HEATED BIOFILM GROWTH IN A PLANAR FRACTURE FOR REDUCTION OF HYDRAULIC APERTURE

By

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Abstract

The objective of this study was to examine the effect of heat on the growth of a biofilm in a parallel glass plate fracture table. Groundwater was collected from a limestone aquifer and amended with a nutrient mixture to stimulate the indigenous microbial population. The amended water was heated to approximately 30°C in an upgradient reservoir attached to the fracture table and recirculated through the 2-m long, 0.6-m wide, parallel glass plate fracture having an approximate fracture aperture of 2000 μm. The fracture was maintained at approximately 10°C to simulate natural in situ groundwater temperature and the upgradient reservoir maintained at 30°C. Geochemical parameters and bacterial counts were measured regularly throughout the biostimulation to monitor biofilm growth in the fracture. Hydraulic tests and tracer experiments completed before and after the biostimulation were used as the primary indicators of the successful bioclogging of the fracture.

Geochemical parameters measured throughout the trial revealed an increasingly reducing environment capable of supporting the development of a diverse biofilm. Direct and indirect bacterial counts revealed the dominant bacteria within the system included common groundwater bacteria pseudomonads, enteric, and slime-forming bacteria. Heterotrophic bacteria were also present in significant concentrations. Visible clusters of biofilm were observed on Day 2 of the trial with a fully-connected biofilm observed by Day 7.

The biofilm impacted the groundwater flow through the fracture resulting in an approximately 2.75-hour delay in the tracer’s breakthrough during the tracer experiment completed on Day 13 of the trial compared to an experiment conducted during the initial stages of biofilm development. Based on the results of the tracer experiment, the biofilm growth reduced the velocity of the groundwater by 9.8%, the fracture aperture by 37.8%, and increased the bulk
dispersivity to 50mm. Recommendations for future work include the application of heated biostimulation at the field scale in a well-characterized, isolated fracture.
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Chapter 1- Introduction

Contaminated fractured bedrock aquifers present a complex technical challenge. Unlike in unconsolidated aquifers, groundwater and contaminant migration in fractured rock is primarily influenced by the presence and interconnectivity of fractures in the subsurface. Furthermore, the concentration gradient between the fracture pathway and the rock matrix may result in matrix diffusion, transferring contaminant into the rock matrix (Tang et al., 1981). Once in the matrix, the diffused mass can serve as a long term contaminant source for decades after the removal of the initial source zone by diffusing back into the fracture (Mutch et al., 1993). Therefore, remedial strategies employed in rock must successfully target both fracture and contaminant mass sequestered in the rock matrix.

Unfortunately, many of the remedial strategies employed in porous media are difficult to successfully adapt to fractured rock in a cost effective manner (NRC, 1994). In situ biostimulation is one remediation technique employed in porous media since the early 1970s that shows some promise in fractured rock applications (Ross and Bickerton, 2002). Biostimulation involves modifying the natural aquifer conditions, typically through nutrient addition, to stimulate the growth of planktonic indigenous microorganisms. Planktonic bacteria can form a structured sessile bacterial community known as a biofilm by excreting extracellular polymeric substances (EPS). EPS are necessary for bacterial adhesion to the geologic material and sequestration of additional nutrients to enable continued bacterial/biofilm growth (Freeze and Cherry, 1979; Fetter, 1999). The formation of the biofilm and continued addition of biomass to the pore spaces or fractures contribute significantly to the clogging of the groundwater and contaminant flow pathways (Vandevivere and Baveye, 1992a, 1992b; Ross et al., 2001).
The benefits of bioclogging at contaminated fractured rock sites are multi-fold. Firstly, it reduces the effective hydraulic conductivity of the geologic media, thereby reducing contaminant transport distances and effectively hydraulically containing the contaminant (Ross and Bickerton, 2002; Hill and Sleep, 2002; Arnon et al., 2005; Ross et al., 2007). Secondly, microbial growth has been shown to penetrate into the rock matrix of porous sedimentary rocks and reduce the back diffusion of contaminant from the matrix into the fracture (Charbonneau et al., 2006). Thirdly, the stimulated microorganisms may be capable of biodeggrading the contaminant depending on the conditions and contaminant of interest (Cookson, 1995; Chapelle, 2001). Finally, the external energy required to stimulate the indigenous microbes is less than that required for other conventional remedial strategies such as pump and treat (NRC, 1994).

Numerous studies in porous media at both the laboratory (Jennings et al., 1995; Kim et al., 2006) and field scales (Cunningham et al., 2003) have shown significant reductions in the effective hydraulic conductivity of the test media following biostimulation. Reductions in conductivity greater than 99% have been achieved in the laboratory and field (Ross et al., 2001; Cunningham et al., 2003). Similar reductions in the effective hydraulic conductivity were achieved in laboratory investigations on the effects of bioclogging on fractured rocks systems (Ross et al., 2001; Hill and Sleep, 2001; Arnon et al., 2005; Castegnier et al., 2006; Ross et al., 2007).

Unfortunately, most laboratory-scale studies using fractured media have been conducted on relatively small-scale fracture systems (e.g., 21 cm wide by 28 cm long (Hill and Sleep, 2002)). In the largest-scale trial conducted to date, Ross et al. (2007) developed a biofilm in a 1500-μm thick planar glass fracture measuring 2 m in length and 0.6 m in width. In the trial, groundwater collected from a shale fracture was amended with a 50 g·L⁻¹ invertose solution over 50 days to stimulate the indigenous bacteria. The system was then starved of nutrients for an
additional 50 days to examine the effects of nutrient starvation on biofilm growth and persistence. Groundwater flow through the fracture was also increased during the final five days of the trial to further examine biofilm stability and persistence. All three phases of the trial were completed at 10°C to simulate natural groundwater temperatures. Evidence of biofilm formation was observed through visual observation and optical density measurements after five days of nutrient addition and by the end of the 100-day trial, the biofilm reached a maximum coverage area of 960 cm². Hydraulic tests indicated a 21% reduction in effective hydraulic conductivity of the fracture following the 100-day trial.

In comparison to porous media, few field-scale studies on biostimulation have been completed in fractured rock environments. Knight (2008) and Bayona (2009) examined the geochemical and hydraulic effects, respectively, associated with biostimulation of an uncontaminated, isolated, dolostone fracture in southern Ontario, Canada. Biological data collected by Knight throughout the three week biostimulation shows significant increases in heterotrophic bacteria counts and an overall diversification of the microbial community following the addition of nutrients to the fracture. Hydraulic testing completed by Bayona prior to and following the biostimulation indicated a maximum reduction in fracture aperture of 28% measured immediately following the biostimulation.

In the early stages of the field trial conducted by Knight and Bayona, the application of heat to the nutrient-amended groundwater showed indications of accelerated growth in the aboveground mixing reservoir. Unfortunately, heating throughout the field trial was not possible due to logistical considerations. However, based on the initial observations and the fact that planktonic bacterial growth rates generally double with each ten degree increase in temperature (Cookson, 1995; Chapelle, 2001), it is hypothesized that combining heating of the groundwater
with nutrient addition as a biostimulation approach may accelerate biofilm development and associated bioclogging in fractured bedrock settings.

The objective of this study is to examine the effect of heat on the growth of a biofilm in a parallel glass plate fracture. Groundwater was collected from a limestone aquifer and amended with a nutrient mixture to stimulate the indigenous microbial population. The amended water was heated to 30°C in an upgradient reservoir attached to the fracture table and recirculated through the 2-m long, 0.6-m wide, parallel glass plate fracture with an approximate fracture aperture of 2000 μm. The fracture was maintained at 10°C to simulate natural in situ groundwater temperature and the upgradient reservoir maintained at 30°C. Geochemical parameters and bacterial counts were measured regularly throughout the biostimulation to monitor biofilm growth in the fracture. Hydraulic tests and tracer experiments completed before and after the biostimulation were used as the primary indicator of the successful bioclogging of the fracture.
1.1 References


Bayona, L. (2009), The effects of *in-situ* simulation of natural biofilm on groundwater flow and back diffusion in a fractured rock aquifer, Queen's University, Kingston, Ontario.


Knight, L. (2008), The effect of biostimulation on geochemical and microbiological conditions in an isolated dolostone fracture, Queen's University, Kingston, Ontario.


Chapter 2 - Literature Review

2.1 Fractured Rock

Groundwater flow and solute transport in fractured rock environments are inherently different from flow and transport in porous media. In porous media, porosity is the primary factor affecting flow and transport. In fractured rock however, open fractures within the rock are significantly more porous than the rock matrix (Mutch et al., 1993) and it is the presence, permeability, and interconnectivity of fractures that dominate groundwater flow and solute transport in fractured media.

2.1.1 Groundwater Flow and Solute Fate and Transport

Groundwater flow through fractured rock is typically described using the Cubic Law (Equation 2.1) to represent flow through a single fracture in laminar conditions (Bear, 1972; Witherspoon, 1980). The Cubic Law is derived from the Navier-Stokes equation which describes the flow of a viscous, incompressible fluid such as groundwater. In the derivation of the Law, parallel plate theory is applied to represent the fracture as two, parallel planar plates as shown in Figure 2.1.

\[
\frac{Q}{\Delta h} = \frac{W \rho g}{L^{12} \mu} 2b^3
\]

(2.1)

Where \( Q \) is flow rate (\( L^3 \cdot T^{-1} \)), \( h \) is hydraulic head (\( L \cdot L^{-1} \)), \( W \) is fracture width (\( L \)), \( L \) is fracture length (\( L \)), \( \rho \) is fluid density (\( M \cdot L^{-3} \)), \( g \) is gravitational acceleration (\( L \cdot T^{-2} \)), \( \mu \) is flow viscosity (\( M \cdot L^{-1} \cdot T^{-1} \)), and \( 2b \) is fracture aperture (\( L \)).
Figure 2-1: Schematic of Parallel Plate Theory as often applied to fracture bedrock hydrogeology (after Bear, 1972).

The Cubic Law was initially derived for open fractures however, Witherspoon et al. (1980) showed the Law valid for closed fractures in which asperities in the fracture touch and the aperture decreases under stress.

Fractured rock environments generally include numerous fractures, some of which are connected. It is through these connected fractures that the majority of groundwater flow occurs. Groundwater is also present within isolated fractures but this water is essentially immobile water since the fractures are not hydraulically connected to the fracture flow. The rock matrix also contains groundwater within the pore spaces of the rock. Since the rock matrix can account for up to 99% of a fractured rock aquifer by volume (Mutch et al., 1993), and the porosity of sedimentary rocks can be as high as 20% (Freeze and Cherry, 1979), a significant amount of water within an aquifer may be sequestered within the rock itself. However, only the pore water present within interconnected pores that are also connected to the fractures will potentially interact with the fracture flow network.

Solute transport within a single fracture may occur via advection, dispersion, and diffusion as described by Equation 2.2 (Tang et al, 1981).

\[
2b \left[ \frac{\partial c}{\partial t} + \frac{v}{R} \frac{\partial c}{\partial z} - \frac{D}{R} \frac{\partial^2 c}{\partial z^2} + \frac{\lambda c}{R} \right] - \frac{2q}{R} = 0 \quad 0 \leq z \leq \infty
\]  

Where \(2b\) is the fracture aperture (L), \(c\) is solute concentration (M·L\(^{-3}\)), \(t\) is time (T), \(v\) is groundwater velocity (L·T\(^{-1}\)), \(z\) is the distance along the length of the fracture (L), \(D\) is the
hydrodynamic dispersion coefficient \((L^2 \cdot T^{-1})\), \(R\) is the retardation coefficient along the fracture wall \((-\cdot)\), \(\lambda\) is the decay constant \((-\cdot)\), and \(q\) is the diffusive flux perpendicular to the fracture axis \((M \cdot L^{-2} \cdot T^{-1})\). Fick’s first law, as shown in Equation 2.3, can be applied as the diffusive flux term.

\[
q = -\theta D' \frac{\partial c'}{\partial x}
\]  

(2.3)

Where \(\theta\) is the rock matrix porosity \((L^3 \cdot L^{-3})\), \(D'\) is the effective diffusion coefficient \((L^2 \cdot T^{-1})\), \(c'\) is the solute concentration in the matrix \((M \cdot L^{-3})\), and \(x\) is the direction into the matrix perpendicular to the fracture axis \((L)\).

As indicated by the diffusive flux term in Equation 2.3, solute present within a fracture may diffuse into the pore water of the rock matrix. This transfer of mass is the result of a concentration gradient between the groundwater within the fracture and the pore water. If the concentration gradient is reversed (i.e., solute-free water is flushed through the fracture), back diffusion will occur and contaminant mass will diffuse from the pore water back into the fracture water (Mutch et al., 1993). The rates of forward and back diffusion are a function of the concentration gradients as well as the effective porosity of the rock matrix (Tang et al., 1981).

### 2.2 Bioclogging in Fractured Rock

Bioclogging is a type of bioremediation that focuses on stimulating biofilm growth in the subsurface to control fluid movement (Ross et al., 2007). The concept of bioclogging was initially applied in the oil industry as a method of plugging high-permeability rock to direct water flooding to lower-permeability areas and push out the oil trapped in the low-permeability zones (Lappin-Scott et al., 1988). However within the last 20 years, bioclogging has been increasingly studied as a remedial approach designed to control contaminant transport by reducing the hydraulic conductivities of impacted aquifers. Several researchers have documented significant
 (>99%) reductions in the hydraulic conductivity of porous media at contaminated sites (Jennings et al., 1995; Cunningham et al., 2003; Kim et al., 2006). Unfortunately, there have been very few studies of bioclogging in fractured rock. This section presents a summary of the work completed to date by researchers in fractured rock.

2.2.1 Laboratory Studies

Laboratory studies of bioclogging in fractured rock can be divided into studies that use rock samples to create an artificial fracture and studies that use parallel glass plates to create an idealized rock fracture. There are two known studies that use bedrock (limestone) samples to create a laboratory-scale fracture (Ross et al., 2001a; Castegnier et al., 2006). Parallel glass plate apparatus were used by Hill and Sleep (2002) and Ross et al. (2007).

Ross et al., (2001a) assessed the potential for indigenous bacteria to clog a limestone fracture following biostimulation using a single-fracture apparatus prepared by cutting a large limestone rock samples into two pieces. The fracture was created by removing a 5-cm wide, 50-cm long, 5000 μm aperture section from one of the two rock pieces before reassembling the rock pieces with silicone sealant and clamps and resaturating the rock. Groundwater collected from a 70-m deep observation well provided indigenous groundwater bacteria that were concentrated and then resuspended in synthetic groundwater. The fluid was then injected into the fracture at a rate of 0.15 mL·min⁻¹ along with 0.88 g·L⁻¹ of molasses. Subsequent inoculations occurred once per week throughout the study. Hydraulic gradient was measured across the fracture using piezometers installed at either end, and the Cubic Law applied to determine the reduction in hydraulic conductivity throughout the trail. Results indicated that a 14% reduction in fracture hydraulic conductivity was noted after eight days with a maximum reduction of greater than 99% noted after 22 days.
Castegnier et al. (2006) used the same laboratory apparatus as Ross et al. (2001a) to evaluate the long-term persistence of a biofilm in a limestone fracture under starvation conditions. The biostimulation phase lasted 43 days with an associated reduction in fracture hydraulic conductivity of greater than 99% in the upgradient and central portions of the fracture. The starvation phase lasted 179 days and the starved biofilm persisted for 129 days following biostimulation. After 50 days of starvation, a series of clogging and unclogging events occurred, resulting in wide fluctuations in both flow rate and hydraulic head measurements.

Hill and Sleep (2002) examined the effects of biofilms on flow and transport through an idealized fracture constructed of two parallel glass plates measuring 21-cm wide, 28-cm long, and sealed together with an aperture of approximately 570 μm. The fracture was inoculated with microorganisms concentrated from top soil and resuspended in a solution containing deionized water and nutrients. This solution was continuously recirculated through the fracture, along with 5 g L⁻¹ glucose for 26 days, at a temperature of approximately 20°C and under a hydraulic gradient of 0.044. Small, less than 5 mm diameter, biomass clusters were visually observed on day 4 of the trial with connecting clusters as large as 1 cm in diameter present by day 7. A continuous biofilm consisting of primarily filamentous bacteria was present in the first half of the fracture by day 12 of the study. Tracer experiments completed throughout the growth phase showed a reduction in the hydraulic conductivity of the fracture of 96.7%, occurring on day 3. Tracer experiments completed prior to and during biofilm growth indicated that both Taylorian and macro dispersion were influencing transport in the fracture before biostimulation, whereas macro dispersion dominated transport during the biostimulation phase.

A larger parallel glass plate apparatus was used by Ross et al. (2007) to examine the development and resistance of a biofilm grown at 10°C during biostimulation, starvation, and variable flow rates. The idealized fracture consisted of two, 0.6-m wide, 2-m long glass sheets separated by a 1500 μm diameter wire along each edge and sealed with silicone sealant.
Groundwater collected from an isolated shale fracture was injected into the fracture via a 76-mm diameter, packered borehole at a velocity of 5 m·d⁻¹. An invertose solution (50 g·L⁻¹ in reverse osmosis water) was also injected into the borehole at a rate of 0.3 mL·min⁻¹ during the 50-day biostimulation phase. The biostimulation phase was followed by an additional 50-day starvation phase, during the last four days of which the groundwater flow rates through the table were increased.

Throughout the trial, biofilm growth and its effect on hydraulic conductivity were measured via daily direct observations using a light transmittance probe, weekly sampling of planktonic bacterial density, continuous ORP monitoring through 14 inline ports in the fracture table, and tracer experiments completed three times over the course of the trial. Notable results include the observation of clustered bacterial growth similar to that observed by Hill and Sleep (2002) near the injection borehole after approximately five days of biostimulation. By day 30, the clusters formed a fully connected, visible biofilm. The biofilm darkened to a brownish colour during the starvation phase and no further changes were noted in the film during increased flow rates.

The tracer experiments conducted on days 2, 50 and 78 involved injecting Lissamine and Bromide tracers into the borehole and monitoring their arrivals at three downgradient sample ports in the fracture table. Model results indicated a 17% decrease in average flow velocity in the fracture between the initial test and the two subsequent tests.

2.2.2 Field Studies

There has been only one known field study involving bioclogging of fractured rock to date. Knight (2008) and Bayona (2009) conducted a joint study in an isolated, uncontaminated, horizontal fracture at a site in southern Ontario. Knight focused on the biological and
geochemical effects of biofilm growth in the fracture during and after biostimulation while Bayona examined the effects of the biofilm on the fracture transmissivity.

In the study, a single horizontal fracture was isolated using a straddle packer system in three boreholes. An injection-withdrawal recirculation system was established between two of the wells with an interwell distance of 4.95 m. Groundwater was pumped from the downgradient well at a rate of 2 Lpm into an insulated, 100-L surface reservoir. The indigenous groundwater bacteria were stimulated through the addition of nutrients to the surface reservoirs between two and four times a day throughout the three week biostimulation phase. The nutrient solution consisted of sodium lactate and commercially available liquid plant fertilizers with a carbon:nitrogen:phosphorous molar ratio of 100:9:4. The target bioavailable carbon concentration in the surface reservoir throughout biostimulation was 8.9 g·L⁻¹. Heaters were also applied to the surface reservoir during 12 days of biostimulation to observe the effects of heat on biological growth. Although accelerated growth was observed in the surface reservoir, the heaters were removed on day 12 due to logistical concerns.

Groundwater samples collected from the withdrawal well before, during and four months after biostimulation were analyzed for both biological (heterotrophic plate counts and denaturing gradient gel electrophoresis (DGGE) analysis) and geochemical (select metals and inorganics) parameters. Biological results indicated significant heterotrophic bacterial growth and increased diversity during biostimulation. Geochemical results for parameters such as dissolved oxygen and other redox-related parameters (nitrate, ferrous iron, sulfate) suggest an anaerobic system, specifically in the zone of sulfate reduction, developed through the biostimulation phase.

Bayona (2009) conducted pulse interference tests prior to, immediately after, and four months after the biostimulation to evaluate the effects of biofilm growth on the hydraulic conductivity of the isolated fracture. Pulse interference tests are conducted by injecting a slug or pulse of water into the injection well and monitoring neighbouring wells for the arrival of the
pulse via changes in hydraulic head. Tests completed between the biostimulation injection well and withdrawal wells indicated a 65% reduction in the transmissivity of the isolated fracture immediately following biostimulation.

2.3 Biofilms

Present in environments and applications ranging from heat exchangers, river beds, water treatment facilities, to the subsurface, biofilms are of interest to researchers from multiple disciplines. Biofilms consist of localized concentrations of microorganisms contained within an extracellular polymeric substance (EPS) matrix. Biofilms can colonize any water-exposed surface, known as the substratum, which is typically an impermeable, non-porous surface although porous, inert, and even food sources can support biofilms (Wilderer and Characklis, 1989).

Biofilms can be composed of single bacterial species or multi-species communities with a heterogeneous distribution of organisms and metabolic activities (White et al., 1999). The composition of a biofilm is constantly changing with components varying based on the microorganisms involved, nutrient conditions, hydrodynamics, ionic species and strength in the surrounding liquid, as well as the phase of the biofilm (Busscher and van der Mei, 1997).

Biofilm phases include initiation, maturation, maintenance and dissolution (Mitik-Dineva et al., 2006). During initiation, planktonic bacteria begin to sense and respond to favourable conditions such as nutrient availability and temperature, enabling their transition from planktonic to sessile bacteria. The maturation phase involves the formation of characteristic biofilm structures (e.g., mushroom) and the production of molecules such as EPS needed to create and maintain a stable biofilm community. Biofilm maintenance generally requires constant nutrient availability and maintaining other environmental conditions essential to biofilm survival (e.g.,
shear stresses, temperature, pH, etc). Insufficient nutrient supply or changing environmental conditions may result in the dissolution or detachment of the biofilm.

2.3.1 Biofilm Components

2.3.1.1 Bacteria

Biofilms can include microorganisms ranging from viruses to bacteria, but bacteria are often the dominant organisms (Characklis et al., 1990a). Their dominance is largely due to their colonizing efficiency related to their small size, rapid growth rates, adaptability to changing environments and their ability to produce EPS. The size of a bacterial cell typically ranges from 0.5 to 3.0 μm depending on the shape of the cell and growth conditions (Cullimore, 1992). The composition of bacteria is estimated by a carbon:nitrogen:phosphorous ratio of 100:20:5 and an estimated cell density of 1.07 g·cm\(^{-3}\) (Characklis et al., 1990a).

Bacteria are classified based on the methods in which they capture energy for metabolism and growth. Organisms that use sunlight as an energy source are termed photosynthetic. Chemolithotrophic bacteric capture their energy from inorganic compounds and carbon dioxide. And heterotrophic bacteria obtain energy by reducing organic carbon substrates. Heterotrophic bacteria are the most ubiquitous of the three groups and the most important type found in biofilms (Characklis et al., 1990a).

Heterotrophic bacteria are further classified based on their interactions with oxygen. For example, aerobic heterotrophs are only able to grow in the presence of oxygen, using oxygen as the final electron acceptors in a series of energy-gaining electron transfers. Anaerobic heterotrophs are only able to grow in the absence of oxygen and use alternate terminal electron acceptors such as sulphate. Another major type of heterotrophic bacteria is facultative anaerobic. These microorganisms can grow with or without oxygen (Baker and Herson, 1994; Chapelle, 2001).
All three types of bacteria, as well as other types of heterotrophic bacteria, are possible within a single biofilm because of the limited diffusion of dissolved oxygen and nutrients through the EPS matrix (Christensen, 1989; Characklis et al., 1990b). A common example is anaerobic bacteria dominating the base film closest to the substratum, where dissolved oxygen concentrations will be limited by molecular diffusion through the EPS, and aerobic bacteria dominating the surface film in contact with the fluid that contains a higher concentration of dissolved oxygen. Heterogeneous and dynamic biofilms result from this varying nutrient and oxygen availability within the film, as well as the spatial arrangement of different bacterial species or even similar species bacteria of different physiological stages.

Numerous researchers have also reported that some bacteria are able to survive in low nutrient conditions by reducing their size to smaller than 0.3 μm and be resuscitated later by adding nutrients to the system (Lappin-Scott et al., 1988; MacLeod et al., 1988; Ross et al., 2001b). Starvation survival is important in fractured rock applications because of the typically low nutrient concentrations in most bedrock aquifers.

2.3.1.2 Extracellular Polymer Substances

The majority of bacterial species produce polymers known as extracellular polymer substances (EPS) (Christensen and Characklis, 1990). In biofilms, the EPS appears as a hydrated substance attached to the microbial cells or as a separate, soluble “slime”. It is often referred to as the glue or sponge-like matrix that holds a biofilm together (Wilderer and Characklis, 1989). The concentration of this gel-like matrix in a biofilm is typically in the range of 1-2% w/v (Christensen and Characklis, 1990) but the matrix can account for up to 50-90% of the biofilm’s organic carbon (Bakke et al., 1984). The EPS is considered to be responsible for many of the physical and chemical properties of the biofilm.

EPS are extremely important in the biofilm formation. The EPS present at the surface of a microbial cell are considered to be a primary factor in the irreversible adhesion of the cell to a
substratum. Allison and Sutherland (1987) showed the importance of EPS in biofilm formation using mutants of fresh-water bacteria. In the study, both non-EPS producing mutants and the mutant capable of producing EPS attached to a glass substratum. However, only the EPS-producing bacteria formed microcolonies or biofilm, suggesting that EPS plays an essential role in the formation of biofilms.

The EPS in biofilms consist almost exclusively of polysaccharides (e.g., glucose, sucrose, glucans, dextrans, etc). The type of polysaccharides is specific to the microorganisms involved and the chemical composition of the EPS has also been shown to vary with the different stages in the bacterial growth cycle (Uhlinger and White, 1983; Christensen and Characklis., 1990). The majority of the polysaccharides comprising the EPS are hydrophilic sugars, so most biofilms are also hydrophilic (Christensen, 1989; Christensen and Characklis, 1990). The EPS is generally anionic and therefore able to provide cation exchange abilities capable of trapping nutrients within the EPS. Organic cations present in the bulk fluid can be trapped by the EPS in the surface film and transported via molecular diffusion through the EPS matrix to microbial cell walls throughout the biofilm (Stolzenbach, 1989). The rate of molecular diffusion in the EPS can therefore control the rate and extent of various processes within the biofilm (e.g., metabolic activity) (Cookson, 1995).

2.3.2 Development

Biofilms are dynamic systems and the accumulation of a biofilm is the result of adsorption and desorption of cells on the substratum, growth of the attached cells, attachment and detachment of cells to and from the biofilm (Cunningham, 1989). The development of a biofilm can be divided into four primary stages discussed in the following sections: Initiation, Maturation, Maintenance, and Dissolution.
2.3.2.1 Initiation

Biofilm growth can begin with the sorption of organic matter in the bulk fluid to the substratum to create a conditioning film on the substratum. This film is created rapidly upon contact between the organics and substratum surface and modifies surface properties such as decreasing hydrophobicity and altering the surface charge. The conditioning film is considered to be a dynamic film, with both reversible and irreversible sorption occurring (Characklis, 1990a).

In fluid systems such as fractured groundwater aquifers, regardless of whether a conditioning film is present or not, biofilm initiation is dependent upon the transport and adhesion or attachment of microbial cells to the substratum. With respect to biofilms, adhesion or attachment are used interchangeably to describe “discrete and sustained association between a bacterium and a surface, or substratum” (Mitik-Dineva et al., 2006). The adhesion or attachment of cells to the substratum occurs by a variety of non-covalent intermolecular forces (e.g., electrostatic, polar, non-polar, hydrogen bonds, hydrophobic, etc) (Christensen, 1989).

The transport and attachment of bacteria in the bulk fluid to the substratum is controlled largely by the concentration of cells in the fluid as well as the hydrodynamic conditions of the system (Christensen, 1989). Higher concentrations of viable cells in the bulk fluid will improve the chance of bacteria hitting and adhering to the substrata (McFeters et al., 1999). In laminar flow conditions, transport of microbial cells can be described by Fick’s first law (Equation 2.3) which states that diffusive flux is proportional to the concentration gradient. Although Fick’s first law is generally applied to the diffusion of soluble compounds, it can also be applied for small particles such as microbial cells by applying the Brownian diffusion coefficient (Characklis, 1990a).

In turbulent conditions, the transportation of microbial cells to the substratum is dependent upon the physical properties of the cell (e.g., size, shape, density). Several transport
mechanisms are possible, including diffusion, gravity, and fluid dynamic forces (Characklis, 1990a).

Surface properties of the bacteria and substrata also play a significant role in the extent of adhesion possible. Several researchers have suggested that the microbial cell’s surface polymers may assist in binding the cell to surfaces by creating a conditioning film on the substrata (Busscher and van der Mei, 1997; Taylor et al., 1998). And rough surfaces generally provide a better attachment than smooth surfaces due to the protection from fluid shear forces.

Environmental factors may also impact the ability of a cell to adhere to a surface. McEldowney and Fletcher (1988) examined the effect of pH and temperature on the ability of different bacteria to permanently adhere to a polystyrene substratum. The researchers showed that common groundwater bacteria *Pseudomonas fluorescens* and *Enterobacter cloacae* showed peaks in adhesion at pH levels between 5.0 and 8.5. The number of microbial cells that permanently adhered to the polystyrene also increased with increasing temperatures, with maximum adhesion occurring between 25 and 50°C.

The number of attached cells varies within one to two orders of magnitude depending on the bacteria, substratum, and ground conditions. In flowing systems, the adhesion of microbial cells results in surface coverage of 0.01-5%. Therefore, greater coverage and higher density films are the result of subsequent growth and colonization of the substratum (Characklis, 1990b; Christensen, 1989). The rate of initial attachment and subsequent growth are the controlling factors in the early development of a biofilm.

2.3.2.2 Maturation

After biofilm initiation, the attached bacteria will continue to colonize the substratum, resulting in patchy clusters of cells as observed in early time films (Stolzenbach, 1989; Hill and...
Sleep, 2002; Ross et al., 2007). As the bacteria continue to colonize the surface and the biofilm grows in coverage and in thickness, the biofilm will have a more defined and spatially consistent structure.

More mature biofilms and colonies typically contain slowly growing cells with areas of greater thickness whereas younger communities have higher overall growth rates that are more spatially uniform (McFeters et al., 1999). Figure 2-2 presents a typical bacteria and biofilm growth curve. Young natural biofilms can also contain as many as 25 to 30 strains of bacteria. With ongoing competition for nutrients as the film matures, this number can be reduced to as few as five or less (Cullimore, 1992). Therefore, spatial variety and vertical stratification are common within more mature biofilms.

![Bacterial growth curve indicating stages of growth with time](image)

**Figure 2-2: Bacterial growth curve indicating stages of growth with time** (Baker and Herson, 1994).

2.3.2.3 Maintenance

Biofilms are dynamic systems and a steady state biofilm is not likely (Gujer and Wanner, 1989). However, as long as the changes in the environment and within the film are minute or are
balanced (e.g., equal cell growth rates and death rates), a system can be considered to be steady state and in maintenance phase. Therefore, biofilm maintenance requires consistent environmental conditions including nutrient concentrations, flow velocity, temperature, pH, etc. Maintaining the growth environment may be difficult in cases where bacterial growth itself creates a toxic environment for continued growth, possible through the production of harmful chemical byproducts or altering the pH of the system.

2.3.2.4 Detachment

Detachment from biofilms is the loss of cells and biomass from the film. There are five distinct mechanisms by which detachment can occur.

1. Predations: Predators in the bulk fluid (e.g., protozoa in septic systems) can graze or harvest the outer surfaces of a biofilm (Christensen, 1989).

2. Stress: Stress on individual cells or sections of the biofilm via a shift in environmental factors (e.g., pH, nutrient shortage) may cause cells to enter a non-attachable phase, shrink, and eventually fall off;

3. Shear: Water velocity shear forces result in the continuous removal of cells and particles from the surface of the film, with increasing velocity resulting in increased film detachment (Christensen, 1989; Powell and Slate, 1982);

4. Sloughing: The periodic loss of large portions of the film is termed sloughing. Unlike the other processes, sloughing is not a continuous process and can remove the entire thickness of a biofilm (Rittman, 1989); and

5. Abrasion: Abrasion where collisions between particles covered in biofilm results in the removal of the film (Christensen, 1989; Rittman, 1989).
The detachment rate is an important characteristic of the biofilm as it will directly affect the stability and longevity of the biofilm. The rate is influenced by the environmental conditions to which the film is exposed as well as the physiological state of the biofilm (Rittman, 1989).

2.3.3 Factors Affecting Growth

2.3.3.1 Bacterial Type

Many different types and strains of bacteria are capable of forming biofilms given the proper growth environment, however, biofilms grown by different organisms are not equal. Some might develop faster, some may be more stable, and they all might have different structures and compositions. As discussed in Section 2.3.1.2, Allison and Sutherland (1987) concluded that EPS production plays a significant role in the initiation and overall development of a biofilm. A microorganism’s ability to secrete EPS will greatly increase, if not control, the microorganism’s ability to form a biofilm. The shape and size of an organism may also impact biofilm development in particular environments (Lappin-Scott et al., 1988; MacLeod et al., 1988).

2.3.3.2 Nutrient Availability

A primary factor affecting bacterial growth is the availability of essential nutrients. The nutritional requirements are based on the typical composition of bacteria, approximated as \( \text{C}_5\text{H}_7\text{O}_2\text{N} \) with minor traces of other elements (e.g., S, Fe, K, Mg, Ca, Zn, Mo, Cu, P, and Mn). Therefore, biomass is approximately 95% by dry weight, carbon, hydrogen, oxygen and nitrogen, with calcium and phosphorus representing 3.5% of the remaining 5% (Cookson, 1995). These elemental needs must be obtained from an organism’s environment and if any of these six essential elements are not available in sufficient quantities, microbial growth will be limited. In a groundwater environment, carbon, nitrogen and phosphorus are typically the limiting nutrients. All three are needed for the synthesis of major molecules such as proteins, lipids, carbohydrates, and nucleic acids (Baker and Herson, 1994).
All living material uses carbon to form the skeleton upon which everything else grows. Organic carbon exists in the subsurface as both living and decaying plant and animal matter, and as contaminants such as trichloroethylene. In environments such as fractured bedrock aquifers, where naturally occurring organic carbon concentrations are too low to sustain bacteria, other carbon sources can be added to the environment (Cookson, 1995). Commonly added sources of carbon include simple sugars (e.g. glucose, lactose), polysaccharides, organic acids and proteins. Microorganisms use nitrogen to biosynthesize protein and nucleic acids, and to promote enzymatic functions in cells (Chapelle, 2001). Nitrogen is also used as a terminal electron acceptor by some respiring bacteria. Microorganisms that degrade contaminants typically require fixed forms of nitrogen such as ammonia, nitrate, nitrite, and organic carbon (Baker and Herson, 1994). Ammonia and nitrate are the most common forms of nitrogen and are typically added to the subsurface as liquid fertilizers.

Phosphorous is also included in many liquid fertilizers as a potassium salt such as $\text{K}_2\text{HPO}_4$ or $\text{KH}_2\text{PO}_4$. It is required by microorganisms to synthesize nucleic acids, cell membranes, and adenosine triphosphate, the energy regulator of the cell (Chapelle, 2001). Phosphorous has low water solubility and its availability to microorganisms may therefore be a limiting growth factor (Baker and Herson, 1994).

The last of the six essential elements, hydrogen and oxygen, are typically supplied by water in the subsurface. Therefore, an organism’s ability to grow or survive is dependent upon the availability of water and subsurface bacterial growth may be limited in zones with low water content.

2.3.3.3 Redox Conditions

In addition to the six essential elements, microorganisms will catalyse oxidation-reduction (redox) reactions to obtain the energy required for cellular growth (Snoeyink and
Redox reactions involve the transfer of electrons from an electron donor to an electron acceptor such as oxygen or nitrate. In general, microorganisms will use sources of carbon (e.g. lactate, trichloroethylene) as the electron donor and will use available electron acceptors preferentially based on which acceptors have the greatest potential for energy release (Larowe and Helgeson, 2007).

One of the principal variables considered to control redox reactions is the redox potential, \( E_h \), also known as the oxidation reduction potential (ORP) (Berkowitz et al., 2008). The ORP of a reaction is related to the Gibbs free energy of formation which measures the enthalpy of a system and indicates whether the reaction releases energy (exergonic) or stores energy (endergonic). An exergonic reaction will have a negative Gibbs free energy of formation, whereas an endergonic reaction will have a positive energy of formation.

In aqueous systems, the Gibbs free energy of formation is converted to the electrochemical potential of the half reaction \( E_h \), or \( E^0 \) when at standard conditions (i.e., 25°C, 1M concentration, 1atm). Equation 2.4 presents the relationship between Gibbs free energy of formation and the standard electrochemical potential.

\[
E^0 = -\frac{\Delta G}{nF} \tag{2.4}
\]

Where \( E^0 \) is the electrochemical potential (M·L²/A·T³), \( \Delta G \) is the Gibbs free energy of formation (M·L²/T²), \( n \) is the number electrons transferred, and \( F \) is Faraday’s constant. A higher \( E^0 \) indicates a greater tendency for reduction to occur and a lower \( E^0 \) indicates a greater tendency for oxidation.

Since microorganisms require energy for cellular functions, a thermodynamic analysis of the environment can be used to predict biological activity in a given system. Table 2.1 presents biologically catalysed reduction half-reactions common to subsurface environments, along with the standard electrochemical potential of each half reaction.
Table 2.1: Electron acceptance reactions common to subsurface environments (modified from Snoeyink and Jenkins, 1980).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$E^o$ (V)</th>
<th>Name of Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$O_{2(g)} + 4H^+ + 4e^- \rightarrow 2H_2O$</td>
<td>+1.23</td>
<td>Aerobic Respiration</td>
</tr>
<tr>
<td>Anaerobic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2(g) + 6H_2O$</td>
<td>+1.24</td>
<td>Denitrification</td>
</tr>
<tr>
<td>$NO_3^- + 10H^+ + 8e^- \rightarrow NH_4^+ + 3H_2O$</td>
<td>+0.88</td>
<td>Nitrate Reduction</td>
</tr>
<tr>
<td>$Fe^{3+} + e^- \rightarrow Fe^{2+}$</td>
<td>+0.77</td>
<td>Iron Reduction</td>
</tr>
<tr>
<td>$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$</td>
<td>+0.24</td>
<td>Sulfate Reduction</td>
</tr>
<tr>
<td>$CO_{2(g)} + 8H^+ + 8e^- \rightarrow CH_4(g) + 2H_2O$</td>
<td>+0.17</td>
<td>Methane Fermentation</td>
</tr>
</tbody>
</table>

The standard electrochemical potential can be converted to the electrochemical potential at non-standard conditions using Equation 2.5 (Drever, 1997).

\[
E_h = E^o - \frac{RT}{nF} \ln Q \tag{2.5}
\]

Where $R$ is a gas constant, $T$ is the temperature and $Q$ is the ratio of chemical activities of reduced species to oxidized species.

Measurements of the redox potential in the environment can be combined with measured concentrations of the electron acceptors identified in Table 2.1 above to determine the redox conditions in the system and possibly identify redox zonation in the subsurface (Chapelle, 2001).

2.3.3.4 pH

Most bacterial growth is generally limited to neutral pHs between 6.0 and 8.0, however, extreme pH environments have also exhibited bacterial growth (Baker and Herson, 1994; Cullimore, 1992). The pH of a growth environment will impact a cell’s ability to function, transport nutrients and electrons across the cell membrane, and the equilibrium of catalyzed
reactions (Baker and Herson, 1994). Therefore, most bacteria maintain an intracellular pH of approximately 7.5. If they are to live and grow in environments with extreme pHs, they must be adapted to their environment (Chapelle, 2001). For example, sulphide-oxidizing bacteria produce sulphuric acid as a by-product and must therefore be adapted to an acidic environment in order to survive. Although some bacteria may be able to adapt to varying pHs, most will reach optimum growth at a specific pH (Cookson, 1995).

In addition to cellular function, the pH of the growth environment will affect the subsurface chemistry with respect to the availability and mobility of nutrients and toxic metals (Baker and Herson, 1994). Increases in pH may result in decreased availability of nutrients such as nitrogen, phosphorous, calcium, magnesium and sodium. A decrease in pH may be accompanied by a decrease in the availability of nitrate. Fortunately, most groundwater environments include natural buffers via the aquifer materials and have neutral pHs such that anthropogenic buffering via carbonate or phosphate addition is not necessary.

2.3.3.5 Water Velocity

Biofilm structure is largely dependent on the flow regime in which the film developed. Biofilms grown in laminar conditions, with lower shear forces, generally develop as patchy clusters of cells that appear to be randomly distributed (Hill and Sleep, 2002; Ross et al., 2007). The films have been shown to be overall heterogeneous with the clusters of cells separated by interstitial voids (Stoodley et al., 1999). The films typically remain stable under moderate increases in flow velocity, however, a sudden substantial increase in fluid velocity and associated shear forces may lead to the detachment of the film.

Fluid shear forces can be significantly higher in turbulent conditions, limiting the ability of planktonic bacteria to successfully attach to the substrata and subsequently reducing the rate of attachment and biofilm development (McMath et al., 1999; Stoodley et al., 1999). In such
conditions, a filamentous biofilm typically results with filamentous bacteria initially colonizing the substrata. The filaments eventually become intertwined and can anchor subsequent non-filamentous growth within the film (Stoodley et al., 1999).

The transport of soluble nutrients required for biofilm growth is also significantly influenced by the groundwater flow velocity, as shown in Equations 2.2 and 2.3. Too slow a flow velocity may lead to suboptimal nutrient delivery and therefore limited growth. The slow removal of bacterial waste products may result in a toxic growth environment and also limit growth. Conversely, an increased flow velocity may reduce the sorption of the nutrients within the EPS matrix of the film and thereby reduce nutrient availability to bacteria within the film.

2.3.3.6 Substrata

The substratum is the surface colonized by the bacteria during the initial development of a biofilm (Wilderer and Characklis, 1989). Many different types of surfaces can serve as a substratum, including porous, nonporous, inert, surface susceptible to corrosion, as well as surfaces that can also serve as a food source for the bacteria. The properties of the substratum like surface roughness and wettability will affect the initial stages of biofilm growth.

Substrata with rougher surfaces are easier than smooth surfaces for bacteria to colonize because of the protection from fluid shear forces offered by the varied topography (Bott, 1999; Mitik-Dineva et al., 2006). The crevices in rougher surfaces also provide protection to bacteria from grazers and abrasion, both of which can lead to biofilm detachment (Wilderer and Characklis, 1989; Verran and Hissett, 1999). Biofilm studies in the laboratory are generally conducted using smooth surfaces to ensure reproducibility, even though most substrata in the environment are rough surfaces.
Surface wettability also impacts the colonization of a substratum by bacteria. Even though microbial cells and their EPS are overall hydrophilic in nature, hydrophobic surfaces are favoured by most bacteria for adhesion (Fletcher and Loeb, 1978; Mitik-Dineva et al., 2006). This counterintuitive preference may be due to the molecular arrangement of hydrophilic and hydrophobic groups within the bacteria (Christensen and Characklis, 1990). Furthermore, as discussed in Section 2.3.2.1 some bacteria are capable of creating a conditioning film to alter the properties of a given substrata and can therefore change the wettability of the surface to ease colonization.

2.3.3.7 Temperature

Bacterial growth is generally possible at temperatures between 0 and 100°C. The minimum growth temperature is limited by the freezing point of water although solute concentrations that lower the freezing point may allow some bacteria to grow at temperatures below zero (Cookson, 1995). Bacterial growth in the deep subsurface has been documented by many researchers (Zobell and Conn, 1940; Morita, 1972) at temperatures ranging from 60 to 105°C, however there is no general agreement regarding the highest documented temperature (Chapelle, 2001).

The temperature range at which individual bacterial strains can function is generally limited. Microorganisms with temperatures ranges between 0 and 20°C are classified as psychrophiles. Mesophiles are microorganisms that function at temperatures ranging from 20 to 40°C. Bacteria that grow at temperatures above 45°C are termed thermophiles (Chapelle, 2001). Most groundwater bacteria are mesophilic as most groundwater used for water supply in the world is between 20 and 30°C. In colder climates, most groundwater bacteria is likely psychrolilic due to colder water temperatures.
Bacterial growth is supported by a series of enzymatically catalyzed reactions and an increase in temperature will increase the rates of the reactions. The rate of reaction will vary exponentially with inverse temperature based on the Arrhenius equation (Oxtoby et al., 1999).

\[
k = A e^{-E_a/RT}
\]  

(2.6)

Where \( k \) is the rate constant, \( A \) is the pre-exponential constant factor, \( E_a \) is the activation energy of the reaction, \( R \) is the gas constant, and \( T \) is the absolute temperature.

Microbial activity generally increases by a factor of two for each ten degree increase in temperature (Baker and Herson, 1994). However, higher temperatures affect the structure of the enzymes and nucleic acids involved in the metabolic reactions necessary for microbial growth and increasing temperatures are favourable only to a point (Chapelle, 2001). The temperature at which the enzymes and nucleic acids are no longer capable of encouraging growth is the maximum cardinal temperature for that bacterial strain. Conversely, the temperature below which growth is not possible is the minimum cardinal temperature. The temperature at which the organism grows at its maximum rate is the optimum cardinal temperature and as shown in Figure 2-3, is slightly skewed from the mid-point of the operational range towards the maximum temperature.
The growth envelope for each bacterial strain, including the optimum temperature, is different. Therefore a shift in the temperature of the growth environment will result in a shift in population diversity (Cullimore, 1992). Furthermore, the minimum, optimum and maximum temperature can be affected by other factors such as the pH and salinity of the environment such that changes in these factors may also impact the bacterial population.

2.3.3.8 Suspended Solids

Along with nutrients and planktonic microbial cells, inorganic suspended solids may be present in fluids such as groundwater. While these inorganic solids may become trapped within the biofilm itself and add to the overall thickness of the film, the solids may also negatively affect biofilm development. High concentrations of suspended solids in the fluid have been shown to decrease biofilm growth rates, most likely due to the additional abrasion caused by the solids (Characklis, 1990a). The inorganic solid might also react with other ions in the fluid, possibly reducing the nutrient availability to the microorganism or significantly altering the geochemical conditions (Cookson, 1995).
2.4 Evaluating Biofilm Development and Bioclogging

Quantifying biofilm development is a difficult task because sampling the biofilm itself is destructive and inhibits future growth. Therefore biofilm development is measured using a combination of indirect measurements including viable and direct counting of planktonic bacteria as well as optical measurements of the biofilm. In general, an increase in planktonic bacteria is desired because it indicates that the environment is capable of supporting growth. Large planktonic populations also increase the number of microbial cells that may colonize the substrata and form a biofilm. The hydraulic effects of bioclogging can be evaluated using groundwater tracer experiments to examine the fate and transport of a solute in the flow system.

2.4.1 Viable Counting

Viable counting methods provide an estimate of the number of viable cells in a given sample that are capable of growth and thereby an indicator of the number of cells potentially able to form a biofilm. An increase in viable cells is also an indication that sufficient nutrients and geochemical conditions exist to support bacterial growth in the system.

There are two general methods that are widely used: the plate counting methods and the most probable number method. Plate counting involves spreading serially diluted samples on a culture media and incubating the sample (Chapelle, 2001). After incubation, the cells are counted microscopically and the results reported in cells per volume of sample.

The most probable number method also involves the serial dilution of samples, however, in liquid growth media instead of an agar-based media. Following incubation, samples with viable cells will exhibit more growth and be cloudy in appearance. Diluted samples that receive no viable cells will be transparent due to a lack of cell growth. The result is reported as greater
than the number of cells present in the largest dilution that turned cloudy following incubation and less than the number of cells present in the smallest dilution that was not cloudy.

The primary concern with both viable counting methods is the dependence upon the organisms’ ability to grow on the selected culture media. For example, heterotrophic plate counting is a common analytical test but targets only heterotrophic bacteria and will not enumerate non-heterotrophic populations.

2.4.2 Direct Counting

Direct counting methods involves individually microscopically counting the number of cells present in a known volume of sample. This method does not distinguish between viable and non-viable cells, therefore providing a total number of cells in the sample. Direct counting methods include the use of fluorescent stains such as acridine orange to dye the cells such that they can be observed and counted under an epifluorescent microscope at a given wavelength.

Measuring the turbidity or optical density of a liquid sample is an alternate direct counting method that is simple, rapid, non-destructive and low cost (Sutton, 1995). Bacteria in liquid cultures are exposed to a light beam in a spectrophotometer and the light is scattered from its original path by the bacteria in suspension. The loss of light from its original path is measured as absorbance (Koch, 1970) and is directly proportional to the number of cells in suspension (Chapelle, 2001). Depending on the size, shape and physiological state of the organism, the deviation of light from its original path will vary, making it difficult to correlate between different media and species (Chapelle, 2001; Koch, 1970; Bakke et al., 2001). Secondary scattering of light may also cause scattered light to reach the detector as well. Additional sources of concern include the cuvette orientation and condition, and the tendency of some organisms to clump and settle (Sutton, 1995). Rigidly controlled conditions and careful wavelength selection can minimize some of these concerns. The standard wavelength range is 420 to 600 nm, with many
researchers tending toward the higher end of the range (Koch, 1970; Ross et al., 2007; Knight, 2008).

2.4.3 Tracer Experiments and Modelling

Tracer experiments are commonly used to evaluate bioclogging and its affect on solute transport properties in both porous and fractured media (Vandevivere and Baveye, 1992b; Hill and Sleep, 2002; Ross et al., 2007; Seifert and Engesgaard, 2007). Tracer experiments typically involve the injection of a tracer into an injection reservoir or well and measurement of the tracer’s arrival at observation point(s). Common tracers used in fractured rock applications include Lissamine FF and Bromide because they have been shown to display limited adsorptive loss (Novakowski, 1985, Lapcevic et al., 2007). Furthermore, no adsorptive loss of the tracers to a biofilm was observed by Ross et al., 2007 and the tracers are considered to be biologically stable (Davis et al., 1995).

Tracer experiments completed in the field can be conducted under natural gradient or forced gradient conditions, with forced gradient tests typically completed in one of three different configurations (injection-withdrawal, radially divergent flow, and radially convergent flow) (Lapcevic et al., 2007).

Laboratory-based tracer experiments are generally less complicated than field-based tests because of the reduced scale of the test media and the associated shorter transport times between the injection and observation points. The flow field is also usually predetermined by the equipment set up and different configurations are generally not required.

Tracer experiment interpretation is conducted using Equation 2.2 for solute transport in a uniform groundwater flow field combined with the appropriate boundary conditions. For the case of a conservative tracer in a parallel plate glass fracture, the retardation, reaction and diffusive flux terms in Equation 2.2 do not apply and the equation becomes
Novakowski (1992a, 1992b) developed a solution to Equation 2.7 for the application where a slug of tracer is injected and observed in a reservoir of known volume and there is continuous mixing in both reservoirs. Although the solution was developed for the interpretation of field tests in porous media it is also applicable for the laboratory case of parallel plate glass fracture with reservoirs on the upgradient and downgradient ends (Figure 2-4). The boundary conditions applied in the derivation of the solution account for mixing in the upgradient reservoir via a mass balance on the fluid entering and leaving the reservoir. Equation 2.8 is the initial boundary condition for the fracture and Equations 2.9 to 2.12 are the initial, boundary, and continuity equations applied for mixing in the upgradient reservoir.

\[
\frac{\partial C}{\partial t} + v \frac{\partial C}{\partial x} - D \frac{\partial^2 C}{\partial x^2} = 0 \quad z \geq 0
\] (2.7)

\[
C(x, 0) = 0 \quad 0 \leq x \leq \infty
\] (2.8)

\[
C_i(t) = C(0, t)
\] (2.9)

\[
V_i \frac{dC_i(t)}{dt} = -\gamma v C_i(t)
\] (2.10)

\[
vC_i(t) = vC(0, t) - D \frac{\partial C(0, t)}{\partial x}
\] (2.11)

\[
C_i(0) = C_o
\] (2.12)

Where \(C\) is the resident concentration (M·L^{-3}), \(x\) is the distance along the fracture (L), \(C_i\) is the upstream reservoir concentration (M·L^{-3}), \(t\) is time (T), \(V_i\) is the volume of the upstream reservoir (L^3), \(\gamma\) is the cross-sectional area for flow (L^2), \(v\) is the average linear groundwater velocity (L·T^{-1}), \(D\) is the coefficient of hydrodynamic dispersion (L^2·T^{-1}), and \(C_o\) is the initial tracer concentration (M·L^{-3}).
Figure 2-4: Schematic diagram showing the configuration of the reservoirs and the fracture table used in tracer experiments (modified from Novakowski, 1992a).

The boundary condition at the downgradient reservoir also accounts for mixing in the reservoir through a mass balance approach. Equations 2.13 and 2.14 represent the downgradient initial and boundary conditions, respectively.

\[
C_e(0) = 0 \quad (2.13)
\]

\[
\frac{V_e}{V} \frac{\partial C(L,t)}{\partial t} = -\gamma D \frac{\partial C(L,t)}{\partial x} \quad (2.14)
\]

Where \(C_e\) is the concentration in the downgradient reservoir (M·L\(^{-3}\)), \(V_e\) is the volume in the downgradient reservoir (L\(^3\)), and \(L\) is the finite length between the upgradient and downgradient reservoirs (L).
2.5 References


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Chapter 3 – Microcosm Experiments

3.1 Introduction

Microcosm experiments were conducted in the laboratory prior to the planar fracture trial to determine the optimal bacterial growth conditions to be applied in the fracture trial. The microcosm experiments were designed to determine if the selected nutrient amendment would stimulate growth of indigenous bacteria collected from a study site in Kingston, Ontario; to determine the oxygen requirements of the bacteria; to evaluate the effect of bedrock on bacterial growth; and to confirm the effect of temperature on bacterial growth.

3.2 Methodology

The microcosm experiments were conducted using groundwater collected from a study site in Kingston, Ontario. The study site is underlain by approximately 4.5 m of overburden overlying limestone bedrock of the Middle-Ordovician Black River Group. The groundwater was collected from a single limestone fracture located approximately 17 m below ground surface and isolated with straddle packers. The water was stored for a maximum of 24 hours at 4°C in 1-L amber glass bottles prior to use in the laboratory.

Sterilized 10-mL glass cuvettes were filled with either nutrient-amended groundwater or groundwater only to evaluate whether the chosen nutrient mixture would stimulate growth of the indigenous bacteria, with the groundwater only cuvettes serving as the control samples. The nutrient mixture was selected based on literature (Cookson, 1995 and Knight, 2008) and previous work conducted at a similar site in southern Ontario and consisted of sodium lactate and liquid fertilizer mixed to yield a solution containing a carbon to nitrogen to phosphorus molar ratio of approximately 100:10:1. Details regarding the nutrient mixture are provided in Appendix A.

Samples of the limestone from the study site, with an average mass of 0.4349 +/- 0.2198 g, were placed in half of the cuvettes containing nutrient-amended groundwater
and half of the cuvettes containing only groundwater to evaluate whether the rock would influence bacterial growth. The size of the rock used was selected to limit the potential for interference with the spectrophotometric method used to evaluate bacterial growth, as discussed below. The samples were collected from the study site at the time of well installation using a diamond core drill rig and cut into smaller pieces using a diamond saw and/or rock hammer. The samples were placed in sterilized containers containing distilled water immediately after drilling and stored in the distilled water at 4°C until used in the microcosm experiments.

To evaluate the effect of anaerobic versus aerobic conditions on indigenous bacterial growth, half of the cuvettes were filled with zero headspace and sealed with a Teflon-lined plastic cap. The remaining cuvettes were left open to encourage an aerobic growth environment. All of the aerobic cuvettes were manually agitated daily throughout the microcosm experiment to promote interaction between the solution and the atmospheric environment. The capped, anaerobic cuvettes were also manually agitated daily as an experimental control.

The cuvettes were placed in water or glycol baths at 4°C, 10°C, 20°C, and 30°C to evaluate the effect of temperature on bacterial growth. Table 3.1 summarizes the various configurations of experimental conditions examined at each of the temperatures of interest. In all cases, duplicate cuvettes were prepared and used in the study. A detailed list of the cuvette contents, including the mass of rock added where applicable, is provided in Appendix B.

Table 3.1: Summary of experimental conditions used in microcosm experiment.

<table>
<thead>
<tr>
<th>Aerobic</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Groundwater only (control);</td>
<td>• Groundwater only (control);</td>
</tr>
<tr>
<td>• Groundwater and rock;</td>
<td>• Groundwater and rock;</td>
</tr>
<tr>
<td>• Groundwater and nutrients;</td>
<td>• Groundwater and nutrients;</td>
</tr>
<tr>
<td>• Groundwater, nutrients and rock.</td>
<td>• Groundwater, nutrients and rock.</td>
</tr>
</tbody>
</table>
Bacterial growth was evaluated throughout the microcosm experiment using a Hach 2800 Spectrophotometer to measure the amount of light transmitted by the contents of each cuvette. The percentage of light transmitted through the cuvette is inversely proportional to the concentration of bacteria in the cuvette. Because the transmitted light does not distinguish between live or dead bacterial cells in the solution, the spectrophotometer is useful for evaluating the bacterial lag and exponential growth phases only and not the stationary and death phase.

A wavelength of 600nm was selected based on a literature review (Koch, 1970; Ross et al., 2007; Knight, 2008). At least once a day for 17 consecutive days, the cuvettes were removed from the temperature baths, allowed to equilibrate to room temperature, and wiped to remove condensation, glycol, etc. before the light transmittance was measured using the spectrophotometer. The end point of the experiment was determined to be when no significant change in transmittance was recorded in any of the cuvettes for at least two consecutive days and this occurred on the sixteenth day.
3.3 Results and Discussion

Thirty-two sample cuvettes, prepared in duplicate for a total of 64 cuvettes, were included in the microcosm experiment as summarized in Table B1 in Appendix B. The results of the experiment are presented in Figures 3-1 through 3-9 and are discussed below. The results presented on Figures 3-2 through 3-9 are the arithmetic mean of the two results obtained at each point in time, with the error bars representing the two measurements obtained.

![Graph showing temperature profiles](image)

**Figure 3-1:** Temperature profiles measured for the 4°C, 10°C, 20°C, and 30°C samples during the microcosm experiment.
Figure 3-2: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing only groundwater (i.e., control samples) and maintained at 4°C, 10°C, 20°C, and 30°C in anaerobic conditions.

Figure 3-3: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing groundwater and rock and maintained at 4°C, 10°C, 20°C, and 30°C in anaerobic conditions.
Figure 3-4: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing nutrient-amended groundwater and maintained at 4°C, 10°C, 20°C, and 30°C in anaerobic conditions.

Figure 3-5: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing nutrient-amended groundwater and rock and maintained at 4°C, 10°C, 20°C, and 30°C in anaerobic conditions.
Figure 3-6: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing only groundwater (i.e., control samples) and maintained at 4°C, 10°C, 20°C, and 30°C in aerobic conditions.

Figure 3-7: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing groundwater and rock and maintained at 4°C, 10°C, 20°C, and 30°C in aerobic conditions.
Figure 3-8: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing nutrient-amended groundwater and maintained at 4°C, 10°C, 20°C, and 30°C in aerobic conditions.

Figure 3-9: Light transmittance results measured during the Microcosm Experiment for the sample cuvettes containing nutrient-amended groundwater and rock and maintained at 4°C, 10°C, 20°C, and 30°C in aerobic conditions.
The results indicate that in general, no significant changes in the percentage of light transmitted through the cuvettes were recorded for any cuvettes that contained groundwater only, regardless of the sample temperature, the inclusion of rock or not, or whether the sample was maintained in an aerobic or anaerobic environment. A slight decline was observed for the average light transmitted by the nutrient-amended samples that contained rock and were maintained at 10°C in anaerobic conditions (Figure 3-5), however, when the standard deviation for these results is considered, the results are comparable to those of similar samples maintained at different temperatures.

Therefore there are insufficient concentrations of the nutrients essential to the growth of the indigenous bacterial population in the groundwater alone and, as anticipated, nutrient addition is necessary to encourage population growth.

Figures 3-8 and 3-9 present the results for the sample cuvettes that included nutrient-amended groundwater and were exposed to atmospheric conditions during the experiment. Figure 3-8 includes the results for the cuvettes that did not include pieces of limestone, whereas Figure 3-9 includes the results for the cuvettes that did contain pieces of rock. Statistical comparison of the results presented in Figures 3-8 and 3-9 completed using t-tests (α=0.05) confirmed that there is a negligible difference in the results obtained for cuvettes that did and did not include pieces of limestone under aerobic conditions. The t-tests were completed by comparing the pair of light transmittance results obtained from both samples at a single point in time (i.e., point-by-point comparison). The limestone pieces included in the cuvettes represented by Figure 3-9 did not appear to alter the solution chemistry such that bacterial growth was affected and are likely chemically inert with respect to the timeframe of the experiment. Therefore, the inclusion of limestone had little to no effect on the growth of the bacteria during the microcosm experiment.
As shown in Figures 3-8 and 3-9, the light transmitted by the samples maintained at 10, 20, and 30°C was reduced by 50 percent as early as Day 8, Day 4, and Day 2, respectively. In other words, samples In comparison, the light transmitted by the 4°C samples decreased by only one third by the end of the 16 day experiment. These results indicate that the growth rate of the indigenous bacteria is directly proportional to the temperature of the growth environment. Furthermore, the results generally agree with the reviewed literature which stated that the bacterial growth rates typically double with every 10°C increase in temperature (Baker and Herson, 1994; Cookson, 1995; Chapelle, 2001).
3.4 References


Knight, L. (2008), The effect of biostimulation on geochemical and microbiological conditions in an isolated dolostone fracture, Queen's University, Kingston, Ontario.


Chapter 4

Planar Fracture Experiment

4.1 Introduction

A parallel plate planar fracture table was used to investigate the effect of groundwater temperature on the rate of biofilm growth and associated bioclogging in a fractured rock environment. Groundwater collected from a limestone aquifer and amended with a nutrient mixture selected to stimulate the growth of the indigenous bacteria was heated to 30°C in an upgradient reservoir attached to the fracture table. Flow was established by circulating flow out of the upgradient reservoir through the fracture table and into a similar reservoir in the discharge end and then back into the heated reservoir. The constant head reservoirs at each end of the fracture table were used to maintain constant flow in the fracture and to represent the equivalent to injection and withdrawal boreholes that would be used in a field application. The fracture table and end reservoir were maintained at 10°C to simulate typical in situ groundwater temperatures and the upgradient reservoir was maintained at 30°C. Geochemical parameters and bacterial concentrations were measured regularly through the biostimulation to monitor biofilm growth in the fracture. Hydraulic measurements and tracer experiments completed during the early and later stages of biostimulation were used to evaluate bioclogging of the fracture.

4.2 Materials and Methodology

4.2.1 Fracture Table Apparatus

The planar fracture consisted of two parallel glass plates each measuring 2.0-m long, 0.6-m wide, and 0.0127-m thick. The plates were separated along their length by a 0.7-mm diameter copper wire sealed in place with marine-grade sealant along each edge, creating a
fracture with an aperture of approximately 2000 \( \mu \)m as determined by hydraulic measurement. A 6.3-L Plexiglas\textsuperscript{TM} constant head reservoir was attached to each end of the table with marine-grade sealant. Figure 4-1 presents a schematic of the fracture table. The table dimensions are presented in Appendix C.

![Figure 4-1: Schematic of planar fracture table used in Fracture Table Experiments.](image)

The upgradient reservoir was encased in 0.025-m thick foam insulation and contained a 50-W aquarium heater with an internal thermostat calibrated to maintain a solution temperature of approximately 30\(^\circ\)C. The table was maintained in a level position in a temperature-controlled room adjusted to approximately 10\(^\circ\)C. The temperatures of the amended groundwater in the upgradient reservoir and in the ambient room were measured continuously throughout the fracture trial using insulated T-type thermocouples (Model 5TC-KK-T-36, Omega Engineering Inc., Laval, Quebec, Canada) and a Campbell Scientific CR10X datalogger.

**4.2.2 Groundwater and Nutrient Conditions**

Groundwater was collected from a single limestone fracture located approximately 17 m below ground surface at the study site in Kingston, Ontario the day before the experiment. The
water was stored for a maximum of 24 hours at 4°C in amber glass bottles prior to being amended with the same nutrient mixture used in the microcosm study and introduced into the fracture table.

Flow through the planar fracture was established by generating a hydraulic head difference between the upgradient and downgradient reservoirs and using a two-head peristaltic pump (Variable Speed Low Flow Pumps, Fischer Scientific, Ottawa, Ontario) to maintain a mean flow rate of approximately 3.0 mL·min⁻¹. The flow rate was selected based on the velocity used in a similar experiment conducted by Ross et al., 2007 and adjusted for the aperture of the fracture used in the fracture trial and the ability of the pumps to maintain a consistent flowrate. Flow calculations are presented in Appendix C.

To ensure an adequate nutrient supply to the growing bacterial population, approximately 102 mL of nutrient mixture was added to the upgradient reservoir every other day during the experiment. The frequency of nutrient addition was based on similar work completed by Knight (2008). An overhead propeller stirrer (IKA® RW 11 “Lab Egg”) was placed in each reservoir to continuously mix the nutrient mixture with the groundwater in the reservoirs/table at a speed of approximately 60 rpm.

4.2.3 Geochemistry

Bacterial growth is highly dependent upon and can also alter the geochemistry of the growth environment (Chapelle, 2001). Therefore, general geochemical parameters such as electrical conductivity, dissolved oxygen, pH, and oxidation-reduction potential were measured in both the upgradient and downgradient reservoirs two to three times a day using a handheld multi-parameter instrument (YSI 556 MPS).

Samples of groundwater were also collected from the upgradient and downgradient reservoirs every day for the first six days of the trial, and every other day thereafter and analysed for selected parameters often used to identify the oxidation-reduction potential zones of
groundwater systems. Concentrations of ferrous iron, nitrate, ammonia, and sulphate were measured in samples collected in duplicate from the upgradient and downgradient reservoirs throughout the fracture table experiment using a HACH 2800 Spectrophotometer. Table 4-1 presents information regarding the HACH analytical powder pillows that were selected based on the parameter concentrations anticipated during the experiment.

**Table 4-0.1: Hach 2800 Spectrophotometer Information for ferrous iron, nitrate, ammonia, and sulphate powder pillows used for geochemical analysis during Fracture Table Experiment.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Powder Pillow Method</th>
<th>Concentration Range (mg/L)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous Iron</td>
<td>8146 – 1, 10 Phenanthroline</td>
<td>0 to 3.000</td>
<td>510</td>
</tr>
<tr>
<td>Nitrate</td>
<td>8171 – Cadmium Reduction</td>
<td>0.1 to 10.0</td>
<td>400</td>
</tr>
<tr>
<td>Ammonia</td>
<td>8155 - Salicyate and Cyanurate Reagent</td>
<td>0 to 0.80</td>
<td>655</td>
</tr>
<tr>
<td>Sulphate</td>
<td>8051 – SulfaVer 4</td>
<td>0 to 70.0</td>
<td>450</td>
</tr>
</tbody>
</table>

The Hach 2800 Spectrophotometer was calibrated using the above-referenced powder pillows and prepared calibration standards. The calibration results are presented in Figures D-1 through D-4 in Appendix D.

**4.2.4 Biology**

Direct measurement of the biofilm is not possible without disturbing the film and influencing future growth. The growth of a biofilm is dependent on having a sufficient number of planktonic bacteria in the bulk fluid to improve the chance of bacteria hitting and adhering to the substrata. Increasing concentrations of planktonic bacteria have been shown to correlate with biofilm growth (McFeters et al., 1999). Therefore, the planktonic bacteria was sampled and analysed to evaluate changes to the bacterial population throughout the experiment. Samples
collected before the experiment (i.e., groundwater collected directed from the study site) as well as samples collected from the upgradient and downgradient reservoirs on the first and seventh day of the experiment were submitted to an accredited commercial laboratory for analysis of heterotrophic bacterial counts, the common groundwater bacteria *Pseudomonas*, and iron bacteria.

In addition, the nature of the developing bacterial community was determined by comparing a sample of raw, unamended groundwater to samples from both the upgradient and downgradient reservoirs using the commercially available Biological Activity Reaction Test (BART™). Samples were tested using BART™ biodetectors for four groups of bacteria commonly present in groundwater: Heterotrophic Aerobic Bacteria (HAB), Iron Related Bacteria (IRB), Sulphate Reducing Bacteria (SRB), and Slime Forming Bacteria (SLYM). Each sample was placed in the biodetectors and monitored daily for the first indication of positive response specific to each type of biodetector. The timing of the response and the type of response observed were then compared to calibration data provided by the manufacturer of the biodetectors to identify the dominant group of bacteria in the population and approximate concentrations.

### 4.2.5 Tracer Experiments

Tracer experiments were performed during the early stages of biofilm development and after the last day of nutrient addition to capture the later growth conditions. Lisamine FF was selected as the tracer because it is biologically stable (Davis et al., 1985), non-toxic (Smart, 1994), and has been used extensively in fractured rock environments (Novakowski et al., 1985, Lapcevic et al., 1995). The tracer experiments were performed by adding a 3.0 mL point injection of 7.25 mg·L⁻¹ concentration into the upgradient reservoir to yield an initial tracer concentration in the upgradient reservoir of 3.4 mg·L⁻¹ for both the early growth and later growth tracer experiments. The increasing tracer concentrations were measure continuously in the downgradient reservoir following the injection of the tracer in the upgradient reservoir. Lisamine FF concentrations were measured using a submersible fluorometer (Turner Designs,
Cyclops-7®) connected to a Campbell Scientific CR10X data logger. The fluorometer has a fluorescence detection limit of less than 0.001 mg·L\(^{-1}\) (Turner, 2007) and was calibrated prior to both tracer experiments using calibration standards prepared from the tracer solution. The calibration results are provided in Appendix E.

To provide for the optimal interpretation of the breakthrough curve, the tracer was injected as a slug and the water withdrawn from the downgradient reservoir was not recirculated through the table. To avoid recirculating the Lissamine FF through the fracture table while maintaining the flow conditions through the table, groundwater was pumped from the downgradient reservoir as effluent at the same flow rate as fresh, amended groundwater was pumped into the upgradient reservoir. The same flow rate of approximate 3.0 mL·min\(^{-1}\) was used during the tracer experiment as was used throughout the experiment.

Because the addition of nutrients or the removal of groundwater from the system for biological or geochemical sampling purposes would either dilute or concentrate the amount of tracer present in the fracture trial, no fluid was added or removed from the system during the tracer experiments to facilitate the analyses of the results. This approach resulted in a shorter early growth tracer experiment (42 hours) in comparison to the later growth experiment (140 hours) because of the need to introduce nutrients to the system during the early stages of the trial to establish/maintain bacterial growth. The later growth experiment was conducted over a longer period of time because it began after the last nutrient injection/sampling event.

4.3 Results and Discussion

This section presents and discusses the results of the planar fracture trial, including the temperature profile measured in the upgradient and downgradient reservoirs, visual observations within the planar fracture, as well as chemical and biological conditions measured in both
reservoirs. The results of the tracer experiments and the associated modeling results are also presented.

4.3.1 Temperature Profile

The average temperature of the groundwater in the upgradient reservoir during the fracture trial was 29.9°C. The temperature ranged from 20.9°C to 33.7°C. Both the minimum and maximum temperatures were recorded within the first day of the trial as the 4°C groundwater was added to the fracture table and the heaters were initially adjusted to match fluctuations in temperature as the chillers in the temperature-controlled room turned on and off.

The temperature of the groundwater in the downgradient reservoir was measured to ensure that ambient room conditions were correctly adjusted to result in a groundwater temperature within the fracture table of 10°C, which is typical of natural conditions. The average temperature of the groundwater in the downgradient reservoir during the fracture trial was 10.5°C. The temperature ranged from 9.5°C to 11.6°C.

The temperature profiles for both the upgradient and downgradient reservoirs are provided in Figure 4-2 below.
4.3.2 Visual Observations

Visual observations of the fluid flowing in the fracture during biostimulation identified a cloudiness to the water in the upgradient reservoir by the end of Day 2 and within the fracture and downgradient reservoir by Day 3. Visible clusters of bacteria were observed in the fracture near the upgradient reservoir on Day 3 with flow lines evident within the growing clusters appearing on Day 4 (Figure 4-3). The formation of patchy clusters during the early stages of biofilm development are a typical structural attachment of biofilms that develop under laminar conditions (Christensen, 1989; Stolzenbach, 1989; Hill and Sleep, 2002). Richter et al. (1999) showed that the development of the biofilm macrostructure along the flow path is more pronounced in biofilms attached to a smooth, glass substrata as opposed to stainless steel or silicon. Similar observations were made by Ross et al. (2007), and the development of the flow lines was attributed to the relatively low roughness and the low hydrophobicity of the glass, as well as the laboratory-controlled flow pattern.
Figure 4-3: Visible clusters of bacteria formed near the upgradient reservoir on Day 4 of the fracture table experiment.

In the present study, the biofilm developed into a visibly connected, spatially consistent biofilm by Day 8 as shown in Figure 4-4. Fully connected biofilms are typical of mature systems (Cullimore, 1992) or of biofilms developed in high nutrient environments such as the film developed in this study (McFeters et al., 1999). The increased temperature of the upgradient reservoir likely also increased the rate at which the microbial clusters connected. The biofilm developed by Ross et al., 2007 using the same fracture table as the present study but using a recirculation system entirely maintained at 10°C took 30 days to form a connected biofilm. And a smaller-scale biofilm developed by Hill and Sleep (2002) in a parallel plate glass fracture maintained at room temperature under constant nutrient injection formed a fully-connected film by Day 7. Therefore, it is apparent that the temperature of the system significantly increased the
rate at which a spatially consistent biofilm that is visible to the naked eye develops.

Figure 4-4: Visible low lines in biofilm near upgradient reservoir on Day 8 of the fracture table experiment.

4.3.3 Geochemistry

A discussion of the geochemical results including electrical conductivity, pH, and oxidation-reduction conditions is presented below.

**Electrical Conductivity**

In general, the electrical conductivity measurements in both the upgradient and downgradient reservoirs increased at a similar rate throughout the experiment (Figure 4-5). Measurements in the upgradient reservoir ranged from approximately $1.5 \times 10^4 \, \mu\text{s}\cdot\text{cm}^{-1}$ at the start of the trial to over $3.8 \times 10^4 \, \mu\text{s}\cdot\text{cm}^{-1}$ at its peak near the end of the trial. Electrical conductivity in the downgradient reservoir was approximately half the value of the upgradient reservoir and ranged from $7.8 \times 10^3$ to $2.0 \times 10^4 \, \mu\text{s}\cdot\text{cm}^{-1}$. These conductivity measurements
were significantly greater than the measured electrical conductivities in the raw groundwater \((1.0 \times 10^3 \mu s \cdot cm^{-1})\) and the nutrient mix \((2.2 \times 10^3 \mu s \cdot cm^{-1})\).

**Figure 4-5: Electrical conductivity profiles measured in the upgradient and downgradient reservoirs during fracture table experiment.**

As shown in Figure 4-5, the addition of nutrients to the upgradient reservoir was immediately followed by an increase in the electrical conductivity measured in that reservoir. Within 24 hours of the addition, the electrical conductivity measurements decreased to values expected within the general increasing trend for the trial. A similar spike in measurements following nutrient addition to the upgradient reservoir was not observed in the downgradient reservoir.

Knight (2008) also observed a slight but steady increasing trend in electrical conductivity with more noticeable increases immediately following nutrient addition during a biostimulation field trial. Increasing dissolved organic carbon concentrations in the upgradient well were also
noted and Knight concluded that electrical conductivity could be used as a general field indicator for dissolved organic carbon and other ions essential for bacterial growth. As the microorganisms withdraw the carbon and other nutrients from their environment, the electrical conductivity of the water will plateau or decline until additional nutrients are added to the water as was shown in the fracture trial (Baker and Herson, 1994).

In comparison to the field trial conducted by Knight, the electrical conductivity response to the consumption of nutrients by the growing microbial population was more evident in the fracture trial. For example, the peak conductivity measurement in the injection well in the field trial was $5.0 \times 10^3 \mu s \cdot cm^{-1}$ compared to over $3.8 \times 10^4 \mu s \cdot cm^{-1}$ in the upgradient reservoir in the fracture trial. In a field trial, it is often difficult to establish a closed system where water and nutrients are contained within the established flow field. Therefore, in Knight’s trial, loss of nutrients to the environment contributed to the declining values of electrical conductivity measured. In the fracture trial, a closed system is much easier to achieve and loss of water and nutrient from the fracture system was limited to evaporation and sampling, suggesting that the variation in electrical conductivity is even more related to microbial growth in the system than was evident in the field trial completed by Knight.

**pH**

Similar pH results were measured in the upgradient and downgradient reservoirs throughout the fracture trial (Figure 4-6). The results are representative of neutral conditions and range from 6.06 to 7.23, indicating the bacteria within the fracture system are neutrophile bacteria (Cookson, 1995). There is no discernible trend in the results and the pH of the two reservoirs are generally within 0.08 of each other. Bacteria are sensitive to the pH of their environment and are also capable of altering the pH of their environment as they grow (Chapelle, 2001). The results suggest that the bacteria present in the reservoirs are not pH-altering bacteria which is consistent
with both the *in situ* groundwater conditions and results obtained in other parallel plate biofilm studies that measured the pH of the systems (Hill and Sleep, 2002).

![Figure 4-6: pH profiles measured in the upgradient and downgradient reservoirs during fracture table experiment.](image)

**Oxidation-Reduction Conditions**

Oxidation-Reduction (Redox) Potential (ORP) was measured regularly in the upgradient and downgradient reservoirs throughout the experiment, as were the concentrations of dissolved oxygen, nitrate, ammonia, ferrous and total iron, and sulphate.

The measured ORP decreased substantially in both the upgradient reservoir (from 210.0 mV to -127.0 mV) and downgradient reservoir (246.0 mV to -34.1mV) during the fracture trial (Figure 4-7). The decreasing ORP is both reservoirs can be attributed to the changing terminal electron accepting conditions and the development of an increasingly reducing environment due to microbial activities throughout the fracture trial.
Figure 4-7: Redox potential profiles measured in the upgradient and downgradient reservoirs during fracture table experiment.

Dissolved oxygen concentrations in the upgradient reservoir decreased from approximately 7.34 mg·L⁻¹ to less than 0.5 mg·L⁻¹ within the first day of the fracture trial and remained at less than 0.5 mg·L⁻¹ for the remainder of the trial (Figure 4-8). Concentrations of dissolved oxygen in the downgradient reservoir increased from 8.95 mg·L⁻¹ to 11.62 mg·L⁻¹ within the first 30 hours before decreasing rapidly to less than 2 mg·L⁻¹, where it remained throughout the fracture trial.

In terms of REDOX conditions, oxygen is the first of the commonly bioavailable electron donors to be reduced (Chapelle, 2001). The sharp decline in the concentration of dissolved oxygen in the upgradient reservoir indicates that oxygen-reducing conditions were only present within the reservoir for approximately one day before the supply of dissolved oxygen was consumed by the growing microbial population and anaerobic conditions were present. Although dissolved oxygen concentrations are generally inversely proportional to temperature, such a rapid decline in dissolved oxygen concentrations in the upgradient reservoir cannot be explained by the...
temperature of the tank alone and it is likely the increased temperature combined with the addition of nutrients to the reservoir accelerated the consumption of oxygen.

![Dissolved Oxygen Profiles](image)

**Figure 4-7: Dissolved oxygen profiles measured in the upgradient and downgradient reservoirs during fracture table experiment.**

The decline in dissolved oxygen concentrations in the downgradient reservoir was delayed in comparison to the upgradient reservoir with the concentrations in the downgradient reservoir beginning to decline by hour 30 of the experiment as opposed to declining immediately in the upgradient reservoir. The 30-hour delay is due to the fact that the nutrients were added to the upgradient reservoir and the travel time for the nutrients in the upgradient reservoir to reach the downgradient reservoir at the beginning of the fracture trial was approximately one day. The additional 6-hour delay is likely a combination of the reduced nutrient concentration in the water from previous microbial activity by the time it reached the downgradient reservoir, and the slower microbial growth rates because of the 10°C temperature in the downgradient reservoir compared to the 30°C upgradient reservoir.
Because anaerobic conditions were achieved in both the upgradient and downgradient reservoirs relatively early in the trial, it is inferred that the fracture was also anaerobic.

Figure 4-9 presents the nitrate concentrations measured in the upgradient and downgradient reservoirs during the experiment. Both reservoirs contained 0.56 mg·L⁻¹ of nitrate on the first day of the trial and the concentration decreased steadily during the first five days of the trial to 0.20 mg·L⁻¹ in the upgradient reservoir and to 0.28 mg·L⁻¹ in the downgradient reservoir. Nitrate concentrations in both the upgradient and downgradient reservoirs increased after the fifth day of the fracture trial to maximum concentrations of 1.45 mg·L⁻¹ and 1.05 mg·L⁻¹ in the upgradient and downgradient reservoirs, respectively.

![Figure 4-8: Nitrate profiles measured in groundwater samples collected from the upgradient and downgradient reservoirs during fracture table experiment.](image)

After oxygen, nitrate is the next most bioavailable electron donor typically present in groundwater environments (Chapelle, 2001). The decreasing nitrate concentrations measured within the first five days of the fracture trial is evidence that nitrate-reducing conditions were
present at this stage of the trial and the growing microbial population was using nitrate as an electron donor. Unfortunately, the concentration of nitrate was measured every other day (i.e., Day 1, Day 3, etc.) and it is therefore not possible to determine whether nitrate reduction began in the upgradient reservoir at the beginning of the trial or only after the supply of dissolved oxygen had been depleted by the end of Day 1.

The faster rate of decreasing nitrate concentration observed in the upgradient reservoir versus the downgradient reservoir is likely the result of increased microbial activity in this reservoir due to the increased temperatures in the reservoir and the addition of nutrients to the upgradient as opposed to the downgradient reservoir.

Nitrate concentrations in both reservoirs increased inexplicably starting on Day 6.

Throughout the fracture trial, concentrations of soluble ferrous iron in the upgradient reservoir increased from 0.07 mg·L⁻¹ to 0.54 mg·L⁻¹ and from 0.07 mg·L⁻¹ to 0.66 mg·L⁻¹ in the downgradient reservoir. In general, ferrous iron concentrations increased at a faster rate in the downgradient reservoir than in the upgradient reservoir as shown in Figure 4-10.
As insoluble ferric iron is reduced to soluble ferrous iron by microbial activity, concentrations of ferrous iron increase (Snoeyink and Jenkins, 1980; Knight, 2008). Unlike in a field trial where additional sources of iron such as the steel well casings or iron-containing rock may be present (Knight, 2008), no external sources of iron were present within the glass fracture table. A nominal concentration of total iron was measured in the nutrient mix added to the fracture system throughout the trial, however it is not known how much of 0.22 mg·L\(^{-1}\) of total iron was ferric or ferrous iron. Therefore, the increasing concentrations of ferrous iron measured during the fracture trial may indicate that an iron-reducing condition was present in the fracture table.

Ammonia and sulphate concentrations were measured during the first six days of the trial. Ammonia concentrations were consistently greater than the method detection limit of 0.80 mg·L\(^{-1}\) (NH\(_3\)-N), likely as a result of the nutrient additions. Sulphate concentrations were consistently
0.0 mg·L\(^{-1}\). Ammonia and sulphate concentrations were not measured after the sixth day of the fracture trial.

4.3.4 Biological

Bacterial plate counts for heterotrophic bacteria, iron related bacteria, and pseudomonads were conducted using a sample of the raw groundwater on Day 1 of the fracture trial, prior to any nutrient addition, and on Day 7 using samples collected from the upgradient and downgradient reservoirs. The concentrations of heterotrophic bacteria, iron related bacteria, and pseudomonads measured in the raw groundwater sample were 1,030 cfu·mL\(^{-1}\), approximately 35,000 cts·mL\(^{-1}\), and 135 cts·100mL\(^{-1}\) respectively. Concentrations of all bacteria measured in both the upgradient and downgradient samples collected on Day 7 were greater than the detection limits of the commercial laboratory, indicating that a sufficient number of serial dilutions were not completed prior to analysis.

A significant increase in the count of heterotrophic bacteria was measured between the raw groundwater sample and the samples from the reservoirs. The result of 35, 150 cts·mL\(^{-1}\) of iron related bacteria in the raw groundwater represents the upper method detection limit of the commercial laboratory used to complete the analysis. The same count was obtained for both the upgradient and downgradient reservoirs, making the results difficult to interpret. An increase in the count of pseudomonads bacteria was also measured between the raw groundwater (135 cts·mL\(^{-1}\)) and the samples from the upgradient and downgradient reservoirs which were classified as “overgrown” by the commercial laboratory, again indicating that sufficient serial dilutions were not prepared.

The results of the BART\(^{\text{TM}}\) detectors are summarized in Table 4-2 and photographs are presented in Appendix F. The approximate concentration of iron related bacteria (IRB test) measured in each of the raw groundwater, the upgradient reservoir, and the downgradient reservoir was the maximum detectable concentration for the commercial units. The biodetectors
responded to the introduction of the samples by turning the solution cloudy and then black in colour, indicating the dominant iron-related bacteria in the samples are pseudomonads and enteric (Droycon Bioconcepts Inc., 2004).
Table 4-2: Results of the BART™ biodetectors for samples of raw groundwater collected at the beginning of the fracture table experiment and from the upgradient and downgradient reservoirs on Day 7 of the experiment.

<table>
<thead>
<tr>
<th>BART™ Kit</th>
<th>Raw Groundwater</th>
<th>Upgradient Reservoir (Day 7)</th>
<th>Downgradient Reservoir (Day 7)</th>
<th>Dominant Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRB – Iron Related Bacteria</td>
<td>&gt;140,000 cfu/mL</td>
<td>&gt;140,000 cfu/mL</td>
<td>&gt;140,000 cfu/mL</td>
<td>Pseudomonads and Enterics</td>
</tr>
<tr>
<td>SLYM – Slime Forming Bacteria</td>
<td>~66,500 cfu/mL</td>
<td>&lt;12,500 cfu/mL</td>
<td>&lt;12,500 cfu/mL</td>
<td>Slime-forming</td>
</tr>
<tr>
<td>HAB – Heterotrophic Aerobic Bacteria</td>
<td>&gt;500,000 cfu/mL</td>
<td>n/a</td>
<td>n/a</td>
<td>Aerobic</td>
</tr>
<tr>
<td>SRB – Sulphate Reducing Bacteria</td>
<td>&gt;5,000 cfu/mL</td>
<td>&gt;700,000 cfu/mL</td>
<td>&gt;700,000 cfu/mL</td>
<td>Dense slime and SRB Consortium</td>
</tr>
</tbody>
</table>

The approximate concentration of slime forming bacteria (SLYM test) was greatest in the raw groundwater in comparison to the samples from the reservoirs. Based on the observed changes in the detectors, the dominant bacteria are slime-forming. Slime-forming bacteria are microorganisms that produce large amounts of slime that can adhere to surfaces as a biofilm (Droycon Bioconcepts Inc., 2004).

Approximate heterotrophic aerobic concentrations in the raw groundwater sample were at the upper limit of the detector kit (>500,000 cfu·mL⁻¹) and based on the response observed, aerobic bacteria was the dominant microorganisms in the sample (Droycon Bioconcepts Inc., 2004). Because the results of the groundwater sample were at the maximum limit of the detector, the test was not conducted on samples from the upgradient and downgradient reservoirs.

Based on the bacterial plate counts and the BART™ results, the microbial population increased significantly between Days 1 and 7 of the fracture trial. The results of both the direct
counting method (i.e., plate counts) and the indirect BART™ method both indicate that of the types of bacteria tested, the iron-related bacteria was present in the unamended groundwater in the greatest concentration. The iron-related bacteria concentration in the groundwater was at the maximum method detection limit for both test methods. Additional growth of this type of bacteria may have occurred during the fracture trial but was not detected by the selected test methods.

The concentration of heterotrophic aerobic bacteria measured in the groundwater by bacterial plate count was significantly lower than that estimated by the BART™ detector. A possible reason for the discrepancy is the length of time the BART™ sample was exposed to atmospheric oxygen in comparison to the bacterial plate count sample which was maintained in a zero headspace bottle prior to analysis by the commercial laboratory. Groundwater collected from bedrock aquifers such as the sample analysed, does not typically exhibit high aerobic bacteria counts due to the relatively low concentrations of dissolved oxygen in the bedrock aquifers. Therefore, the bacterial plate count result is considered to be more representative of true groundwater conditions, however, the BART™ results are likely more representative of the water used for the experiment.

An increase of three orders of magnitude in the concentration of heterotrophic aerobic bacteria was observed between the raw groundwater sample and the samples collected from the upgradient and downgradient reservoirs as analyzed by the plate counts. Although pseudomonas aeruginosa was measured separately from the heterotrophic bacteria by direct counting methods and is identified as the dominant iron-related bacteria by the BART™ detectors, it is also an aerobic bacteria. And like the general heterotrophic aerobic bacteria concentrations, the concentration of pseudomonas aeruginosa also increased significantly between the raw groundwater sample and the reservoir samples to the point where the sample was classified as “overgrown” by the commercial laboratory.
The increase in aerobic bacterial growth measured through direct and indirect bacterial enumeration methods is likely a result of a combination of several factors. The addition of the nutrients and the temperature increase associated with the fracture table are experiment-controlled factors. Another potential factor is exposure to the atmospheric oxygen during the collection of the groundwater from the site, the fracture trial itself, and the subsequent sampling process. The dissolved oxygen in the groundwater at the beginning of the experiment was approximately 2.5 mg·L\(^{-1}\) (upgradient reservoir) to 4.0 mg·L\(^{-1}\) (downgradient reservoir) greater than the average concentration of 4.85 mg·L\(^{-1}\) measured in the field during groundwater sampling. The additional dissolved oxygen in the water may have initially accelerated the aerobic population growth in the table beyond what could be reasonably anticipated in a field environment. However, as discussed in Section 4.2.3.3, concentrations of dissolved oxygen in both the upgradient and downgradient reservoirs decreased rapidly and the potential impacts of increased concentrations would have only impacted the early stage growth.

### 4.3.5 Tracer Experiments and Modelling

The effect of biofilm growth on groundwater flow through the fracture was assessed using tracer experiments completed during the initial stages of biofilm development (i.e., Day 1-3) and at the end of the fracture trial (i.e., Day 13-15). For the tracer experiments a 3.0-mL point injection of 7.25 mg·L\(^{-1}\) of Lissamine FF was injected into the upgradient reservoir and the breakthrough concentrations were measured in the downgradient reservoir. The early growth tracer experiment was terminated after only 42 hours because the addition of nutrient mixture to the upgradient reservoir on Day 3 diluted the concentration of Lissamine in the system. The later growth tracer experiment continued for 140 hours because the last nutrient addition to the system coincided with the start of the later growth tracer experiment on Day 13 and the concentration of Lissamine in the fracture table was constant throughout the tracer experiment.
The biofilm impacted the groundwater flow through the fracture resulting in an approximately 2.75-hour delay in the tracer's breakthrough compared to the test conducted during the initial stages of biofilm development (Figure 4-11). The biofilm also caused an increase in the dispersion of the tracer as evidenced by the shape of the curve, primarily the shallower slopes of the rising and falling limbs and the slightly lower peak normalized concentration as shown on Figure 4-11.

Figure 4-10: Results from the early and later growth tracer experiments conducted as part of the fracture table experiment.

The tracer data was modeled using equation (32) from Novakowski (1989) where a slug of tracer is injected and observed in a reservoir of known volume and there is continuous mixing in both reservoirs. Although the solution was developed for the interpretation of field tests in porous media it is also applicable for the laboratory case of parallel plate glass fracture with reservoirs on the upgradient and downgradient ends. The boundary conditions applied in the derivation of the solution account for mixing in the upgradient reservoir via a mass balance on the
fluid entering and leaving the reservoir. Equation 4.1 is the initial boundary condition for the fracture and equations 4.2 to 4.5 are the initial, boundary, and continuity equations applied for mixing in the upgradient reservoir.

\[ C(x, 0) = 0 \quad 0 \leq x \leq 0 \]  
(4.1)

\[ C_i(t) = C(0, t) \]  
(4.2)

\[ V_i \frac{dC_i(t)}{dt} = -\gamma v C_i(t) \]  
(4.3)

\[ v C_i(t) = v C(0, t) - D \frac{\partial C(0, t)}{\partial x} \]  
(4.4)

\[ C_i(0) = C_o \]  
(4.5)

Where \( C \) is the resident concentration \((M \cdot L^{-3})\), \( x \) is the distance along the fracture \((L)\), \( C_i \) is the upstream reservoir concentration \((M \cdot L^{-3})\), \( t \) is time \((T)\), \( V_i \) is the volume of the upstream reservoir \((L^3)\), \( \gamma \) is the cross-sectional area for flow \((L^2)\), \( v \) is the average linear groundwater velocity \((L \cdot T^{-1})\), \( D \) is the coefficient of hydrodynamic dispersion \((L^2 \cdot T^{-1})\), and \( C_o \) is the initial tracer concentration \((M \cdot L^{-3})\).

The boundary condition at the downgradient reservoir also accounts for mixing in the reservoir through a mass balance approach. Equations 4.6 and 4.7 represent the downgradient initial and boundary conditions, respectively.

\[ C_e(0) = 0 \]  
(4.6)

\[ V_e \frac{\partial C(L, t)}{\partial t} = -\gamma D \frac{\partial C(L, t)}{\partial x} \]  
(4.7)

Where \( C_e \) is the concentration in the downgradient reservoir \((M \cdot L^{-3})\), \( V_e \) is the volume in the downgradient reservoir \((L^3)\), and \( L \) is the finite length between the upgradient and downgradient reservoirs \((L)\).

The model results were determined to be unique as the groundwater velocity positions the peak of the curve with respect to time, the fracture aperture determines the slope of the falling
limb, and the dispersivity positions the initial arrival of the tracer and the slope of the rising limb. Sensitivity analyses were conducted by manipulating each variable and while maintaining a fixed value for the other two variables in order to determine the accuracy of the fitted parameters. The results of the model simulations and the sensitivity analyses are summarized in Table 4-3 below for each of the early growth and later growth tracer experiments. The model fits are presented in Figures 4-12 and 4-13. Figures illustrating the sensitivity analyses are provided in Appendix I.

Table 4-3: Summary of the groundwater velocity, dispersivity and fracture aperture results from the early growth and later growth tracer model results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early Growth</th>
<th>Later Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundwater Velocity</td>
<td>$6.12 \pm 0.12 \text{ m\cdot day}^{-1}$</td>
<td>$5.52 \pm 0.19 \text{ m\cdot day}^{-1}$</td>
</tr>
<tr>
<td>Dispersivity</td>
<td>$1.0 \times 10^{-3} \text{ mm}$</td>
<td>$50 \pm 30 \text{ mm}$</td>
</tr>
<tr>
<td>Fracture Aperture</td>
<td>$2050 \pm 50 \mu\text{m}$</td>
<td>$1275 \pm 50 \mu\text{m}$</td>
</tr>
</tbody>
</table>

Figure 4-11: Comparison of the experimental tracer and tracer model results from early growth tracer experiment conducted as part of the fracture table experiment.
Figure 4-12: Comparison of the experimental tracer and tracer model results from later growth tracer experiment conducted as part of the fracture table experiment.

Based on the results of the tracer experiments, the biofilm growth reduced the velocity of the groundwater and the fracture aperture by 10% and 38%, respectively, and increased the bulk dispersivity to 50 mm from effectively zero. These results were obtained after a 13-day growth period with the application of heat to the upgradient reservoir.

Table 4.4 presents a comparison of the results from the present study to the two-dimensional fracture trial completed by Ross et al., (2007).

Table 4.4: Comparison of results from present one-dimension, heated fracture trial to two-dimensional, not heated trial completed by Ross et al., 2007.

<table>
<thead>
<tr>
<th>Study</th>
<th>Temperature (°C)</th>
<th>No. of Days</th>
<th>Velocity</th>
<th>Aperture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>30 (10)</td>
<td>13</td>
<td>-10%</td>
<td>-38%</td>
</tr>
<tr>
<td>Ross et al., (2007)</td>
<td>10</td>
<td>50</td>
<td>-21%</td>
<td>-11%</td>
</tr>
</tbody>
</table>
There are several differences between the present study and that conducted by Ross et al., namely the use of groundwater from different study sites, the use of continuous substrate injection by Ross et al., and that the present study is a one-dimensional trial versus the two-dimensional trial by Ross et al. Although one-dimensional systems like the present study for the flow system to migrate through the biofilm and not around the closed region as is possible in two-dimensional systems which are more representative of field-scale applications (Thullner, 2010), the results of this study are promising and indicate that the application of heat can accelerate biofilm growth in a large-aperture fracture to reduce the fracture aperture and groundwater velocity and may accelerate bioclogging in field applications as well.

The significantly increased bulk dispersivity value measured in the fracture trial is generally consistent with results achieved by others in porous media (Seifert and Engesgaard, 2007) and in parallel plate studies (Hill and Sleep, 2002; Ross et al, 2007). Unfortunately, a more complete and direct comparison to the parallel plate studies is difficult because the studies conducted by others involved either multiple tracer experiments completed at different velocities to evaluate the effect of Taylorian dispersion (Hill and Sleep, 2002), or a two-dimensional model to differentiate between longitudinal and transverse dispersion (Ross et al., 2007).

Boschan et al. (2008) presented Equation 4.8 below relating the variation of the normalized dispersivity in a fracture to the Peclet number $Pe$.

$$\frac{l_d}{2b} = \alpha_G + \alpha_T Pe$$  \hspace{1cm} (4.8)

Where $l_d$ is the dispersivity (L), $2b$ is the average fracture aperture (L), $\alpha_G$ is macrodispersion ($L^2$·T$^{-1}$), and $\alpha_T$ is Taylor dispersion ($L^2$·T$^{-1}$). $Pe$ is the Peclet number equal to $v2b/D_m$ where $v$ is the average groundwater velocity ($L$·T$^{-1}$) and $D_m$ is the molecular diffusion assumed to be negligible in the application of Equation 4.8 for $Pe$ greater than 1.
Applying Equation 4.8 to the model results, Taylor dispersion dominates the early growth tracer experiment as anticipated for a smooth, parallel plate fracture. Taylor dispersion still plays a significant role in the solute transport for the later growth tracer experiment, however the effect of the macrodispersion term is greater for the later growth in comparison to the early growth tracer. These results are consistent with the general conclusions made by others regarding the increased in mixing and macrodispersion with flow through a biofilm. The calculations are presented in Appendix J.

Although Lissamine has been used extensively as a tracer in fractured rock studies and shown to be conservative (Novakowski et al., 1985; Lapcevic et al., 1999) and is considered biologically stable (Davis et al., 1985) there have been mixed results in biofilm studies by others regarding whether Lissamine is adsorbed by a developing biofilm and later released during sloughing of the biofilm (Seifert and Engesgaard, 2007; Bayona, 2009). Bioadsorption of the tracer was not observed in the present study based on the good model fits obtained for both the early and later biofilm growth tracer experiments using a model that did not account for retardation of the tracer within the biofilm.

4.4 Conclusions

A parallel plate planar glass fracture table was used to investigate the effect of groundwater temperature on the rate of biofilm growth and associated bioclogging in the fracture. Conditions suitable for biofilm development were present within the fracture and the developed biofilm reduced the groundwater velocity in the planar fracture as well as the aperture of the fracture. The biofilm developed within the fracture, beginning with the growth of clusters of bacteria near the upgradient reservoirs within the first few days of biostimulation followed by the development of a fully connected biofilm by the seventh day of the trial. Based on the results of the tracer experiment, the biofilm growth reduced the velocity of the groundwater by 9.8%, the
fracture aperture by 37.8%, and increased the bulk dispersivity to 50mm. The conclusions are presented below.

1. Chemical analyses conducted on samples collected from the upgradient and downgradient reservoirs indicates that increasingly reducing conditions developed throughout the trial with the supply of dissolved oxygen exhausted in both reservoirs within the first 30 hours of the trial. Nitrate and ferric iron were also reduced to some extent during the experiment.

2. The electrical conductivity results in both reservoirs revealed an overall increasing trend, with the results measured in the upgradient responding directly to the addition of nutrients with an immediate increase in conductivity followed by a steady decline as the nutrients are consumed by the growing microbial population. This result is consistent with observations made by Knight (2008) and indicates that electrical conductivity is a good indicator of the nutrient load in the bulk fluid of the system.

3. Both direct and indirect counting of bacteria common to groundwater indicated that all types of bacteria monitored exhibited increasing concentrations throughout the biostimulation. Based on the results of the BART™ biodetectors, the dominant types of bacteria included pseudomonads, enteric, slime-forming bacteria. Heterotrophic bacteria were also present in significant concentrations. These types of bacteria are consistent with the reducing conditions indicated by the geochemical analyses and are generally considered capable of forming biofilms.

4. The application of heat during the biostimulation trial to induce bioclogging in a parallel plate planar glass fracture was successful at the laboratory scale. Assuming suitable conditions for biofilm development can be achieved in a field setting (i.e., nutrient
availability, laminar flow conditions, presence of the requisite indigenous bacteria, etc.)

the approach presented in the present study shows promise in field applications.
4.5 References


Knight, L. (2008), The effect of biostimulation on geochemical and microbiological conditions in an isolated dolostone fracture, Queen's University, Kingston, Ontario.


Chapter 5 - General Conclusions and Recommendations

5.1 General Conclusions

The objective of this study was to examine the effect of heat on the growth of a biofilm in a parallel glass plate fracture table. Groundwater collected from a limestone aquifer was amended with a nutrient mixture consisting of approximately 100:10:1 molar ratio of carbon to nitrogen to phosphorous to stimulate the indigenous microbial population. A microcosm experiment was completed prior to the planar fracture trial and confirmed that the selected nutrient mixture was capable of stimulating the indigenous groundwater bacteria under aerobic conditions. Furthermore, growth rates increased with increasing temperatures as high as 30°C.

In the fracture trial, the amended groundwater was heated to 30°C in an upgradient reservoir attached to the fracture table and recirculated through the 2-m long, 0.6-m wide, parallel glass plate fracture at an approximate flow rate of 3.0 mL·min⁻¹ for thirteen days. The approximate aperture of the fracture at the beginning of the biostimulation trial was 2000 μm. The fracture was maintained at 10°C to simulate natural in situ groundwater temperature and the upgradient reservoir maintained at 30°C.

Electrical conductivity was measured regularly in both the upgradient and downgradient reservoirs, with a generally increasing trend observed in both reservoirs. The electrical conductivity measurements in the upgradient reservoir spiked with the addition of nutrients to the reservoir and declined as the growing microbial population withdraw the nutrients from the water. The electrical conductivity measurements in the upgradient reservoir were therefore used as a general indicator of low nutrient availability in the fracture system.

The biofilm developed within the fracture, beginning with the growth of clusters of bacteria near the upgradient reservoirs within the first few days of biostimulation followed by the
development of a fully connected biofilm by the seventh day of the trial. Based on results of a comparable study conducted by Ross et al. (2007), heating the upgradient reservoir in the present study accelerated the development of the fully connected biofilm by as many as 23 days.

Geochemical parameters monitored throughout the biostimulation revealed increasingly reducing conditions including the depletion of dissolved oxygen in the upgradient reservoir within the first 24 hours of the trial and within the first 30 hours in the downgradient reservoir. Evidence of nitrate and ferric iron reduction were also observed in both reservoirs.

Both direct and indirect counting of bacteria common to groundwater indicated that all types of bacteria monitored exhibited increasing concentrations throughout the biostimulation. Based on the results of the BART™ biodetectors, the dominant types of bacteria included pseudomonads, enteric, slime-forming bacteria. Heterotrophic bacteria were also present in significant concentrations. These types of bacteria are consistent with the reducing conditions indicated by the geochemical analyses and are generally considered capable of forming biofilms.

The biofilm impacted the groundwater flow through the fracture resulting in an approximately 2.75 hours delay in the tracer’s breakthrough during the tracer experiment completed on Day 13 of the trial compared to an experiment conducted during the initial stages of biofilm development. Based on the results of the tracer experiment, the biofilm growth reduced the velocity of the groundwater by 9.8%, the fracture aperture by 37.8%, and increased the bulk dispersivity to 50mm. In a similar study conducted by Ross et al. (2002) using the same fracture table maintained at 10°C, the velocity was decreased by 21.2% and the aperture was reduced by 11.3% after 50 days of biostimulation. Therefore, the results of the present study are comparable but were achieved in approximately a quarter of the time through the application of heat to the upgradient reservoir.
The application of heat during the biostimulation trial to induce bioclogging in a parallel plate planar glass fracture was successful at the laboratory scale. Assuming suitable conditions for biofilm development can be achieved in a field setting (i.e., nutrient availability, laminar flow conditions, presence of the requisite indigenous bacteria, etc.) the approach presented in the present study shows promise in field applications.

5.2 Recommendations

5.2.1 Laboratory Investigations

Future laboratory scale investigations would be of use to examine the effect of temperature on bioclogging in a fracture system with a groundwater velocity greater than 5 m·d\(^{-1}\). \textit{In situ} groundwater velocities in fractured bedrock vary considerably from site to site, and since biofilm development varies with flow conditions, the efficacy of increased temperatures on bioclogging may be different in more turbulent flow than was used in the present study.

In the present study, a complete evaluation of the change in dispersivity as a result of bioclogging was not possible because of the one-dimensional model used and the lack of tracer experiments conducted at multiple velocities. Either of the aforementioned approaches would be useful to develop an improved understanding of the affect of bioclogging on transport processes in a large-scale fracture, particularly when coupled with an examination of biofilm growth in more turbulent conditions where Taylorian dispersion are thought to be more prevalent.

The 2000 µm aperture used in the present study represents the larger end of the spectrum of apertures encountered in the field environment. Although the wire used in the construction of the fracture aperture had a diameter of 700 µm, the application of marine sealant on such a larger scale fracture affected the final aperture of the glass fracture. It is recommended that the fracture
table be constructed in a manner to ensure a smaller fracture to examine the effect of temperature on bioclogging in smaller fractures that may be encountered in the field.

Although the present study revealed that it is possible to develop a biofilm at a faster rate by increasing the temperature of the fluid circulated through the fracture, the long term persistence of the biofilm under starvation conditions was not examined for logical reasons. This information would be of interest as long term injections of nutrients at the field scale is both expensive and impractical.

5.2.2 Field-Scale Investigations

Based on the success of the present study, field-scale biostimulation combined with the application of heat is the next logical step. Because biofilm growth is highly dependent on the delivery of nutrients to the target fracture(s), successful field scale investigations will require a detailed site characterization to ensure adequate nutrient and heat delivery. Furthermore, it is recommended that the field-scale trial be conducted using a single isolated fracture before increasing the complexity to the scale of a fracture network.
Appendix A: Nutrient Mix Calculations and Analytical Results
Nutrient Mix Calculations

**Target Concentrations:**

- 10 g/L carbon
- 100:10:1 carbon:nitrogen:phosphorous ratio

**Volume of sodium lactate solution required for 10g/L of carbon determined as follows:**

- Sodium lactate (60% w/w) $C_3H_5NaO_3 = 112.0598$ g/mol

\[
\frac{60 \text{ g sodium lactate}}{100 \text{ g solution}} \times \frac{36.0321 \text{ g/mol carbon}}{112.0598 \text{ g/mol sodium lactate}} \times 1.3 \times \frac{1}{251} = 251 \text{ g carbon/L solution}
\]

\[
M_1 = 250.78 \text{ g/L}
\]

\[
M_2 = 10 \text{ g/L}
\]

\[
V_2 = 1 \text{ L}
\]

\[
V_1 = \frac{M_2 V_2}{M_1} = \frac{(10 \text{ g/L})(1 \text{ L})}{250.78 \text{ g/L}} = 40 \text{ mL}
\]

Therefore, 39.88mL of sodium lactate added to 960.12 mL of H$_2$O (1L− 39.88mL) will result in a 10g/L carbon concentration.

**Volume of Liquid Fertilizers Required for 100:10:1 C:N:P determined as follows:**

Convert 10g/L carbon to moles

\[
\frac{10 \text{ g/L}}{12.0107 \text{ g/mol}} = 0.83259 \text{ mol carbon/L}
\]

For nitrogen and phosphorous, need

\[
(0.083259 \text{ mol nitrogen})(14.0067 \text{ g/mol}) = 1.166 \text{ g nitrogen}
\]

\[
(0.0083259 \text{ mol phosphorous})(30.9738 \text{ g/mol}) = 0.2579 \text{ g phosphorous}
\]
Appendix A

Table A-1: Information for liquid fertilizer used in microcosm and fracture table experiments to amend the groundwater.

<table>
<thead>
<tr>
<th>Liquid Fertilizer (N-P-K)</th>
<th>LiquaFeed (12-4-8)</th>
<th>Liquid Growth (10-10-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution Density</td>
<td>1.134 g/mL</td>
<td>1.28 g/mL</td>
</tr>
<tr>
<td>Nitrogen Concentration*</td>
<td>136.080 g/L (12% w/w)</td>
<td>128 g/L (10% w/w)</td>
</tr>
<tr>
<td>Phosphorous Concentration*</td>
<td>19.791 g/L (4% w/w as P₂O₅)</td>
<td>55.86 g/L (10% w/w)</td>
</tr>
</tbody>
</table>

*Conversion from % w/w to g/L similar to carbon conversion above

To achieve a 10 g/L carbon concentration and a ratio of 100:10:1 of C:N:P, the following is required for every one litre of groundwater in the system:

Table A-2: Volumes of nutrient mix components used per litre of groundwater to amend the groundwater in the microcosm and fracture table experiments.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume Added (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Lactate (60% w/w)</td>
<td>41.92</td>
</tr>
<tr>
<td>LiquaFeed (12-4-8)</td>
<td>6.62</td>
</tr>
<tr>
<td>Liquid Growth (10-10-10)</td>
<td>2.52</td>
</tr>
</tbody>
</table>
Figure A-1: Photograph of products used in nutrient mix preparation.
## Table A-3: Analytical results for raw groundwater and nutrient mix

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Raw Groundwater</th>
<th>Nutrient Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophic Plate Count</td>
<td>cfu/mL</td>
<td>1030</td>
<td>n/a</td>
</tr>
<tr>
<td>Iron Bacteria</td>
<td>cts/mL</td>
<td>35,150</td>
<td>n/a</td>
</tr>
<tr>
<td>Alkalinity (as CaCO₃)</td>
<td>mg/L</td>
<td>388</td>
<td>n/a</td>
</tr>
<tr>
<td>Carbonate (as CaCO₃)</td>
<td>mg/L</td>
<td>&lt;3</td>
<td>n/a</td>
</tr>
<tr>
<td>Bicarbonate (as CaCO₃)</td>
<td>mg/L</td>
<td>388</td>
<td>n/a</td>
</tr>
<tr>
<td>pH</td>
<td>pH units</td>
<td>7.32</td>
<td>7.0</td>
</tr>
<tr>
<td>Conductivity</td>
<td>µmho/cm</td>
<td>1050</td>
<td>2164</td>
</tr>
<tr>
<td>Colour</td>
<td>TCU</td>
<td>&lt;2</td>
<td>n/a</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>10.1</td>
<td>n/a</td>
</tr>
<tr>
<td>Fluoride</td>
<td>mg/L</td>
<td>0.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Chloride</td>
<td>mg/L</td>
<td>73</td>
<td>n/a</td>
</tr>
<tr>
<td>Nitrite (N)</td>
<td>mg/L</td>
<td>&lt;0.1</td>
<td>n/a</td>
</tr>
<tr>
<td>Nitrate (N)</td>
<td>mg/L</td>
<td>0.9</td>
<td>n/a</td>
</tr>
<tr>
<td>Sulphate</td>
<td>mg/L</td>
<td>63</td>
<td>n/a</td>
</tr>
<tr>
<td>Ammonia + Ammonium (N)</td>
<td>mg/L</td>
<td>&lt;0.05</td>
<td>47.8</td>
</tr>
<tr>
<td>o-Phosphate (P)</td>
<td>mg/L</td>
<td>0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hardness (as CaCO₃)</td>
<td>mg/L</td>
<td>414</td>
<td>n/a</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg/L</td>
<td>79.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Copper</td>
<td>mg/L</td>
<td>&lt;0.002</td>
<td>n/a</td>
</tr>
<tr>
<td>Iron</td>
<td>mg/L</td>
<td>1.71</td>
<td>0.22</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mg/L</td>
<td>52.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Manganese</td>
<td>mg/L</td>
<td>0.006</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>mg/L</td>
<td>2.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Sodium</td>
<td>mg/L</td>
<td>38.7</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Zinc</td>
<td>mg/L</td>
<td>0.089</td>
<td>2.30</td>
</tr>
<tr>
<td>Anion Sum</td>
<td>meq/L</td>
<td>11.2</td>
<td>n/a</td>
</tr>
<tr>
<td>Cation Sum</td>
<td>meq/L</td>
<td>10.1</td>
<td>n/a</td>
</tr>
<tr>
<td>% Difference</td>
<td>%</td>
<td>5.21</td>
<td>n/a</td>
</tr>
<tr>
<td>Ion Ratio</td>
<td>AS/CS</td>
<td>1.11</td>
<td>n/a</td>
</tr>
<tr>
<td>TDS (ion sum calc.)</td>
<td>mg/L</td>
<td>549</td>
<td>n/a</td>
</tr>
<tr>
<td>Conductivity (calc.)</td>
<td>µmho/cm</td>
<td>963</td>
<td>n/a</td>
</tr>
<tr>
<td>Langelier Index (25°C)</td>
<td>S.I.</td>
<td>0.337</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**Note:**

n/a = Not Analysed
Appendix B: Microcosm Experiment
Table B-1: Sample cuvette information for microcosm experiment.
### Appendix B

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Anaerobic/Aerobic</th>
<th>Sample ID</th>
<th>Groundwater</th>
<th>Nutrient Mixture</th>
<th>Rock Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Anaerobic</td>
<td>20W1</td>
<td>√</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20W2</td>
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<tr>
<td></td>
<td></td>
<td>20S1</td>
<td>√</td>
<td>√</td>
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<tr>
<td></td>
<td></td>
<td>20S2</td>
<td>√</td>
<td>√</td>
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<td></td>
<td>20WR1</td>
<td>√</td>
<td>-</td>
<td>√</td>
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<tr>
<td></td>
<td></td>
<td>20WR2</td>
<td>√</td>
<td>-</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20SR1</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20SR2</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>20WO1</td>
<td>√</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20WO2</td>
<td>√</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20SO1</td>
<td>√</td>
<td>√</td>
<td>-</td>
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<td></td>
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<td>20SO2</td>
<td>√</td>
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<td></td>
<td></td>
<td>20WRO1</td>
<td>√</td>
<td>-</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20WRO2</td>
<td>√</td>
<td>-</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20SRO1</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20SRO2</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>30</td>
<td>Anaerobic</td>
<td>30W1</td>
<td>√</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
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<td></td>
<td></td>
<td>30WR1</td>
<td>√</td>
<td>-</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30WR2</td>
<td>√</td>
<td>-</td>
<td>√</td>
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<td></td>
<td></td>
<td>30SR1</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30SR2</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>30WO1</td>
<td>√</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30WO2</td>
<td>√</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30SO1</td>
<td>√</td>
<td>√</td>
<td>-</td>
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<td>√</td>
<td>√</td>
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<td></td>
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<td>30WRO1</td>
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<td>30WRO2</td>
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<tr>
<td></td>
<td></td>
<td>30SRO1</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30SRO2</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>
Appendix C: Fracture Table Information
Appendix C

Table C-1: Fracture table dimensions.

<table>
<thead>
<tr>
<th>Part of Table</th>
<th>Length (m)</th>
<th>Width (m)</th>
<th>Height/Aperture² (m)</th>
<th>Volume (m³)</th>
<th>Volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upgradient Reservoir</td>
<td>0.0525</td>
<td>0.61</td>
<td>0.115</td>
<td>3.68 x 10⁻³</td>
<td>3.68</td>
</tr>
<tr>
<td>Fracture</td>
<td>2.10</td>
<td>0.55</td>
<td>2050 x 10⁻⁶</td>
<td>2.37 x 10⁻³</td>
<td>2.37</td>
</tr>
<tr>
<td>Downgradient Reservoir</td>
<td>0.0525</td>
<td>0.61</td>
<td>0.110</td>
<td>3.52 x 10⁻³</td>
<td>3.52</td>
</tr>
</tbody>
</table>

Notes:
1. Height based on measured head of water in the reservoir, not the actual capacity of the reservoir.
2. Aperture based on results of Early Growth tracer model.

Groundwater flow rate through fracture table:
Target average velocity = 5 m·d⁻¹ (based on rate used by Ross et al., 2007)

\[
Q = vA \\
Q = (5m \cdot d)(2050\mu m)(0.55m) \\
Q = 5.6375 \times 10^{-3} m \cdot d^{-1} \\
Q = 3.9 mL \cdot min^{-1}
\]

Therefore, groundwater should be pumped into the upgradient reservoir/pumped out of the downgradient reservoir at a rate of approximately 3.9 mL·min⁻¹.

A rate of 3.9 mL·min⁻¹ could not be consistently maintained by the pumps used in the fracture trial, therefore a rate of approximately 3.0 mL·min⁻¹ was used. This rate corresponds to an average velocity of 3.83 m·d⁻¹.
Appendix D: Spectrophotometer Calibration Curves
### Table D-1: Hach 2800 Spectrophotometer Powder Pillow Information.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Powder Pillow Method</th>
<th>Concentration Range (mg/L)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous Iron</td>
<td>8146 – 1, 10 Phenanthroline</td>
<td>0 to 3.000</td>
<td>510</td>
</tr>
<tr>
<td>Total Iron</td>
<td>8008 – FerroVer</td>
<td>0.02 to 3.000</td>
<td>510</td>
</tr>
<tr>
<td>Nitrate</td>
<td>8171 – Cadmium Reduction</td>
<td>0.1 to 10.0</td>
<td>400</td>
</tr>
<tr>
<td>Ammonia</td>
<td>8155 - Salicyate and Cyanurate Reagent</td>
<td>0 to 0.80</td>
<td>655</td>
</tr>
<tr>
<td>Sulphate</td>
<td>8051 – SulfaVer 4</td>
<td>0 to 70.0</td>
<td>450</td>
</tr>
</tbody>
</table>

### Figure D-1: Calibration curve for Hach ferrous iron powder pillow.

\[
y = 0.226x \\
R^2 = 0.8381
\]
Appendix D

Figure D-2: Calibration curve for Hach nitrate powder pillow.

Figure D-3: Calibration curve for Hach ammonia powder pillow.
Figure D-4: Calibration curve for Hach sulphate powder pillow.
Appendix E: Fluorometer Calibration Results
Figure E-1: Calibration curve for fluorometer used in tracer experiments.
Appendix F: BART™ Photographs
Figure F-1: BART\textsuperscript{TM} IRB, SLYM, HAB, SRB Biodetectors (L-R) containing raw groundwater (i.e., no nutrient addition) on Day 1 of the BART\textsuperscript{TM} trial.

Figure F-2: BART\textsuperscript{TM} IRB, SLYM, HAB, SRB Biodetectors (L-R) containing raw groundwater (i.e., no nutrient addition) on Day 2 of the BART\textsuperscript{TM} trial.
Figure F-3: BART™ IRB, SLYM, HAB, SRB Biodetectors (L-R) containing raw groundwater (i.e., no nutrient addition) on Day 4 of the BART™ trial.

Figure F-4: BART™ IRB, SLYM, HAB, SRB Biodetectors (L-R) containing raw groundwater (i.e., no nutrient addition) on Day 6 of the BART™ trial.
Figure F-5: BART™ IRB, SLYM, HAB, SRB Biodetectors (L-R) containing raw groundwater (i.e., no nutrient addition) on Day 8 of the BART™ trial.

Figure F-6: BART™ IRB, SLYM, HAB, SRB Biodetectors (L-R) containing sample collected from Upgradient Reservoir on Day 7 of the fracture table experiment on Day 2 of the BART™ trial.
Figure F-7: BART™ IRB, SLYM, HAB, SRB Biodetectors (L-R) containing sample collected from Downgradient Reservoir on Day 7 of the fracture table experiment on Day 2 of the BART™ trial.
Appendix G: Modelling Methodology
Modelling Methodology

The early and later growth tracer experiments were modeled to quantify the affect of biofilm development on groundwater flow velocity, fracture aperture and hydrodynamic dispersion within the fracture table. An analytical, one dimensional advection-dispersion model was applied because of the relatively uniform flow conditions and the use of the conservative tracer Lissamine FF. The governing equation is give by

\[
\frac{\partial C}{\partial t} + v \frac{\partial C}{\partial x} - D_L \frac{\partial^2 C}{\partial x^2} = 0 \quad (x \geq 0)
\]

Where \( C \) is resident tracer concentration, \( t \) is time, \( v \) is the average groundwater velocity, \( x \) is the flow direction, and \( D_L \) is the hydrodynamic dispersion coefficient. The hydrodynamic dispersion coefficient is related to the longitudinal dispersivity \( \alpha_L \) and is given by

\[
D_L = v \alpha_L \quad (G.2)
\]

The advection-dispersion equation is valid throughout the model domain with the exception of near the upgradient and downgradient reservoirs where the effects of mixing within the reservoirs must be considered (Palmer, 1988; Moench, 1989). The effect of mixing in upgradient and downgradient reservoirs is well described by Novakowski (1992a) using a mass balance on the fluid leaving the reservoir and entering the domain for the upgradient boundary condition and leaving the domain and entering the reservoir for the downgradient boundary condition.
The following equations describe the boundary conditions on the upgradient reservoir and formation side of the boundary and the initial condition for a slug input of tracer at time zero that is continuously and physically mixed at $t > 0$.

\[ V_u \frac{d C_u(t)}{dt} = -\gamma v C_u(t) \quad \text{(G.3)} \]

\[ v C_u(t) = v C(0, t) - D_L \frac{\partial C(0, t)}{\partial x} \quad \text{(G.4)} \]

\[ C_u(0) = C_0 \quad \text{(G.5)} \]

Where $V_u$ is the volume of the reservoir, $C_u$ is the concentration in the reservoir, the subscript $u$ denotes the upgradient reservoir, and $\gamma$ is the cross-sectional area through which the groundwater is flowing (i.e., fracture aperture).

Conversely, the following equations describe the conservation of mass in the downgradient reservoir including the initial and continuity conditions.

\[ V_d \frac{d C_d(t)}{dt} = \gamma \left[ v C(L, t) - D_L \frac{\partial C(L, t)}{\partial x} \right] \quad \text{(G.6)} \]

\[ C_d(0) = 0 \quad \text{(G.7)} \]

\[ C_d(t) = C(L, t) \quad \text{(G.8)} \]

Where the subscript $d$ denotes the downgradient reservoir and $L$ is the distance downgradient from the upgradient reservoir.

The full derivation of the analytical solution is provided in Novakowski (1992a) and the model was validated in Novakowski (1992b) using column experiments.
Appendix G

To model the early and later growth hydraulic tracer experiments, the solution was numerically inverted using the De Hoog et al. algorithm (1982). To fit the model to the laboratory data, the data was visually compared to the model output and the model adjusted until a visual match was obtained.
Appendix H: Sample Tracer Model Input
Sample Tracer Model Input File
(Early Growth – Base Case Model)

2 IPLC: 2 FOR FINITE ;1 FOR SEMI-INFINITE SOLN
1 IPX: 1 FOR CONC - TIME; 2 FOR CONC - DIST
28 IPI: CODE FOR SOLUTION; SEE COMMENTS
0 ITEST: CODE FOR ANALYTICAL INVERSIONS
0 ISWITCH: 0 FOR TD/XD; 1 FOR TD
0 ISWITCH2: 0 FOR XD/TD OR XD/PE; 1 FOR XD
1 ISW2: 0 FOR DIM. DIST.; 1 FOR REAL DIST.
2.55E-1 V: GROUNDWATER VELOCITY
1.0 RET: RETARDATION FACTOR
0.001 ALPHA: DISPERSIVITY
2.1 XL: FINITE DOMAIN LENGTH
3.6E-3 VOLUM: VOLUME UPPER RESERVOIR
3.6E-3 VOLL: VOLUME LOWER RESERVOIR
1.0 XPOR: POROSITY
11.0E-4 XAREAU: CROSS-SECTIONAL AREA UPPER RESERVOIR
11.0E-4 XAREAL: CROSS-SECTIONAL AREA LOWER RESERVOIR
1 NR: NUMBER OF DISTANCE POINTS MINUS ONE
2.1 XSTART: START DISTANCE
2.1 FX: STOP DISTANCE
50 NT: NUMBER OF TIMES MINUS ONE
0.0 TSTART: START TIME
73.0 ST: STOP TIME
1.000E-6 ERROR:
0.000000 ALPHA:
0.799930 TFACT:
16 NTERM:
Appendix I: Model Sensitivity Analyses
Figure I-1: Velocity sensitivity analysis results for early growth tracer experiment model.

Figure I-2: Aperture sensitivity analysis results for early growth tracer experiment model.
Figure I-3: Dispersivity sensitivity analysis results for early growth tracer experiment model.

Figure I-4: Velocity sensitivity analysis results for later growth tracer experiment model.
Figure I-5: Aperture sensitivity analysis for later growth tracer experiment.

Figure I-6: Dispersivity sensitivity analysis for later growth tracer experiment model.
Appendix J: Dispersion Calculations
Dispersion Calculations

Equation (2) from Boschan et al., 2008:

\[ \frac{l_d}{2b} = \alpha_G + \alpha_T Pe \]  

Where \( l_d \) is dispersivity, \( 2b \) is the fracture aperture, \( \alpha_G \) is macrodispersion, \( \alpha_T \) is Taylor dispersion equal to \( 1/210 \) for Newtonian fluids (Boschan et al., 2008) and \( Pe \) is the Peclet number where

\[ Pe = v \cdot 2b \]

Early Growth:
\( l_d = 1.0 \times 10^{-6} \) m
\( 2b = 2050 \times 10^{-6} \) m
\( v = 7.08 \times 10^{-5} \) m/s

Rearranging Equation J.1 and substitute parameters above
\( \alpha_G = 4.878 \times 10^{-4} \) m

Later Growth:
\( l_d = 0.05 \)
\( 2b = 1275 \times 10^{-6} \) m
\( v = 6.39 \times 10^{-5} \) m/s

Rearranging Equation J.1 and substitute parameters above
\( \alpha_G = 1.52 \times 10^{-8} \) m