dependent on both the dissolved species and the porosity of the porous media. In the same manner, τ is a function of the flow path length through the porous media and the ability of the dissolved species (or bacteria) to move along this flow path. The representative *P. fl.* m18 radius is substantially larger than the Cl^- radius, approximately 1000 times larger. Thus, there would have been fewer flow paths available for the movement of *P. fl.* m18 than the Cl^-. Accordingly, the τ value for the *P. fl.* m18 would be less than the τ value for the Cl^-. Assuming the same τ value for both Cl^- and *P. fl.* m18 would have resulted in a slightly higher than actual D_e for *P. fl.* m18. Nevertheless, it provided a good conservative starting point for additional predictive modelling.

Literature values for bacterial D_o range from 8.7×10^{-11} m^2·sec^{-1} (Berg and Brown, 1972) to 1.74×10^{-13} m^2·sec^{-1} (Rijnaarts et al., 1993). Assuming a τ value of 0.30,

breakthrough curves were modelled with an equivalent range of bacterial D_e values (2.6×10^{-11} and 5.2×10^{-14} $m^2\cdot sec^{-1}$, respectively). The porosity determined from the gravimetric mass measurement (0.40) and measured dimensions of the physical model (Fig.'s 3.5.3.1 and 3.5.3.2) were used in the modelling simulations. Model simulations showed that the *P. fl.* m18 breakthrough concentrations found using a D_e value of 5.2×10^{-14} $m^2\cdot sec^{-1}$ would be below the minimum detectable bacterial concentrations during the time frame of the double reservoir diffusion experiments. Therefore, it was questionable that the *P. fl.* m18 breakthrough results were the result of bacterial diffusion alone.

A simulation of the *P. fl.* m18 double reservoir diffusion results using a D_e of 2.6×10^{-11} $m^2\cdot sec^{-1}$ (based on the D_o value of Berg and Brown, 1972) is plotted on Fig. 4.2.3.3. Though the simulation closely resembles the *P. fl.* m18 results, the D_o value found by Berg and Brown (1972) and used in this simulation, was for a motile bacteria. The bacterial strain used in the double reservoir diffusion tests, *P. fl.* m18, was a non-motile bacteria, verified before and after the diffusion tests by microscopic examination of a wet mount slide preparation. A D_o value as high as 2.6×10^{-11} $m^2\cdot sec^{-1}$ for *P. fl.* m18 would have been very unlikely. The D_o for *P. fl.* m18 would likely be at least two orders of magnitude lower than the motile D_o value of Berg and Brown (1972).

An estimate was made of the D_e value required to yield the *P. fl.* m18 breakthrough results found in this investigation. The best-fit model for the *P. fl.* m18 breakthroughs,

assuming diffusion was the only transport mechanism, was found when $D_e = \sim 1 \times 10^{-11}$ $m^2 \cdot sec^{-1}$ (Fig. 4.2.3.3). There was a large difference between this best-fit value and other stated D_e values for non-motile bacteria ($\sim 1 \times 10^{-13}$ $m^2 \cdot sec^{-1}$, from Rijnaarts et al., 1993). The unusually high best-fit D_e value for *P. fl.* m18 and the irregular shape of the *P. fl.* m18 breakthrough data indicated that other gradients were likely present in the double reservoir diffusion cells. Possible gradients included: temperature, elevation, bulk liquid counter-diffusion backflow (Russel et al., 1989), and pressure.

Modelling indicated that the *P. fl.* m18 concentration gradients and estimated bacterial diffusion coefficients were not large enough to cause the early *P. fl.* m18 breakthrough. Elevation and thermal gradients between the collection and source reservoirs were unlikely causes of the early *P. fl.* m18 breakthrough results. Each half-cell was at the same elevation and located in the same laboratory. Therefore, there would be negligible elevation and thermal gradients driving flow through the sample ring. Bulk flow of liquid in response to the net diffusive flux of *P. fl.* m18 and the Cl^- would be across the sample ring, in the opposite direction of the net *P. fl.* m18 and Cl^- flux. This would be a hindrance to the net diffusion of Cl^- and *P. fl.* m18, not a driving potential through the sample ring. However, pressure gradients could be formed in the diffusion models, likely caused by the sampling method used to determine the *P. fl.* m18 concentrations in the reservoirs. Pressure gradients across the sample ring could cause bulk fluid movement through the sample ring.

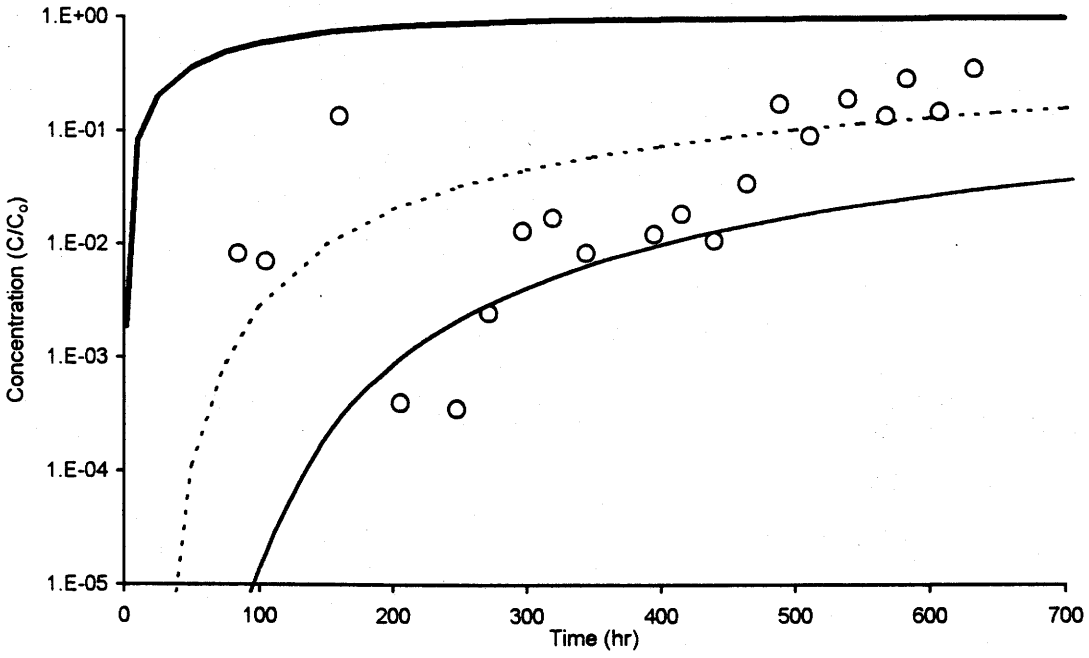


FIGURE 4.2.3.3. Simulations of the *P.fl.* m18 breakthrough results from the double reservoir diffusion tests. The *P.fl.* m18 breakthrough data (circles) is compared to simulations with D_e values of: 6×10^{-10} $m^2\cdot sec^{-1}$ (thick solid line), 2.6×10^{-11} $m^2\cdot sec^{-1}$ (dashed line), and 1×10^{-11} $m^2\cdot sec^{-1}$ (thin solid line).

Mathematical simulations were conducted to determine an equivalent velocity through the sample ring that would match the breakthrough data of *P. fl.* m18. The modelling was done by varying the velocity through the sample ring and using a fixed D_e value (7.3×10^{-14} $m^2 \cdot sec^{-1}$, from the $D_B(\phi)$ estimate). The best-fit advective-diffusive model for the bacterial breakthrough was found using an average linear velocity (Freeze and Cherry, 1979) of 1×10^{-9} $m \cdot sec^{-1}$. If the velocity was caused by the pressure gradient alone, an equivalent pressure drop across the sample ring can be calculated. A hydraulic conductivity of ~1 $cm \cdot sec^{-1}$ was estimated (Shepherd, 1989) using the average sand grain size determined from the sieve analysis. Assuming a permeable length of 1 cm through the sample ring, the equivalent pressure drop across the sample ring was calculated with Bernoulli's Law to be $\Delta P = 1\times10^{-3}$ Pa (Vennard and Street, 1982). Though no pressure differential measurements were made, available pressure measuring devices were evaluated for use in this application. The lowest pressure difference measurable at the time of the experiments using a Druck® pressure transducer (Druck Inc., New Fairfield, CT) was $\Delta P = 7.5$ Pa. This was well above the needed pressure differential measurement. Thus, it is probable that a pressure gradient influenced the movement of bacteria through the sample ring, but the equivalent pressure differential was below the minimum value measurable using available instrumentation. This modelled velocity is a representative velocity and while this was called an average linear velocity, the system can not be considered to be under steady-state conditions.

The P_e number has been used to demonstrate relative magnitudes of diffusion or advective-dispersion for fluid flowing through porous media (Perkins and Johnston, 1963). No reference has been found that used the P_e number to describe fluid flow conditions for bacterial transport. In Figure 4.2.3.4, the P_e number was used to explain the flow conditions that likely occurred during the *P. fl.* m18 double reservoir diffusion experiment. A dispersivity of 1×10^{-4} m or the straight-length path/100 (Gelhar, 1986; Gillham and Cherry, 1982) was used along with the previously estimated average linear fluid velocity and D_e value (1×10^{-9} m·sec^{-1} and 7.3×10^{-14} m^2·sec^{-1}, respectively) to determine the coefficient of D_h and the P_e number. A representative pore diameter of 1×10^{-3} m was estimated for the quartz sand (Krone et al., 1958) used in the double reservoir diffusion tests. Accordingly, the D_h value and P_e number determined for the *P. fl.* m18 results were: 2×10^{-13} m^2·sec^{-1} and 14, respectively. Comparatively, the D_h value and P_e number for the Cl^- results (6×10^{-10} m^2·sec^{-1} and 1.7×10^{-3}, respectively) have been calculated using the Cl^- D_e value and the same estimates for dispersivity and particle size. The results for Cl^- and *P. fl.* m18 from the double reservoir diffusion test are plotted on Figure 4.2.3.4.

The P_e number for the Cl^- data is well within the region defined as diffusion-dominated. This provides an understanding of the lack of velocity influence on the Cl^- movement, but not the *P. fl.* m18. However, there was a significant difference between the Cl^- and the *P. fl.* m18 D_o values. The D_o value for *P. fl.* m18 was approximately three orders of magnitude lower than that for Cl^-. Correspondingly, the large magnitude of the *P. fl.* m18 P_e value found during this

study indicates that diffusion-dominated conditions for the transport of *P. fl.* m18 were not likely present (Fig. 4.2.3.4). Thus with the same physical model and technique, maintaining diffusion-dominated conditions for Cl^- diffusion was attainable while not attainable for *P. fl.* m18.

Further investigation indicated that the Cl^- breakthrough would be unaffected by the small pressure-induced velocity that influenced the *P. fl.* m18 breakthrough results. The Cl^- results from the double reservoir diffusion test were simulated with two distinct scenarios: a combined advective-diffusive case and a diffusion only case. The applied simulation conditions were: combined advective-diffusive flow ($v = 1\times10^{-9}$ m·sec^{-1} and a dispersivity of 1×10^{-3} m and $D_e = 6.1\times10^{-10}$ m^2·sec^{-1}) and diffusion only ($D_e = 6.1\times10^{-10}$ m^2·sec^{-1}). Modelling results indicated there was a negligible percent difference between the diffusion case and the combined advective-diffusive case on the Cl^- breakthrough curves. After 800 hr, the maximum percent difference was 0.2%. Under the conditions of this experiment a velocity $>1\times10^{-7}$ m·sec^{-1} (yielding a hydrodynamic dispersion (Freeze and Cherry, 1979) of 7.1×10^{-10} m^2·sec^{-1}) would be required to make a noticeable change to the effective diffusion of Cl^- through the sample ring. Hence, the estimated velocity influencing the bacterial diffusion would have negligible effect on the diffusion of Cl^- during the course of the experiments.

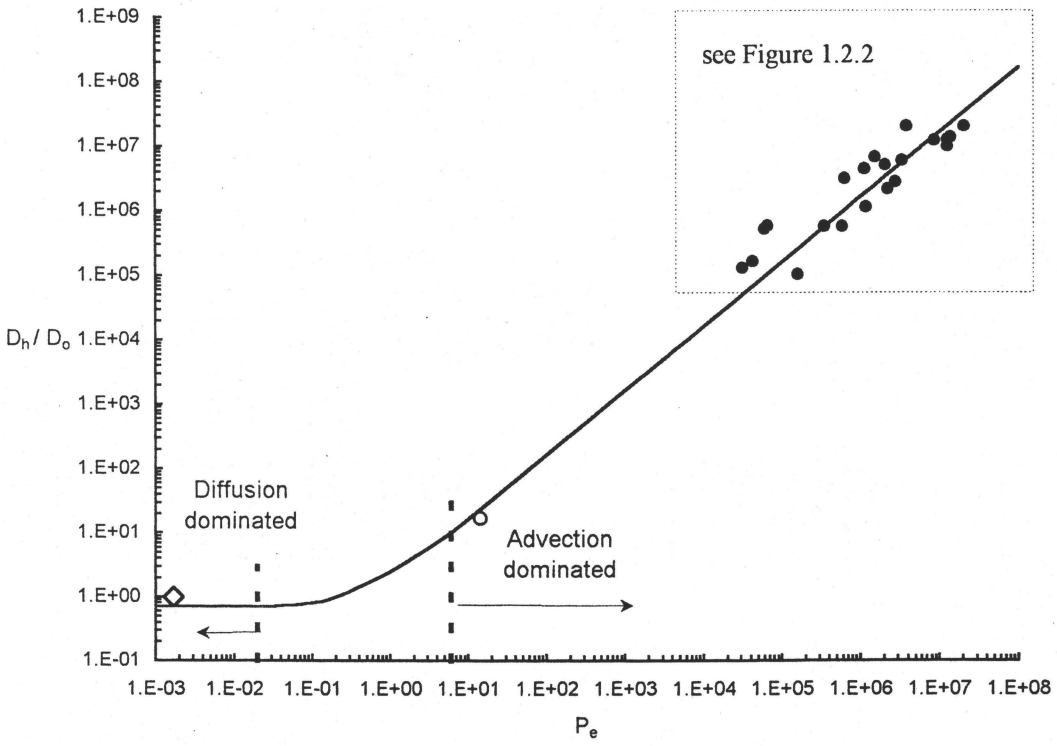


FIGURE 4.2.3.4. The Peclet plot showing the results from the *P. fl.* m18 double reservoir diffusion tests. Peclet numbers for: the Cl^- (plotted as a diamond) and *P. fl.* m18 (plotted as a circle) double reservoir diffusion results and the literature values shown in Fig. 1.2.2 (plotted as solid circles).

Measuring and accounting for a (likely) variable pressure gradient with a pressure and concentration gradient coupled advective-diffusive model would have been overly complex. Because it was assumed that the sampling caused the pressure gradient, methods were sought to reduce the pressure gradients. Since previous bacterial experiments (Hendry et al., 1999) have shown the need for repeated sampling to gather representative bacterial concentrations, multiple sampling was necessary and sampling at the close of the experiment, as provided in the single reservoir diffusion test, would not provide sufficient information for analytical modelling. Two possible solutions to the pressure gradient problem were: reduce the sampling frequency or shorten the duration of the experiments. In earlier tests conducted for this study, reducing the sampling frequency did little to reduce early breakthrough of the *P. fl.* m18. Thus, shortening the time period of the experiments was the only option left to fix the pressure gradient problem.

If the duration of the experiments was shortened, the cumulative sampling effects would be reduced. The double reservoir diffusion experiments could be shortened by either reducing the time to detection of the bacteria in the collection reservoir or decreasing the travel path of the bacteria. The travel path could have been shortened by either reducing the straight-line distance through the sample ring or increasing the τ value (Fetter, 1992). The thickness of the sample rings used in all of the double reservoir diffusion tests was 10 mm. Sample rings <10 mm thick, though easily constructed, proved to be too difficult to pack with sand and did not seal properly with

the two half-cells. By increasing the average particle size of the sand, the τ value increased. However, the τ value determined from the Cl^- modelling (0.30) was already large and changes to the sand size would not have a large impact. The next alternative was to decrease the collection reservoir volume. This would decrease the time required to detect the bacteria in the collection reservoir and increase the sensitivity of the bacterial measurements. Two approaches were tested to reduce the collection reservoir volume. First, an impermeable plug fixed into the collection reservoir or second, marbles packed into the collection reservoir. The two approaches reduced the collection reservoir volume by 38 and 41%, respectively. A volume decrease of 41% in the collection reservoir was modelled assuming a $D_e = 7.3\times10^{-14}$ $m^2 \cdot sec^{-1}$. The 41% porosity reduction increased the predicted Cl^- concentrations by an average of 50% over a time period of 500 hr. This was not enough of an improvement to warrant further development.

Although the double reservoir diffusion model was tested with only one bacterial strain in a suspension, it is unlikely that results would have been much different for any of the other bacterial strains. The key problem to be overcome in all bacterial diffusion experiments was minimizing the sampling effects on the experimental system. The physical model and method were tested with a Cl^- tracer and performed satisfactorily, but the concurrent *P. fl.* m18 diffusion test was not successful. This was explained by the difference in magnitude of the D_o values for Cl^- and *P. fl.* m18. Controlling experimental conditions (i.e., systematic and experimental errors) for the duration of time required for measurable bacterial diffusion to occur was a difficult

task. Although motile bacteria have been shown to have effective diffusion rates much greater than non-motile bacteria, the motile bacteria would still be exposed to the same potential for sampling-induced error. A candidate for testing in the double reservoir model would likely have to have a diffusion coefficient of the same order of magnitude as Cl^- or allow for passive sampling with minimal disturbance to the diffusion experiment in progress. D_o values measured by DLS clearly indicated that bacterial diffusion coefficients were much smaller than known Cl^- D_o values. At this point, experiments with the double reservoir diffusion model were halted.

Most previous bacterial sorption studies have used either pulsed or finite bacterial concentrations. Many of these bacterial transport studies have proposed the use of a two-site sorption model or sorption with both reversible and irreversible components (Bales et al., 1991; Bales et al., 1997; Harvey and Garabedian, 1991; Hendry et al., 1997; Hendry et al., 1999; Hornberger et al., 1992; Lindqvist et al., 1994). The rapid breakthrough of the *P. fl.* m18 data prevented observation of sorption effects using diffusion-dominated conditions. Though a modelled average velocity through the sample ring was presented, it could not be verified to be a constant velocity. Thus steady-state conditions could not be assumed in the double reservoir diffusion tests. Still, some estimations were made of possible sorption effects using diffusion-dominated conditions.

In previous bacterial transport column studies (e.g., Bales et al., 1989; Gannon et al., 1991b; Hendry et al., 1999; Wollum II and Cassel, 1978), decreases in fluid velocity

have caused a reduction in peak bacterial output concentration. This decrease has been attributed to increased bacterial sorption to the porous media within the columns. In the double reservoir diffusion tests, a constant bacterial source concentration was maintained in the source reservoir throughout the experiment. Because of this difference in source concentration, breakthrough results from previous investigations are not readily comparable. As well, because of the problems with the double reservoir diffusion tests, *P. fl.* m18 breakthrough data could not be modelled to determine sorption effects on the diffusive transport of *P. fl.* m18. However, predictive modelling was done to estimate possible bacterial sorption effects during the double reservoir diffusion tests.

The breakthrough of *P. fl.* m18 was simulated with an irreversible sorption function (POLLUTE™). Addition of a decay rate of 400 hr was used to simulate a small bacterial irreversible sorption rate (0.0025 hr^{-1}) in the double reservoir diffusion tests. The value of this irreversible sorption was much smaller than previously modelled values of irreversible sorption (0.062 hr^{-1}, Hendry et al., 1999). However, it provided a good indication of the effects of sorption on the diffusive transport of bacteria in our experiments. Modelling the *P. fl.* m18 movement through the sample ring with this irreversible sorption rate yielded a reduced peak breakthrough concentration (Fig. 4.2.3.5). Importantly, this low irreversible sorption modelled value reduced the expected bacterial concentration in the collection reservoir below the detection limit used in the double reservoir diffusion experiments. Therefore under solely diffusion-dominated conditions, the irreversible sorption of the *P. fl.* m18 may have

been reduced to non-detectable concentrations. As well, in our experiments a constant bacterial source concentration was maintained in the source reservoir throughout the diffusion experiments. It was not know whether the bacterial concentrations in the collection reservoir would have reached the detectable bacterial concentration limits during the time of the experiment (~800 hrs) under ideal diffusion-dominated conditions.

Also presented in Fig. 4.2.3.5 are *P. fl.* m18 simulations incorporating an equilibrium sorption exchange mechanism, a K_d function (Freeze and Cherry, 1979). K_d functions (0.55 to 6.11 $cm^3 \cdot g^{-1}$) have been used to describe changing bacterial sorption kinetics with a varied fluid ionic strength in batch tests (Mills et al., 1994). A K_d function was used to describe sorption of bacteria to the porous media during the double reservoir diffusion test (K_d = 1 $cm^3 \cdot g^{-1}$). Addition of sorption to the simulated diffusive transport of the *P. fl.* m18 through the sample ring caused the simulated breakthrough to be attenuated (or shifted to the right). Thus, sorption of *P. fl.* m18 to the quartz sand in the double reservoir diffusion test would have retarded the *P. fl.* m18 breakthrough data, not advanced the breakthrough. Therefore, the breakthrough of the bacteria through the sample ring would likely have been reduced in peak concentration (because of irreversible sorption) and retarded (because of equilibrium sorption), but not arrested (Fig. 4.2.3.5). These model predictions indicated that bacterial sorption in

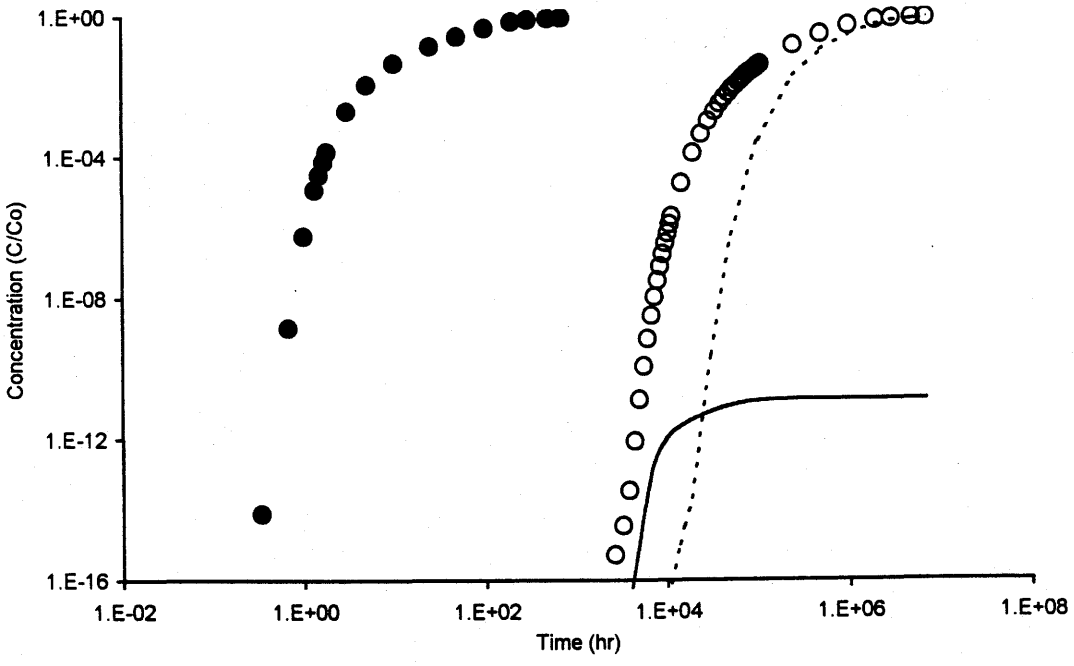


FIGURE 4.2.3.5. Simulated Cl^- (solid circles) and *P.fl.* m18 (circles) breakthrough results from the double reservoir diffusion tests. Also presented are simulations of *P.fl.* m18 breakthrough results using an equilibrium sorption function (K_d = 1 $cm^3 \cdot g^{-1}$, dashed line) and an irreversible sorption function (equivalent to 0.0025 hr^{-1}, solid line).

the double reservoir test would be a hindrance to the earlier arrival of the bacterial breakthrough, not advance it. However, problems with the double reservoir diffusion tests prevented observation of diffusion-dominated *P. fl.* m18 sorption relationships.

CHAPTER 5

CONCLUSIONS

Previous investigations have determined that low groundwater velocity conditions are prevalent in the subsurface. A full understanding of bacterial transport and attenuation in the subsurface requires an understanding of the movement of bacteria using these low velocity and diffusion-dominated conditions. In this thesis, bacterial diffusion was investigated by modifying and developing laboratory techniques to quantify bacterial diffusion. Three techniques were used in this investigation: dynamic light scattering measurements, the diffusing sphere technique, and the double reservoir diffusion technique. Five bacteria were bacterial strains were used in these mono-culture experiments: *Pseudomonas fluorescens* 840406-E, *Pseudomonas fluorescens* m18, *Klebsiella oxytoca*, *Burkholderia cepacia* G4PR1, and a *Pseudomonas* isolate. Based on research conducted as a part of this thesis, the following conclusions were made:

1 Bacteria have measurable free-water diffusion coefficients. The free-water diffusion coefficients determined for all bacteria were $\sim 1 \times 10^{-13}$ $m^2 \cdot sec^{-1}$. However, the size of bacteria (~1 μm) is at the upper limit of where particles are affected by

Brownian movement (Smoluchowski, 1917). Hence, the size of bacteria will likely inhibit bacterial diffusion from becoming the dominant transport mechanism in most laboratory investigations. The results do not clearly indicate whether bacterial diffusion will play an important role in the transport and attenuation of bacteria in the subsurface.

2 Bacterial sizes are approximately 1000 times greater than molecules of dissolved solutes. Correspondingly, measured free-water diffusion coefficients for bacteria were three orders of magnitude less than known free-water diffusion coefficients for dissolved solutes. As well, estimates of free-water bacterial diffusion coefficients determined by both the Einstein-Stokes equation and the volume-fraction modified Einstein-Stokes equation agreed with measurements made using dynamic light scattering. Therefore, the Einstein-Stokes equation and the volume-fraction modified Einstein-Stokes equation provided a good estimate of bacterial free-water diffusion coefficients.

3 The bacterial diffusion experiments showed that bacterial diffusion studies should be conducted with an understanding of interfering gradients (e.g., growth, motility, temperature, and sampling induced-pressure gradients). Adequate control, minimization, and/ or measurement of these factors is necessary to obtain successful results in bacterial diffusion experiments.

4 Modelling of a design prior to physical experimentation is frequently useful for confirmation of physical model and method adequacy and gauging expecting results. Predictive modelling may have been a useful indicator of the sensitivity of the double reservoir diffusion model to experimental conditions. However, it is unlikely that

prior modelling would have indicated the, yet to be defined cause of the, sinking in the diffusing sphere technique.

5 Bacterial gravimetric densities obtained with Percoll™ density gradient centrifugations should be used carefully. Bacterial densities appeared to be closely related to bacterial growth rates and mean cell age. Growth rates of bacteria were determined from the rate of change of (increasing) turbidity of growing bacterial cultures. Faster growing strains were older at the time of preparation of bacterial suspensions. These older bacteria were denser, as determined by density gradient centrifugation. It is not clear whether this potential gravimetric cell density and mean cell age relationship can be extended to nutrient-limited conditions, like those found in the subsurface.

6 A representative bacterial radius for *Pseudomonas* isolate 5 (*P.* 5) was determined with the dynamic light scattering technique and the Einstein-Stokes equation. The calculated *P.* 5 radius was similar to estimates found using bacterial dimensions from phase-contrast microscopy (0.93 and 0.92 μm, respectively). This indicated that very little clumping of *P.* 5 occurred during the time between the preparation of the bacterial suspension and dynamic light scattering measurements of the bacterial suspension (~36 hr). However, there was a significant difference between the size determinations from the calculated estimates and phase-contrast microscopy measurements for *Klebsiella oxytoca* and *Burkholderia cepacia* G4PR1 (percent difference 82 and 172%, respectively). This suggested that bacterial clumping may be strain dependent and that bacterial transport and attenuation are likely influenced by strain characteristics.

7 The diffusing sphere technique allowed measurement of fluorescein free-water diffusion coefficients. However, the diffusing sphere technique could not be successfully applied to determine bacterial diffusion coefficients. Sinking of the stained sphere of bacteria prevented measurement of bacterial diffusion using the diffusing sphere technique. No explanation could be found for this sinking. The test tube settling experiment results (Section 4.1.3) did not indicate that settling would be problem. It is suggested that the low value for bacterial free-water diffusion coefficients may play a key role in this discrepancy between the success of the fluorescein measurements and the sinking of the stained bacteria.

8 In these double reservoir diffusion experiments, diffusion-dominated conditions were attained the dissolved solute, while not attained for the bacteria. Diffusion-dominated conditions are a function of the physical system and the diffusing species measured. Estimates of Peclet numbers for bacterial transport in a porous media were approximately three orders of magnitude greater than for a dissolved solute. Likely, diffusion-dominated conditions for the bacteria could not be achieved because of the difference in Peclet (P_e) number. Thus, diffusion-dominated conditions for bacterial studies are much more difficult to maintain than for dissolved solutes.

9 The P_e number and P_e plot provided a good characterization of the transport conditions in bacterial transport and attenuation investigations. The P_e number calculated using the equivalent velocity determined for *P. fl.* m18 double reservoir diffusion results was a good indicator of the non-diffusive conditions likely present during this experiment. The P_e number will likely be important tool for gauging diffusion-dominated conditions in future bacterial transport investigations.

10 Determination of effective bacterial diffusion coefficients through a porous media is one of the most difficult challenges facing researchers in this field. Two separate methods were investigated: the diffusing sphere technique and the double reservoir diffusion method. Both methods performed well for dissolved solutes, but did not work for studies of bacterial diffusion.

CHAPTER 6

FUTURE CONSIDERATIONS

The primary purpose of this thesis was to study bacterial transport and attenuation under diffusion-dominated conditions. Three separate techniques were used to examine the diffusive movement of bacteria: dynamic light scattering measurements, the diffusing sphere technique, and the double reservoir diffusion technique. As a result of the research conducted in this thesis, four areas that require further investigation are identified. They are:

1 In subsequent investigations of bacterial diffusion, better delineation of the factors affecting the gravimetric density of bacterial cells is necessary. While the bacterial cell density [$M \cdot L^{-3}$] determinations appeared indicative of bacterial characteristics, further investigation of the culture preparation method effect on the bacterial gravimetric density should be conducted. Bacterial strain growth rate, growth stage at the time of testing, and clumping of cells during the rinsing procedure, as well as clumping of the bacteria in suspension as a function of time may also play an important role in determining bacterial gravimetric density.

2 Both motile and non-motile bacteria are present in the subsurface. Previous authors have showed substantially different rates of movement between motile and non-motile bacteria. A comparative investigation of the free-water diffusion coefficients of motile and non-motile bacteria that incorporates motility should be performed. The determined free-water bacterial diffusion coefficients would then be an enhanced diffusion coefficient and be representative of both Brownian movement and motility.

3 Clumping of bacteria will increase the representative bacterial radii. Accordingly, the bacterial diffusion coefficient should decrease. This is predicted by the Einstein-Stokes equation and the volume-fraction modified Einstein-Stokes equation. Further investigation is necessary to quantify clumping rates and correlate them with diffusion rates.

4 Results from this study could not clearly show an effect or lack of effect of the nucleic stain used to label bacteria in the diffusing sphere experiments. A closer examination of the affect of nucleic stains on the physiology of bacteria is necessary. The continued use of nucleic stains (e.g., SYTO 9, Molecular Probes Inc., Eugene, OR) requires an improved understanding of its affects on bacterial cell physiology.

CHAPTER 7

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APPENDICES

APPENDIX A:

DIFFUSING SPHERE TECHNIQUE: DATA

Fluorescein

Data set: ser3h1_477

Conditions:

a' (um)	251
r (um)	477
D_o (m^2*sec^{-1})	4.2E-10

Measured data		Modelled data	
Time (sec)	$C/C_o*C_o/C_{MAX}$	Time (sec)	$C/C_o*C_o/C_{MAX}$
0	0.018	0.0001	0.00
2.5	0.058	5.25	0.00
7.5	0.058	7	0.02
12.5	0.236	8.75	0.04
17.5	0.355	10.5	0.08
22.5	0.464	12.25	0.13
27.5	0.596	14	0.18
32.5	0.680	15.75	0.24
37.5	0.777	17.5	0.29
42.5	0.855	19.25	0.35
47.5	0.912	21	0.40
52.5	0.950	22.75	0.46
57.5	0.985	26.25	0.55
62.5	0.993	28	0.60
67.5	1.000	29.75	0.64
72.5	0.982	31.5	0.67
77.5	0.970	33.25	0.71
82.5	0.990	35	0.74
87.5	0.951	38.5	0.80
		40.25	0.82
		42	0.85
		43.75	0.87
		45.5	0.89
		47.25	0.90
		49	0.92
		50.75	0.93
		54.25	0.95
		56	0.96
		57.75	0.97
		59.5	0.98
		61.25	0.98
		64.75	0.99
		66.5	0.99
		68.25	1.00
		70	1.00
		73.5	1.00
		75.25	1.00
		78.75	1.00
		80.5	1.00
		84	0.99
		87.5	0.99

Fluorescein — Data set: try12_dist1, 297

Conditions:

a' (um)	40
r (um)	297
D_o (m^2*sec^{-1})	9.0E-11

Measured data		Modelled data	
Time (sec)	$C/C_o*C_o/C_{MAX}$	Time (sec)	$C/C_o*C_o/C_{MAX}$
0	0.000	0.0001	0.00
5	0.000	5	0.00
15	0.001	10	0.00
25	0.010	15	0.00
35	0.066	20	0.00
45	0.164	25	0.01
55	0.299	30	0.02
65	0.441	35	0.05
75	0.548	40	0.10
85	0.682	45	0.16
95	0.745	50	0.22
105	0.813	55	0.29
115	0.899	60	0.37
125	0.899	65	0.44
135	1.000	70	0.51
		75	0.57
		80	0.63
		85	0.69
		90	0.74
		95	0.78
		100	0.82
		105	0.85
		110	0.88
		115	0.91
		120	0.93
		125	0.95
		130	0.96
		135	0.98

Fluorescein		Data set: ser3f_319	
Conditions:			
a' (um)	147		
r (um)	319		
D_o (m^2*sec^{-1})	3.0E-10		
Measured data		Modelled data	
Time (sec)	$C/C_o*C_o/C_{MAX}$	Time (sec)	$C/C_o*C_o/C_{MAX}$
0	0.068	0.0001	0.00
2.5	0.097	4	0.00
7.5	0.187	6	0.03
12.5	0.359	10	0.16
17.5	0.536	12	0.26
22.5	0.679	14	0.35
27.5	0.808	18	0.53
32.5	0.897	20	0.60
37.5	0.958	22	0.67
42.5	0.996	26	0.78
47.5	0.999	28	0.83
52.5	1.000	30	0.87
57.5	0.985	34	0.93
62.5	0.950	36	0.95
67.5	0.949	38	0.96
72.5	0.917	42	0.99
77.5	0.891	44	0.99
82.5	0.837	46	1.00
87.5	0.808	52	1.00
92.5	0.770	54	0.99
97.5	0.731	58	0.98
102.5	0.691	60	0.98
107.5	0.652	62	0.97
112.5	0.619	66	0.95
		68	0.94
		70	0.93
		74	0.91
		76	0.90
		78	0.89
		82	0.87
		84	0.86
		86	0.85
		90	0.83
		92	0.82
		94	0.81
		98	0.79
		100	0.77
		102	0.76
		106	0.74
		108	0.73
		110	0.73
		112	0.72

Fluorescein Data set: ser4b_325

Conditions:

a' (um)	159
r (um)	325
D_o (m^2*sec^{-1})	6.2E-10

Measured data		Modelled data	
Time (sec)	$C/C_o*C_o/C_{MAX}$	Time (sec)	$C/C_o*C_o/C_{MAX}$
0	0.005	0.0001	0.00
2.5	0.188	0.75	0.00
7.5	0.534	1.5	0.00
12.5	0.802	2.25	0.01
17.5	0.945	3	0.04
22.5	1.000	3.75	0.09
27.5	1.000	4.5	0.15
		5.25	0.22
		6	0.29
		6.75	0.36
		7.5	0.43
		8.25	0.50
		9	0.56
		9.75	0.62
		10.5	0.67
		11.25	0.72
		12	0.76
		12.75	0.79
		13.5	0.83
		14.25	0.86
		15	0.88
		15.75	0.91
		16.5	0.93
		17.25	0.94
		18	0.96
		18.75	0.97
		19.5	0.98
		20.25	0.98
		21	0.99
		21.75	1.00
		22.5	1.00
		23.25	1.00
		24	1.00
		24.75	1.00
		25.5	1.00
		26.25	1.00
		27	0.99
		27.75	0.99

P.fl. m18
Data set: ser6e_268

Conditions:

a' (um) 251
r (um) 477

Measured data

Time (sec)	$C/C_o*C_o/C_{MAX}$
0	0.025
3	0.063
6	0.192
9	0.380
12	0.536
15	0.662
18	0.756
21	0.818
24	0.928
27	0.956
30	1.000
33	0.925
36	0.823

P.fl. 840406-E
Data set: aser2b_235

Conditions:

a' (um)	147
r (um)	235

Measured data

Time (sec)	C/C_o*C_o/C_{MAX}
0	0.008
3	0.019
6	0.060
9	0.117
12	0.174
15	0.260
18	0.288
21	0.386
24	0.497
27	0.523
30	0.614
33	0.645
36	0.677
39	0.729
42	0.789
45	0.820
48	0.831
51	0.809
54	0.790
57	0.788
60	0.869
63	0.883
66	0.927
69	0.958
72	0.946
75	0.978
78	0.971
81	0.944
84	1.000

P.fl. 840406-E
Data set: aser2g_244

Conditions:

a' (um) 148
r (um) 244

Measured data

Time (sec)	C/C_o*C_o/C_{MAX}
0	0.023
5	0.048
10	0.088
15	0.222
20	0.353
25	0.480
30	0.653
35	0.767
40	0.895
45	0.911
50	0.891
55	0.929
60	0.948
65	1.000

P.fl. 840406-E
Data set: aser2e_222

Conditions:

a' (um)	142
r (um)	222

Measured data

Time (sec)	C/C_o*C_o/C_{MAX}
0	0.004
10	0.101
20	0.366
30	0.577
40	0.747
50	0.790
60	0.957
70	0.924
80	0.763
90	0.892
100	0.959
110	0.929
120	1.000
130	0.976
140	0.891

P.fl. m18
Data set: aser4a_360

Conditions:

a' (um) 129
r (um) 360

Measured data

Time (sec)	C/C_o*C_o/C_{MAX}
0	0.000
5	0.054
10	0.205
15	0.427
20	0.600
25	0.753
30	0.899
35	0.997
40	1.000
45	0.978

PR1
Data set: aser9i_217

Conditions:

a' (um)	129
r (um)	360

Measured data

Time (sec)	C/C_o * C_o/C_{MAX}
0	0.026
3	0.137
6	0.270
9	0.440
12	0.615
15	0.785
18	0.870
21	0.965
24	1.000

APPENDIX B:

DOUBLE RESERVOIR DIFFUSION CELL TECNIQUE: DATA

Recorded data for known Cl- concentrations

Known Cl- conc. ($mg*L^{-1}$)	Daily average of the datalogger voltage drop ratio reading							
	Half-cell #1	Half-cell #2	Half-cell #3	Half-cell #4	Half-cell #5	Half-cell #6	Half-cell #7	Half-cell #8
0	0.67	0.67	0.69	0.67	0.69	0.72	0.62	0.67
5.00	0.61	0.61	0.63	0.61	0.62	0.66	0.56	0.61
9.99	0.58	0.58	0.60	0.58	0.60	0.63	0.53	0.58
19.96	0.53	0.52	0.55	0.53	0.54	0.58	0.46	0.51
34.88	0.42	0.41	0.44	0.42	0.44	0.47	0.37	0.42
49.75	0.38	0.38	0.40	0.39	0.41	0.44	0.34	0.38
65.58	0.34	0.34	0.36	0.34	0.36	0.39	0.30	0.34
74.44	0.32	0.32	0.33	0.32	0.33	0.37	0.28	0.32
99.01	0.27	0.27	0.29	0.27	0.29	0.32	0.24	0.27

Cl- calibration data for datalogger voltage drop readings

Datalogger reading	Calibrated chloride concentration from datalogger voltage drop ratio reading ($mg*L^{-1}$)							
	Half-cell #1	Half-cell #2	Half-cell #3	Half-cell #4	Half-cell #5	Half-cell #6	Half-cell #7	Half-cell #8
0.27	100	100	99	99	99		100	100
0.29	91	91	97	89	96		95	90
0.31	82	82	88	81	87	100	90	81
0.33	73	71	79	72	79	93	85	72
0.35	65	63	71	65	71	84	78	64
0.37	58	56	63	57	63	76	71	57
0.39	51	49	56	50	56	68	65	50
0.41	45	43	49	44	49	61	59	43
0.43	39	37	43	38	43	54	53	37
0.45	33	31	37	32	37	48	46	31
0.47	28	26	32	27	32	42	39	26
0.49	23	22	27	23	27	36	33	22
0.51	19	18	23	19	22	31	27	18
0.53	15	14	19	15	18	26	22	14
0.55	12	11	15	12	15	22	18	11
0.57	9	9	12	9	12	18	14	8
0.59	7	7	9	7	9	15	11	6
0.61	5	5	7	5	7	12	8	5
0.63	4	4	5	4	5	9	6	3
0.65	3	3	4	3	4	7	4	3
0.67	3	3	3	3	3	5	3	3
0.69	3	3	3	3	2	4	3	3
0.71	3	4	3	3	3	3	3	4
0.73	4	5	3	4	3	2		5

Cl- data: experiment 03,21,97			*Continued*		
Time (hr)	Average C/C_o for the four double reservoir diffusion cells	Standard Deviation for the four double reservoir diffusion cells	Time (hr)	Average C/C_o for the four double reservoir diffusion cells	Standard Deviation for the four double reservoir diffusion cells
0.1	0.000		77	0.514	0.062
5	0.082	0.023	78	0.521	0.061
20	0.257	0.104	82	0.543	0.062
30	0.267	0.066	85	0.544	0.053
35	0.316	0.064	88	0.554	0.054
40	0.348	0.057	92	0.563	0.056
41.5	0.355	0.054	95	0.573	0.056
42	0.355	0.053	99	0.586	0.058
42.5	0.356	0.052	103	0.604	0.060
43	0.356	0.051	107	0.606	0.056
43.5	0.359	0.050	112	0.621	0.059
44	0.362	0.049	117	0.640	0.063
44.5	0.366	0.049	122	0.653	0.062
45	0.369	0.049	128	0.667	0.060
45.5	0.371	0.050	134	0.687	0.064
46	0.371	0.050	141	0.717	0.057
46.5	0.371	0.052	148	0.724	0.055
47	0.373	0.052	155	0.738	0.057
47.5	0.375	0.052	163	0.746	0.055
48	0.378	0.052	172	0.765	0.056
48.5	0.381	0.051	181	0.791	0.057
49	0.384	0.051	190	0.803	0.058
49.5	0.388	0.054	201	0.830	0.053
50	0.391	0.056	212	0.850	0.046
50.5	0.394	0.059	224	0.868	0.048
51	0.397	0.061	237	0.886	0.044
52	0.403	0.063	251	0.897	0.043
53	0.408	0.061	265	0.911	0.045
54	0.413	0.061	281	0.921	0.042
55	0.418	0.062	298	0.930	0.037
56	0.421	0.064	316	0.945	0.038
57	0.426	0.067	335	0.959	0.026
58	0.430	0.068	356	0.964	0.022
59	0.433	0.065	378	0.978	0.018
61	0.427	0.063	401	0.981	0.010
62	0.428	0.064	426	0.991	0.011
63	0.433	0.064	453	0.991	0.009
65	0.450	0.067	482	1.000	0.000
67	0.467	0.067	513	1.000	0.000
69	0.478	0.069			
71	0.485	0.068			
73	0.496	0.063			
75	0.504	0.061			

Modelled Cl- data for experiment: 03,21,97			
Concentrations in C/C_o			
	D_e values in m^2*sec^{-1}		
Time (hr)	5.0E-10	6.0E-10	7.0E-10
2	0.000724	0.001869	0.003745
10	0.06163	0.08254	0.1032
25	0.166	0.2022	0.2363
50	0.3086	0.3617	0.4101
75	0.4268	0.4893	0.5443
100	0.5247	0.5914	0.648
150	0.6732	0.7385	0.7899
200	0.7753	0.8326	0.8747
300	0.8938	0.9314	0.9554
400	0.9498	0.9719	0.9841
500	0.9763	0.9885	0.9943
600	0.9888	0.9953	0.998
700	0.9947	0.9981	0.9993

P.fl. m18 data for the double reservoir diffusion experiment
Reservoir concentrations in $(CFU*mL^{-1})/(CFU*mL^{-1})$

Time (hr)	Stock ($CFU*mL^{-1}$)	Diffusion Cells #1 and #2	#3 and #4	#5 and #6	#7 and #8	Average	Standard deviation
84.5	1.10E+07	8.22E-03	8.22E-03	8.22E-03	8.22E-03	8.22E-03	0.0E+00
105		7.64E-03		6.49E-03		7.06E-03	8.1E-04
160.25	9.50E+05	1.16E-02	2.00E-03	3.11E-01	2.21E-01	1.36E-01	1.5E-01
205.5	1.33E+07	8.96E-04	3.54E-04	1.04E-05	3.39E-04	4.00E-04	3.7E-04
248	2.57E+07	1.95E-05	1.95E-05	1.28E-03	9.34E-05	3.54E-04	6.2E-04
272	6.33E+07	1.30E-04	7.58E-03	1.39E-03	8.37E-04	2.48E-03	3.4E-03
297.25	6.60E+07	1.29E-03	1.09E-02	3.86E-02	2.41E-03	1.33E-02	1.7E-02
319.75	4.45E+07	3.42E-03	9.98E-03	4.55E-02	1.01E-02	1.72E-02	1.9E-02
344.75	5.70E+07	5.89E-03	1.60E-02	3.83E-05	1.18E-02	8.42E-03	7.0E-03
395	9.30E+07	4.84E-03	1.04E-02	2.67E-02	7.53E-03	1.24E-02	9.8E-03
415.75	9.50E+07	5.37E-03	1.37E-02	4.91E-02	6.84E-03	1.87E-02	2.1E-02
440	8.40E+07	5.00E-03	2.08E-02	9.76E-03	8.21E-03	1.10E-02	6.9E-03
464.5	5.10E+07	8.24E-03	2.75E-02	8.43E-02	1.96E-02	3.49E-02	3.4E-02
489.25	3.72E+07	3.65E-02	1.84E-01	4.07E-01	7.39E-02	1.75E-01	1.7E-01
511.5	2.34E+07	4.44E-02	8.89E-02	1.84E-01	5.04E-02	9.19E-02	6.4E-02
539.75	1.47E+07	2.93E-02	3.74E-02	6.80E-01	3.33E-02	1.95E-01	3.2E-01
568	9.10E+06	4.29E-02	8.02E-02	3.52E-01	7.58E-02	1.38E-01	1.4E-01
583.5	3.60E+06	7.50E-02	3.17E-01	6.56E-01	1.22E-01	2.92E-01	2.6E-01
607.5	3.10E+06	3.81E-02	7.68E-02	4.52E-01	2.68E-02	1.48E-01	2.0E-01
633.25	1.16E+06	8.19E-02	3.10E-01	8.10E-01	2.21E-01	3.56E-01	3.2E-01
655.5	2.20E+05	2.28E+00	6.55E-01	4.55E+00	4.64E+00	3.03E+00	1.9E+00
679.75	2.90E+05	6.38E-01	3.28E+00	3.59E+00	5.76E-01	2.02E+00	1.6E+00
708.5	5.30E+05	3.66E-01	7.09E-01	1.94E+00	6.98E-01	9.29E-01	6.9E-01
731.5	6.00E+05	1.52E+00	2.67E+00	6.33E+00	1.60E-01	2.67E+00	2.6E+00
752	4.40E+05	6.59E-01	1.75E+00	2.09E+00	1.02E+00	1.38E+00	6.6E-01
775.75	7.00E+05	6.00E-01	1.94E+00	2.61E+00	1.00E+00	1.54E+00	9.1E-01
825.5	1.70E+05	1.18E+00	9.12E+00	6.76E+00	6.00E+00	5.76E+00	3.3E+00

Modelled *P.fl.* m18 data for the double reservoir diffusion experiment

Concentrations in C/C_o

	D_e values in m^2*sec^{-1}		
Time (hr)	1.00E-11	2.60E-11	7.30E-14
2	8.03E-43	1.701E-26	
10	2.22E-19	4.078E-14	
25	1.372E-14	3.695E-07	
50	8.475E-09	0.0001127	
75	1.108E-06	0.0008988	
100	0.0000139	0.002799	
150	0.0002021	0.00993	
200	0.0008576	0.02039	
250	0.002167	0.03289	6.228E-46
300	0.004173	0.04655	1.182E-41
350	0.006832	0.06081	1.13E-38
400	0.01007	0.07535	1.777E-36
450	0.01379	0.08997	8.697E-35
500	0.01792	0.1045	2.027E-33
550	0.02239	0.119	4.567E-32
600	0.02713	0.1332	1.729E-30
650	0.03208	0.1473	5.357E-29
700	0.03722	0.1612	1.04E-27
750	0.04249	0.1749	1.331E-26
800	0.04786	0.1883	1.213E-25

Sorption modelling of the *P.fl.* m18 data from the double reservoir diffusion experiment Concentrations in C/C_o		
Time (hr)	K_d = 1	Decay rate 400 hr
8.50E+02		
1.70E+03	2.93E-41	8.56E-23
3.40E+03	4.71E-30	4.40E-18
6.80E+03	2.31E-20	1.48E-13
1.02E+04	2.90E-17	1.12E-12
1.36E+04	1.03E-15	2.24E-12
1.53E+04	3.45E-15	2.77E-12
1.70E+04	9.16E-15	3.28E-12
1.87E+04	2.17E-14	3.76E-12
3.06E+04	2.60E-10	6.58E-12
5.10E+04	6.57E-07	9.75E-12
1.02E+05	3.34E-04	1.30E-11
2.55E+05	2.26E-02	1.41E-11
5.10E+05	1.22E-01	1.41E-11
1.02E+06	3.36E-01	1.41E-11
2.04E+06	6.29E-01	1.41E-11
3.06E+06	7.93E-01	1.41E-11
5.10E+06	9.36E-01	1.41E-11
7.14E+06	9.80E-01	1.41E-11

APPENDIX C:

A REVIEW PAPER: AQUEOUS DIFFUSION OF BACTERIA

PROBLEM BACKGROUND

The awareness that contaminants in the subsurface pose a threat to drinking water supplies has been an issue for a number of years. By 1897 (Rafter, 1897), it was known that proper sewage disposal is vital to community health. Rafter proposed general guidelines (filter material type, maximum sewage irrigation rates, and an effluent quality guideline) for sewage disposal in the United States. Still, in 1998 a thorough understanding of bacterial transport in the subsurface is not possible because of the lack of knowledge of the mechanisms controlling bacterial transport and attenuation in the subsurface.

In 1902 Slichter recounted a site history emphasizing the importance of understanding groundwater flow (Slichter, 1902). He reported that cesspools had contaminated down gradient wells, causing 18 cases of typhoid fever within a city district. Slichter proved there was a need for a better understanding of bacterial transport. Moreover, the push for more subsurface and groundwater flow research was also accelerated by the demand for more water sources (Meinzer, 1932; Meinzer, 1939).

Transport and attenuation of conservative species in groundwater, and their controlling mechanisms, are now generally well understood (Fetter, 1992; Perkins and Johnston, 1963). This large accumulation of work studying the transport of conservative species

provides useful parameter estimations for modelling contaminant transport in the subsurface (Anderson, 1979; Crank, 1956; Cussler, 1984; Ogata, 1970; Shackelford, 1994).

While the knowledge of conservative species transport has advanced, the study of bacterial transport is lacking. Even before 1906 (Dole, 1906), bacterial tracers were being developed and used for groundwater problems. However, the understanding of bacterial transport in the subsurface is dependant upon extrapolation from conservative species transport knowledge and gross controlling factors for bacterial transport (Bitton and Harvey, 1992; Harvey, 1991). The broad concepts presented 100 years ago are still applied today in Saskatchewan (1996), and by other provincial and state governing agencies (Macaulay, 1997).

PREVIOUS WORK

The numerous factors controlling bacterial transport and attenuation in the subsurface make bacterial transport experiments very complex. One of the first bacterial transport field experiments attempted to determine how near a well may be located to a broken sewer line without it becoming contaminated (Ditthorn and Luerssen, 1909).

Since 1909, the study of bacterial transport has defined some of the factors controlling bacterial transport in the subsurface.

There are many published reviews of bacterial transport in the subsurface (Abu-Ashour et al., 1994; Bitton and Harvey, 1992; Corapcioglu and Haridas, 1984; Crane and Moore, 1984; Gerba et al., 1991; Harvey, 1996; Keswick et al., 1982; Lawrence and Hendry, 1996; McDowell-Boyer et al., 1986; Peterson and Ward, 1987; Peterson and Ward, 1989; Viraraghavan, 1978; Yates and Yates, 1988). Table 1 presents some of the factors controlling bacterial attenuation and transport in the subsurface.

Table 1. Mechanisms controlling bacterial transport and attenuation in the subsurface adapted from Bitton and Harvey (1992) Crane and Moore (1984), and Yates and Yates (1988).

Microbial conditions	Origin and type of microorganism and physiological state.
Media properties	Groundwater velocity, water content, average pore/grain size, pore/grain size distribution, tortuosity, porosity, surface characteristics, and organic content.
Environmental conditions	Temperature, presence of soluble organics, ionic strength, pH, and local groundwater chemistry.

Harvey et al. (1995) showed through field and laboratory tests that bacteria cultured in the lab and later placed in a subsurface environment showed large differences in transport and attenuation compared to native subsurface bacteria.

Sharma and McInerney (1994) showed that the bacteria cell size and pore size ratio may be a controlling factor, although McDowell-Boyer et al. (1986) indicated that straining would not be a consideration for cell/ pore ratios <5%. Numerous authors showed that bacterial transport is strongly influenced by the organic content of the porous media and the media surface characteristics (Gannon et al., 1991a; Gross and Logan, 1995; Johnson et al., 1996; Krone et al., 1958; Rijnaarts et al., 1996b; Scholl and Harvey, 1992).

Some bacteria are motile and capable of moving through liquids. Motile bacteria move by rotating their corkscrew-shaped flagella. Chemotaxis, bacterial migration in response to a chemical gradient, has been studied in the laboratory (Barton and Ford, 1995; Ford et al., 1991; Harwood et al., 1989; Rivero et al., 1989). Numerous models attempt to predict a chemotactic transport rate as a function of attractant gradient and motility of the bacteria (Brosilow et al., 1996; Rivero et al., 1989; Segel, 1977).

Experiments by McCaulou et al. (1995) showed that a temperature increase (from 4 to 18°C) caused a decrease in irreversible bacterial sorption in sand packed columns. In column experiments by Johnson et al. (1996), decreased ionic strength of the fluid medium (0.07 to 10^{-5} M) caused a 2 orders of magnitude decrease in the fraction of bacteria irreversibly sorbed in the columns. This reduction is in agreement with other investigations of ionic strength effects on bacterial attenuation (Gannon et al., 1991b; Gross and Logan, 1995).

Column experiments conducted under controlled laboratory conditions have defined some of the dominant mechanisms controlling bacterial transport (Harvey et al., 1997). Unfortunately, much of the work focuses on experiments conducted with high fluid velocities (Table 2) that do not reflect typical groundwater velocities found in the subsurface (Bales et al., 1997; Freeze and Cherry, 1979).

Table 2. Average fluid velocities used in some column experiments.

Velocity ($m \cdot day^{-1}$)	References
1.9 to 11.7	(Bales et al., 1989)
0.5, 2.4, and 10.8	(Camper et al., 1993)
8.6	(Gannon et al., 1991b)
0.5	(Harvey et al., 1995)
8.6, 18.3, and 20.2	(Hendry et al., 1997)
0.11 to 3.2	(Hendry et al., 1999)
10.5	(Hornberger et al., 1992)
4.4	(Lindqvist and Bengtsson, 1995)
5.8	(McCaulou et al., 1995)
5.2	(Rijnaarts et al., 1996b)
17.3	(Rijnaarts et al., 1996a)
2.7	(Smith et al., 1985)
1	(Toran and Palumbo, 1992)
17.4	(Wollum II and Cassel, 1978)

Diffusion in the subsurface

In 1959 Blackwell recognized that diffusion was the dominant dispersion mechanism under most reservoir conditions (Blackwell et al., 1959). However, most bacterial transport experiments have been conducted at high groundwater velocities and very little work is conducted using diffusion controlled conditions (Ford et al., 1991; Reynolds et al., 1989; Rijnaarts et al., 1993; Sharma and McInerney, 1994). Previous research either has not quantified bacterial diffusion coefficients, has incorrectly represented the physical problem in the mathematical solution, or has not properly addressed some of the controlling mechanisms of bacterial transport in the experiments. Using a stopped-flow diffusion chamber, Ford et al. (1991) determined bacterial motility and chemotaxis transport rates ($1.1x10^{-9}$ and $8x10^{-9}$ $m^2 \cdot sec^{-1}$, respectively), with no measurement of bacterial diffusion. In the companion paper, Ford and Lauffenburger (1991) presented mathematical modelling of the bacterial motility and chemotaxis experiments; however, the mathematical solution they used did not fully represent their physical problem. The authors assumed a finite source diffusing into an infinite volume, instead of the finite volume found in their experiment (Crank, 1956).

Reynolds et al. (1989) examined, using static flow conditions, the transport of anaerobic bacteria in sand packed nutrient-saturated tubes. The authors found bacterial growth penetration rates of >0.01 $m \cdot day^{-1}$ through the tubes. In similar experiments, Sharma et al. (1993) found faster growth penetration rates of bacteria (0.1 $m \cdot day^{-1}$) through nutrient saturated sand packed columns. The laboratory

determined growth penetration rates found by these authors demonstrate that growth induced transport rates are more than enough to overwhelm diffusion controlled conditions ($P_e > 6x10^1$ (Fetter, 1992)). Sharma and McInerney (1994) filled columns with nutrient saturated porous media and monitored bacterial movement by sampling the distal column end using a syringe technique similar to Reynolds et al. (1989). While the static flow column is simple, sampling is likely to affect the static flow conditions necessary for diffusion studies.

The diffusion of isolated particles in a suspension can be estimated using the Einstein-Stokes equation (Smoluchowski, 1917). Yao et al (1971) and Rijnaarts et al. (1993) applied the equation to bacterial experiments and showed a good correlation between data from experiments and estimates from the Einstein-Stokes equation. Further development of the Einstein-Stokes equation by Batchelor (1976) showed that the Einstein-Stokes equation may be made applicable to cases where the concentration of the suspended particles is high enough that the particles can not be considered discreet.

Rijnaarts et al. (1993) measured free-water diffusion coefficients and bacterial radii of Coryneform bacteria and Pseudomonads using dynamic light scattering (Pusey and Tough, 1985). Free-water diffusion coefficients D_o represent the Brownian movement in the fluid medium, usually water. Calculated estimates of free-water bacterial diffusion coefficients were found using the bacteria radii and the Einstein-Stokes equation (Einstein, 1926)

$$D_B = \frac{kT}{6\pi\mu P}$$

Eq. 1

where k is the Stefan-Boltzmann constant (1.38×10^{-23} kg·cm^2·sec^{-2}·Kelvin^{-1}), T is the temperature (Kelvin), μ is the viscosity of the surrounding fluid medium (kg·cm^{-1}·sec^{-1}), and P is the radius of the spherical particle (cm). Estimates of the bacterial diffusion coefficients are presented in Table 3 along with measured free-water bacterial diffusion coefficients (Rijnaarts et al., 1993).

Table 3. Estimates of bacterial diffusion coefficients using Einstein-Stokes's equation D_B and free-water diffusion coefficients of bacteria D_o from Rijnaarts et al. (1993).

Strain	D_B (m^2·sec^{-1})	D_o (m^2·sec^{-1})
Coryneform strain DSM 6685	5.4×10^{-13}	5.90×10^{-13}
Rhodococcus sp. strain C125	1.9×10^{-13}	1.74×10^{-13}
Rhodococcus erythropolis A177	2.3×10^{-13}	1.95×10^{-13}
Corynebacterium sp. strain DSM 44016	4.7×10^{-13}	3.78×10^{-13}
Gordona sp. strain DSM 44015	3.4×10^{-13}	2.61×10^{-13}
Pseudomonas fluorescens p62	3.8×10^{-13}	3.75×10^{-13}
Pseudomonas putida mt2	3.8×10^{-13}	3.65×10^{-13}

Some research describes bacterial motility as a series of run and tumble events (Berg and Brown, 1972). Slater et al. (1981) used dynamic light scattering measurements to correlate the run duration, the angle change, and the swimming velocity of motile bacteria. In auxiliary experiments, Slater et al. measured bacterial diffusion

coefficients for motile and non-motile bacteria using dynamic light scattering. The authours determined bacterial diffusion coefficients 3 orders of magnitude greater for motile than for non-motile bacteria ($4.6x10^{-13}$ and $3.6x10^{-10} m^2 \cdot sec^{-1}$, respectively).

The goal of this study is to better understand the role of bacterial diffusion in bacterial transport. The specific objective of this thesis is to determine free-water bacterial diffusion coefficients for select motile and non-motile bacteria. Three independent methods were investigated and applied to achieve this objective: the double reservoir diffusion model, the diffusing sphere technique, and dynamic light scattering.

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