Bacterial plume dynamics in the marshland upwelling system employed in the near freshwater conditions

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BACTERIAL PLUME DYNAMICS IN THE MARSHLAND UPWELLING SYSTEM
EMPLOYED IN THE NEAR FRESHWATER CONDITIONS

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Civil Engineering
in
The Department of Civil and Environmental Engineering

by
Benjamin K. Addo
Bsc., Kwame Nkrumah University of Science and Technology, 2002
December, 2004
To the Late Maxwell Adjei Addo, My Beloved Father

Who Gave All He Had Towards My Education
Acknowledgements

Working towards completing this document has been a great experience, and one that offered me the opportunity to interact many a people whose advice and constructive criticisms has shaped up my approach to problem solving. Firstly I am grateful to the Almighty God for the wisdom and blessings bestowed on me. I would like to thank my family, especially my mum for her support, encouragement and prayers. Being thousands of miles away, their words have gone a long way to make my dreams come true. To Angelica, Thanks.

My thanks and gratitude goes to my major advisor, Dr. Kelly Rusch, for helping me develop my research skills and giving me the chance to prove myself. Special thanks to Drs. Malone and Pardue for being great mentors that I could always fall on. To the MUS team and all my friends, when you wake up to the sunrise, just know that it is Benjamin Addo saying thanks to you all. This research was made possible by the Louisiana Department of Environmental Quality. To them and to all those I cannot mention here, I say thank you.
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Abstract

The marshland upwelling system (MUS) was installed in a floatation marsh along the banks of the Bayou Segnette Waterway, Louisiana. This site was characterized by native groundwater of low salinity regime. Previous studies focused on the removal of fecal pathogens from settled/raw and secondarily treated wastewater under high saline groundwater conditions. The objectives of this research were to: 1) quantify the impact of natural die-off on bacterial removal within the MUS, 2) determine bacterial retardation rates in laboratory-repacked sandy loam soil columns, and 3) evaluate the effectiveness of the MUS in removing fecal pathogens from settled, raw wastewater.

Varying salinities and temperatures were used to investigate the inactivation rates for fecal coliforms. Rapid inactivation was recorded for each temperature studied, followed by a much slower die-off process. The higher temperature (25°C) was more detrimental to fecal coliform survival than the 20°C study. Increasing salinity concentrations was not detrimental to fecal coliform survival.

Continuous injection experiments performed in one-dimensional columns packed with sandy loam soils from the field recorded bacterial retardation factors of between 4.7 and 7.7 with respect to the conservative wastewater tracer. Higher limitations to bacterial transport are expected under field-scale conditions.

The MUS was evaluated under three separate injection schemes: a high flow/low temperature loading (HFLT), a 0.95 L/min and a 1.9L/min studies injecting for 15 minutes every hour. The 0.95 L/min flowrate was most suited for bacterial removal. The injection depth employed impacted fecal coliform removal rates. Mean influent concentrations of 92,510±489,614 MPN/100mL were reduced to effluent concentrations of 4.0±7.6 MPN/100mL (observed in the 2.7 m wells). Four-log reductions in influent concentrations were observed within a one-meter radial distance from the injection point. Overall removal followed a first-order decay
relationship with respect to vector distance. Removal rate constants for fecal coliforms ranged from 2.0 -4.0 m$^{-1}$, and that for *E. coli* ranged from 1.7-4.0 m$^{-1}$. 
Chapter One: Global Introduction

1.1 Background

The natural resources abundant in the coastal areas of Louisiana have resulted in the construction of thousands of fishing and hunting camps in sensitive marsh areas. This development brings with it serious environmental impacts associated with the improper disposal of wastes generated from such camps. A majority of the camps are built directly over surface water bodies home to shellfishing, a resource that provides economic benefits for the state of Louisiana. The economic impact of jobs related to this natural resource and the benefits that could accrue to the state makes it prudent that this industry be maintained.

Abundance of this natural resource and the biological diversity offered to coastal dwellers, however, comes with a trade off. Indiscriminate waste disposal practices and the arising dangers posed to aquatic life and human health have caused the industry to suffer setbacks in the past decade (BTNEP, 1999). Domestic wastewater produced by coastal camps is discharged into surrounding water bodies/marshlands virtually untreated. A report on wastewater disposal practices in the Barataria-Terrebonne estuary stated that a considerable number of camps were not equipped with any type of community or onsite wastewater treatment system (BTNEP, 1999), further contributing to the decline in water quality. Such practices cause the release of organic matter, nutrients and pathogens into surrounding water bodies, resulting in serious impacts on the oyster industry. Occurrence and concentration of pathogenic organisms in raw wastewater depend on the sources contributing to the wastewater, the existence of infected persons in the population, and the environmental factors that influence pathogenic survival rates (USEPA, 2002). The 1995 shellfish register for classified growing waters lists the top five pollution sources contributing to harvest limitations in shellfish growing waters as: urban runoff, upstream sources, wildlife, individual wastewater treatment systems, and municipal wastewater treatment plants (NOAA, 1997).
The Barataria-Terrebonne estuary in Louisiana is one of the nation’s leading oyster producing areas, supporting large sectors of the state’s economy (BTNEP, 1999). On an annual basis, the marshes in Louisiana alone produce approximately 544 million kilograms of commercial fish and shellfish (USEPA, 2000a), a catch that was worth $244 million in 1991. In fact the total value of all fishery and wildlife enterprises to the Louisiana economy for the year 2002 was $593 million (AGSUM, 2002). Oyster production in Louisiana averaged 5.7 million kilograms per year from 1997 to 2001, representing the largest and most valuable oyster resource nationwide. This value accounted for greater than 50% of production for the Gulf of Mexico, and 37% of all oysters landed in the United States (LDWF, 2002). Commercial landings had a dockside value of $27.5 million in 2001. Productive shellfish growing waters are however diminishing at an alarming rate. A decline in the classified acreage and trends in estuaries by drainage area was recorded in 1995, with a harvest limitation of 41% for the Barataria Bay, and 13% for the Terrebonne and Timbalier Bays (NOAA, 1997).

Fecal coliform bacteria, which are used as indicators for other enteric bacteria and viruses, serve as the monitoring parameter by which the suitability of a water body for shellfish harvesting is determined. Approved shellfish growing waters are required to have a fecal coliform median or geometric mean most probable number (MPN) of 14 or less/100 mL, and not more than 10 percent of the samples analyzed exceeding a MPN of 43/100mL for a five-tube decimal dilution test. Restricted waters on the other hand are required to have a geometric mean MPN of fecal coliform not exceeding 88/100 mL, and not more than 10 percent of the samples exceeding 260/100mL for a 5–tube decimal dilution test (NOAA, 1997). To further protect consumers, a guideline for shellfish meats of 230 fecal coliforms/100g was established. A less stringent criterion for fecal coliform concentrations in bathing waters set by the EPA is a logarithmic mean of 200/100mL, based on a minimum of five samples taken over a 30-day period, with no more than 10% of the total samples exceeding 400/100mL (Viessmann Jr. and Hammer, 1998). Among the efforts to improve the water quality, and subsequent restoration of deteriorating shellfish
growing waters, include connecting residences with failing or malfunctioning onsite systems to a sewage collection system.

Conventional onsite wastewater treatment systems are recognized as potentially viable, low-cost, long-term, decentralized approaches to wastewater treatment if they are planned, designed, installed, operated, and maintained properly (USEPA, 1997). A 1999 housing survey conducted by the U.S. Census Bureau indicated that approximately 22% of the estimated 102 million occupied homes in the United States are served by onsite systems (USCB, 1999). Of these onsite treatment systems, nearly all, regardless of daily wastewater flow rate or strength, use septic systems as the sole means of treatment or as a pretreatment step (USEPA, 2002). Often used as an alternative to centralized wastewater treatment systems, septic systems can help reduce the risk of groundwater contamination and waterborne disease outbreaks often associated with disposal of untreated sanitary wastes, when properly sited, operated and maintained (USEPA, 1999).

Septic systems are typically composed of two individual units, the septic tank and the absorption field. The septic tank serves as the means of primary solids removal by sedimentation and typically operates under anaerobic conditions (Hagedorn, 1984). The absorption field treats the nutrients and pathogens present in the effluent from the septic tank (BTNEP, 1999). Bacterial transport through the absorption field is controlled by the porosity of the soil and the degree of saturation with water.

Incipient rainfall generally lowers the ionic strength of the pore fluids, thus promoting bacterial transport through soils (Logan and Rogers, 2000; Bitton and Harvey, 1992; Gannon et al., 1991; Zyman et al. 1988; Corapcioglu and Haridas, 1985; Gerba and Bitton, 1984; Lamka et al., 1980), a phenomenon which is best explained by a reduction in the electrostatic forces of attraction for the negatively charged bacterial cells (existing as colloidal suspensions). Gerba et al. (1975) concluded in their study on the fate of wastewater bacteria and viruses in soil that 2 to 3 months was sufficient for reduction of pathogenic bacteria to negligible numbers once they had
been applied to the soil. Brown et al. (1979) noted that most fecal coliform bacteria and coliphage viruses were removed within the first 30 cm of travel in unsaturated soils (8, 41, and 80% sand) beneath absorption trenches, with occasional migration of up to 120 cm, before removal.

However, the implementation of septic systems in coastal areas can adversely affect system operation and potentially lead to the release of untreated wastes (USEPA, 1999; Hagedorn and McCoy, 1979). Many of the systems currently in use do not provide the level of treatment necessary to adequately protect public health and surface and groundwater quality (USEPA, 2000b). System failure can occur as a result of a high water table, poor soil conditions, and/or influent flow exceeding the adsorptive capacity of the soil. Clogging of the soil matrix results in surface ponding (flooding) due to the continual application of wastewater. Lack of proper septic system maintenance and incomplete treatment due to anaerobic conditions are among other reasons assigned for such failures.

A 1999 survey of onsite wastewater treatment systems in the Barataria-Terrebonne estuarine system listed limited uplands, high water tables, minimal elevation and clayey soils as limitations in the applicability of traditional onsite wastewater treatment systems, such as septic systems (BTNEP, 1999). A survey conducted in 1996 stated that pathogens and nutrients were the most common causes of impairment among Louisiana estuaries (LDEQ, 1996). This survey also stated the leading sources of such impairments to emanate from septic tanks, municipal point sources, and pasture land. Monitoring of onsite/decentralized wastewater treatment systems nationwide has revealed that approximately 15.1 billion liters of wastewater are released daily by these systems (USEPA, 2000b). Malfunctioning septic systems have been associated with the degradation of 32 percent of all harvest-limited shellfish growing waters nationwide (NOAA, 1997). More than half of the existing systems are over 30 years old, and 10% of all systems fail each year (USEPA, 2003). Overflows from septic tanks were estimated to be responsible for 42% of the outbreaks and 71% of the illness caused by using untreated groundwater in non-municipal systems (Craun, 1981).
The concept of natural systems for wastewater treatment is gaining increasing popularity around the world as an alternative to conventional wastewater treatment systems. The technical feasibility of using wetlands to treat wastewater depends on wastewater characteristics, process performance capabilities, process design, operation and maintenance, discharge standards, and in some cases, site-specific environmental factors (Kadlec and Knight, 1996). The high degree of biological activity in most wetland ecosystems provides the ability to transform common pollutants typically found in domestic wastewater into biochemically useful nutrients to further increase biological productivity.

1.2 Marshland Upwelling System

The marshland upwelling system (MUS) was developed as a total wastewater treatment system that overcomes limitations associated with the use of conventional wastewater treatment systems in coastal areas. The MUS was designed to operate in saturated, anaerobic conditions. The system consists of the following components: 1) a primary collection/distribution tank, 2) an injection pump connected to a programmable timer, 3) the soil matrix that acts as the receptacle and treatment media for the untreated waste, 4) an injection well, and 5) a monitoring well for regulatory purposes. Selected well depths and the injection schemes employed are dependent on site-specific conditions and the operational criteria determined from different experimental systems to be most ideal for effective treatment.

Domestic wastewater (black and gray) from the primary collection/distribution tank is intermittently injected into the saline substrata by means of a progressive cavity pump (high pressure, low flow) (Figure 1.1). Effective operation of the system is dependent on a number of factors including: the soil matrix, the salinity regime of the groundwater at the site, and the injection flow rate and frequency. The salinity regime of the groundwater in coastal areas makes it unusable as a potable water source. Injection pressures are monitored to help detect possible system/soil clogging (depicted by a gradual and consistent increase in injection pressures), and channelization (depicted by a sudden decrease in injection pressure during an injection cycle).
Figure 1.1 The MUS consists of primary and secondary injection wells, and one monitoring well positioned at the most ideal depth for contaminant removal.

Excessive pressure build-up resulting from active wastewater injection may cause the development of preferential flow paths that promote the transport of wastewater to the marsh surface. The system is however designed to naturally assuage pressure build-up due to the intermittent injection, which allows for a gradual dissipation of pressure that accumulates in the subsurface during active wastewater injection.

During an injection cycle, wastewater is directed radially from the base of the injection well by pump-driven forces. Displacement of bacteria in this phase is primarily controlled by the injection flow rate (Richardson, 2002). Once injection has ceased, the wastewater plume is subject to buoyancy forces derived from the density difference between the injected wastewater and the native saline groundwater. This density difference forces the injected wastewater towards the ground surface, utilizing the native soils as a filter for biochemical and physical processes (Watson Jr. and Rusch, 2002a; Watson Jr. and Rusch, 2001; Watson Jr. 2000; Streamlau, 1994). Changes in background salinity concentrations resulting from tidal movements and/or infiltration of water into the subsurface can alter the density-dependent transport of the injected wastewater. For instance, tidal movements from surrounding water bodies may cause the influx of freshwater,
causing a reduction in background salinity concentrations. Resulting density gradients promote increased lateral transport of the wastewater plume. Advective forces resulting from bulk groundwater flow may also cause increased lateral wastewater transport. The gradual and upward percolation of wastewater due to buoyancy forces, bacterial filtration by the soil matrix, and/or biodegradation of solids, results in an effluent of improved quality (Richardson, 2002; Watson Jr. and Rusch, 2002b). The filtering capability of soil promotes the effective removal of most pathogenic organisms present in sewage, including bacteria, viruses, protozoa, and parasitic worm (Zoeteman, 1985).

1.2.1 MUS Study Site Characterization

Two MUS systems are in operation along the Bayou Segnette Waterway, Louisiana, which is south of Westwego and Northwest of the Town of Jean Lafitte. Bayou Segnette is a 2.3 square kilometer, bald cypress swamp, and a U.S. Army Corps of Engineers maintained waterway that is 19.6 kilometers long from the head of the natural bayou at Westwego to the end of the man-made reach at Bayou Villars, which intersects with the Gulf Intracoastal Waterway near Lafitte. The Bayou Segnette State Park is located in Jefferson Parish and is part of the Barataria-Terrebonne Estuarine System in the Mississippi Deltaic Plain of South Central Louisiana. There are approximately 150 camps along the Bayou Segnette Waterway. Those camps that have a treatment system are using the three-cell segmented holding tank system followed by a chlorine contact chamber approved by the Louisiana Department of Health and Hospitals (LDHH). Most of the camps, however, discharge their waste untreated into the marsh, which eventually washes into the bayou and other surface waters.

The goal of this study was to evaluate the MUS under near freshwater conditions. The subsurface salinity regimes (0-10 ppt compared to salinity levels as high as 35 ppt for previous MUS installations), if proved favorable, will provide a further boost to the MUS as a more credible alternative to conventional onsite wastewater treatment systems. Two systems were installed: 1) a single camp system, and 2) a four-camp cluster system. The single camp system,
located on the east side of the bayou, is occupied permanently, providing a continual source of wastewater from three residents. Located on the west side of the bayou is the cluster camp system, which is connected to four camps (Figure 1.2). These camps are used intermittently throughout the year, with the summer months being the period of most usage.

The single camp system consists of two injection wells: a primary injection well set at a depth of 4.3 m, and a secondary injection well (designed to inject in the event of clogging of the primary well) installed at a depth of 6.1 m. Hydraulic dysfunction resulting from wastewater injection in previous MUS studies necessitated the use of a back-up injection well for the current MUS installed. The secondary injection well is connected to the primary injection well via a pressure relief valve, which will trigger in the event of excessive pressure build-up during an injection cycle, sending wastewater to the secondary injection well.

Figure 1.2 The MUS study site consists of two systems: one serving a cluster of four camps which is used on a week-end basis and another system serving a single live-in camp which is occupied all year round. Positioning of the two systems with respect to the surrounding water bodies is as shown.
Thirty-eight monitoring wells positioned at varying vertical and lateral distances from the injection wells were installed at the single-camp study site. Specific monitoring well depths monitored were 2.7 m, 3.9 m, 4.3 m, 4.6 m, 6.1 m, and 7.6 m. A schematic showing the positioning of monitoring wells relative to injection wells for the single camp system is shown in Figure 1.3. A black 2840-liter polyethylene tank was used for wastewater collection and distribution, a size determined from the frequency of camp usage and the associated wastewater generation potential.

Figure 1.3 The single camp system of the MUS consists of primary and secondary injection wells surrounded by thirty-eight monitoring wells at depths of 2.7 m, 3.9 m, 4.3 m, 4.6 m, 6.1 m, and 7.6 m. The walkway by which the site is accessed is delineated.
Wastewater (black and gray) from the single camp gravity drains into the primary collection/distribution tank and is injected down the marsh via a progressive cavity pump (high pressure, low flow) (Figure 1.4). The primary collection/distribution tank provides quiescent conditions for settleable and floatable organic solids to settle out prior to active wastewater injection. Connected to the pump is a voltage regulator, which facilitates precise control over the flow rate. The pump is automatically turned on at set time intervals via a multi-channel programmable timer and a float switch. The float switch, set approximately 0.3 m above the effluent line from the primary collection/distribution tank, prevents the pump from running dry when the water level in the tank is below a set minimum. A check valve was incorporated into the design configuration to prevent the creation of a vacuum in the piping after active wastewater injection has ceased. This vacuum, if created, would suck soil into the injection pipe and subsequently lead to clogging.

Figure 1.4 The MUS is equipped with a progressive cavity pump that triggers when the water level in the primary collection/distribution tank reaches a set minimum. The process schematic of the MUS showing the plumbing works, flow and pressure monitoring devices are as shown.
The second study site, the cluster camp system, consists of two injection wells, each at a depth of 6.3 m, and thirty-two monitoring wells positioned radially from the injection wells at various depths. Specific monitoring well depths monitored were 0.6 m, 1.5 m, 2.7 m, 3.1 m, and 3.9 m. A schematic showing the positioning of monitoring wells relative to injection wells is shown in Figure 1.5. Both injection wells are open for injection during an injection cycle. Excessive pressure build-up will result in flow being channeled to the injection well with the least pressure, allowing for a gradual dissipation.

Figure 1.5 The cluster camp system of the MUS consists of primary and secondary injection wells surrounded by thirty-two monitoring wells at depths of 0.6 m, 1.5 m, 2.7 m, 3.1 m, and 3.9 m.

Wastewater from each of the four camps is channeled into individual 114-liter lift stations located at the back of the camps, with connections to the main collection line. The lift stations
stations are equipped with pumps that automatically turn on when the water reaches a set minimum level, pumping the water into the primary collection/distribution tank from where it is injected into the subsurface soils of the marsh. During an injection cycle, wastewater preferentially moves down the injection well that provides the least amount of resistance to flow. The preferred injection well down which wastewater moves is dependent on plume movement within the subsurface in a preceding injection cycle, and the density dependent transport and the associated buoyancy forces (slug input scenario).

1.2.1.1 Construction and Installation of Injection and Monitoring Wells

The MUS injection well consists of an open-ended 1.9 cm diameter PVC pipe surrounded by a 5.1 cm diameter casing. The two pipes (inner and outer PVC sections) are connected above the marsh surface using a 1.9 cm threaded female slip by 5.1 cm male adapter. Connected to the top of the injection well are series of 1.9 cm diameter adapters and 90s that hold a manual pressure-monitoring device (Figure 1.6a). The pressure-monitoring device provides a means of monitoring injection pressures during an injection cycle. The upper section of the 5.1 cm casing protruding above the marsh surface is shaped into a 5.1cm tee fitted with a pressure relief valve. The pressure relief valve links the primary and secondary (back-up) injection wells via a 1.9 cm flexible PVC fitting.

The monitoring well essentially consists of a 5.1 cm diameter PVC pipe/casing buried at a specific depth (selected depth is dependent on site-specific geo-environmental conditions relating to water conductivity). Enclosed in the casing is a 1.9 cm PVC pipe with its end connected to a 0.3 m length well screen with 0.25 mm slits (Figure 1.6b). Use of the well screen is with the intent of eliminating solids build-up as water samples are grabbed from the well. A 1.9 cm diameter female thread by male adapter connects the inner and outer casings. Each monitoring well is equipped with a separate flexible norprene tubing to reduce the likelihood of cross contamination during sampling.
Installation of monitoring wells and the injection wells were performed by hydraulic injection, a common practice of well injection in coastal areas. In this process, water, usually drawn from the Bayou, is injected down the 5.1 cm diameter PVC casing and the applied water pressure concurrently washes out solids from within. The removed solids come up from the top of the casing against the water pressure, and the pipe is subsequently pushed down with the eventual removal of solids. The 1.9 cm pipe (with screen) is then inserted into the casing and surrounded by clean inert sand (20-40 mesh). The purpose of using the sand was to increase the hydraulic conductivity of the region immediately surrounding the screen and to prevent fine-textured materials from clogging the well. The monitoring well is allowed to rest for a minimum period of two weeks to allow the subsurface water quality to return to previous conditions before well installation.
1.2.2 Previous MUS Studies

Previous studies on the MUS were undertaken at two sites: Port Fourchon, Louisiana, and Moss Point, Mississippi. In all, three separate evaluations were performed on settled, raw and secondarily treated wastewater, in marsh environments that formed part of the Scatlake soil series, a soil formation common to the coastal salt marshes of the Gulf of Mexico. The Louisiana Universities Marine Consortium (LUMCON) campsite in Port Fourchon, Louisiana, was the site of the first MUS evaluated for retention of fecal bacteria using supernatant wastewater associated with an extended aeration, activated sludge package plant. Supernatant wastewater is defined as secondarily-treated wastewater (effluent from a secondary clarifier) with low BOD and suspended solids concentrations. The camp lies within the Barataria-Terrebonne Estuary, a historically significant oyster-producing area (NOAA, 1997). The marshland is described as *Spartina Marsh* with surface soils consisting of dark, highly organic, saturated strata. Installation of the injection wells and monitoring wells revealed an impermeable soil layer at a depth of 4.6 meters (Streamlau, 1994). This project was executed for one and a half years, between February 1994 and March 1995. The reason for selecting secondarily treated wastewater as the source of supply for this study was to help establish a proof-of-concept for the system as the low influent suspended solids and BOD loadings reduced the potential for clogging problems (Streamlau, 1994). During this initial proof-of-concept study, approximately 575,320 L of secondarily treated wastewater were injected into the MUS. Mean fecal coliform counts of 1600 colonies/100 mL were reduced to less than 2 colonies/100 mL within 2.4 vector meters from the point of injection (Streamlau, 1994).

A second MUS evaluation, also performed at the LUMCON site, used settled, raw wastewater as the source and was investigated for the removal of fecal pathogens. Both studies proved successful for removal of fecal pathogens establishing a basis for further evaluation under differing hydrogeological and geo-environmental settings (Watson Jr. and Rsuch, 2002a; 2001). System operation extended from December 1998 to December 2000, over which a total of 28,930
L of wastewater were injected. Flowrates of 0.9, 1.9, and 3.8 L/min injecting for 30-minutes every three hours were tested. System performance is presented in Table 1.1, where four-to-five order reductions in fecal coliform and \textit{E. coli} concentrations were achieved for 1.5 m and 3.0 m well depths.

Table 1.1 Geometric mean bacterial concentrations at various depths and flowrates

<table>
<thead>
<tr>
<th>Injection Flowrate (L/min)</th>
<th>Fecal Coliform Conc. (col./100mL)</th>
<th>\textit{Escherichia coli} Conc. (col./100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent 3.0 m Wells 1.5 m Wells</td>
<td>Influent 3.0 m Wells 1.5 m Wells</td>
</tr>
<tr>
<td>0.9</td>
<td>351,502 4.1 3.6</td>
<td>154,432 2.7 2.4</td>
</tr>
<tr>
<td>1.9</td>
<td>1,041,552 5.3 3.3</td>
<td>243,120 2.8 2.6</td>
</tr>
<tr>
<td>3.8</td>
<td>953,541 5.0 3.7</td>
<td>215,665 3.3 2.6</td>
</tr>
</tbody>
</table>

* – fecal coliform concentrations are reported by arithmetic means

The third MUS evaluation was executed in a saline \textit{Juncus} marsh within the Grand Bay National Estuarine Research Reserve (NERR), Moss Point, Mississippi. This system was evaluated for approximately two years (June 2001 – May 2003). Flowrates of 1.9, 2.8, and 5.5 L/min at an injection frequency of 30 minutes every three hours were tested, in addition to a flowrate of 2.8 L/min, injecting at 15 minutes every hour. This system was evaluated for its effectiveness in the removal of fecal pathogens, CBOD$_5$ and nutrients. The marsh surface at this site also consisted of dark, highly organic soils consistent with the Scatlake series. The system consisted of one injection well 3.8 m deep, surrounded by 21 nested monitoring wells at depths of 1.5 m, 2.3 m, and 3.0 m. 71,559 L of settled, raw wastewater was injected into the marsh subsurface over the course of the two-year study period. Geometric mean fecal coliform concentrations ranging from 55,269 ± 2,218,016 colonies/100 mL were reduced to 2.7 ± 14.1 colonies/100 mL at the effluent of the system (1.5 m monitoring wells) (Richardson and Rusch, 2004). Fontenot (2003) and Richardson (2002) reported that the MUS installed at the Mississippi site could remove organic and ammonia nitrogen as well as fecal bacteria. Fecal coliform removal was mainly attributable to filtration, and the high adsorption capacity of the \textit{insitu} material was believed to have contributed to the high removal of nitrogen. Predicted distances of travel for the removal of fecal coliform from the injected wastewater to NSSP standards are shown in Table
1.2. Overall, the Grand Bay MUS was predicted to meet NSSP standards over a shorter distance of travel than the LUMCON MUS, which required an additional vector meter of travel. Detailed evaluations of previous MUS studies are outlined in Watson Jr. and Rusch, (2002a; 2001) and Richardson (2002).

Table 1.2 Predicted fecal coliform concentration as a function of vector distance for the Grand Bay and LUMCON MUS

<table>
<thead>
<tr>
<th>Injection Flowrate (L/min)</th>
<th>First Order Rate Constant (m⁻¹)</th>
<th>Predicted Surface Concentration ranges (col/100mL)</th>
<th>Predicted Travel Distance Ranges (m)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9ᵃ</td>
<td>6.6</td>
<td>0-0.0001</td>
<td>0.73-2.03</td>
</tr>
<tr>
<td>1.9ᵇ</td>
<td>6.0</td>
<td>0-0.0012</td>
<td>0.81-2.24</td>
</tr>
<tr>
<td>2.8ᵃ</td>
<td>5.8</td>
<td>0-0.0026</td>
<td>0.84-2.32</td>
</tr>
<tr>
<td>2.8ᶜ</td>
<td>5.6</td>
<td>0-0.0055</td>
<td>0.87-2.40</td>
</tr>
</tbody>
</table>

ᵃRichardson and Rusch, 2004; ᵇWatson Jr. and Rusch, 2001; ᶜInjection frequency of 15 minutes every hour; ᵈDistance required to reduce overall geometric mean fecal coliform concentrations to less than 14 MPN/100mL.

1.3 Bacterial Transport Mechanisms and Removal

Microorganisms have varying degrees of stability within the environment. Their survival and transport in soils and aquifers are controlled by a number of factors: climate (e.g., temperature, rainfall), type of soil or aquifer material (e.g., texture, pH, water holding capacity, cation exchange capacity), pore fluid properties (e.g., chemistry, saturation, and type of pathogen) (Gerba and Bitton, 1984). The attachment of bacteria to the surface of solid aquifer materials may result in prolonged survival rates as constant deposition leads to the development of a biofilm (Mathess and Pekdeger, 1985). Investigations with autochthonic bacteria indicated that such attachments were most intensive in the exponential growth phase. Attached bacteria are protected from external forces, such as the shear forces induced from overall groundwater flow. Bacterial growth within the developed biofilm zones may be enhanced if they encounter a high nutrient supply source (Mathess and Pekdeger, 1985).

Bacterial retention in porous media has been attributed to several mechanisms including straining or filtration at pore constrictions, sedimentation in the pores, diffusion in pores not contributing actively to the transport of water, and adsorption (Yates and Yates, 1988;
Corapcioglu and Haridas, 1984). Possible interaction among these mechanisms of bacterial transport may act to make them operationally indistinguishable (Gannon et al., 1991). Adsorption of bacteria onto solid surfaces in the porous medium may reduce the dimensions of the pore constrictions and thereby enhance straining. While small bacteria (with size within the range 500-1000 nm) are adsorbed onto soil particles, larger bacteria are rather immobilized in soils by physical straining and filtration (Bouwer, 1984).

1.3.1 Decay and Growth

Microbial populations in wastewater are highly variable and dependent on the human population contributing to a waste stream. Extensive research into bacterial survival in the subsurface environment has entailed modeling efforts that have employed development from mass balance principles: the major sources and sinks of fecal coliform bacteria. Two groups of microorganisms can be differentiated when the survival of bacteria in groundwater is considered: 1) Allochthonic pathogenic microorganisms (pathogenic bacteria and enterotoxine producing bacteria), which enter groundwater due to contamination, and 2) Autochthonic ground water microorganisms, which flourish under favorable ecological conditions developing high population densities ($>10^3$/mL) (Matthess and Pekdeger, 1985).

Wastewater injected into the subsurface environment of the marshland upwelling system results in the introduction of allochthonic bacteria. The introduced bacteria are usually eliminated by natural processes, but may survive for a period of 1-7 days (Matthess and Pekdeger, 1985). After this period, the elimination of bacteria may be approximated by an exponential function that can be used to estimate the order of magnitude of the number of bacteria at any time [Merkli (1975) as cited by Mathess and Pekdeger (1985)]:

$$C(t) = C_o e^{-\lambda(t-t_0)}$$

where $t \geq t_0$, and $t_0 \leq 7$ days

$C(t) =$ concentration at time $t$

$C_o =$ initial concentration
\[ \lambda = \text{elimination constant} = \frac{(\ln 2)}{d_{50}} \]
\[ d_{50} = \text{half-life of microorganism} \]
\[ t_o = \text{time at which decay starts} \]
\[ t = \text{time of measurement} \]

The survival of microorganisms in the subsurface environment is also dependent on their location within the soil profile. Enteric microorganisms located at the soil surface will undergo a fairly rapid inactivation rate due to the combined effects of sunlight, antagonisms and drying (Gerba, 1985). Microorganisms located in the aerobic zone would have prolonged survival times. Soil moisture, pH, and availability of organic matter can indirectly influence survival rates by regulating the growth of antagonistic organisms.

In conducting \textit{in situ} and laboratory experiments on estimates of coliform mortality rates, Mancini and Ridgewood (1978) observed an optimum pH for fecal coliform survival to be between 6 and 7, with a rapid decline above and below these values. In addition, acid reactions were observed to cause higher inactivation rates compared to alkaline reactions: an observation that was also made by Cuthbert et al. (1950). Other authors have observed the optimum pH for bacterial growth to be between 6.5 and 7.5, with very minimal survival rates at pH levels above 9.5 or below 4.0 (Metcalf and Eddy, 2003).

High temperatures generally connote high bacterial death rates. This is because rates of microbial reactions increase with increase in temperature. This increase place added demands on nutrients available in a system leading to its continual depletion if the nutrients are not renewed (as is usually the case in dilute natural systems). The ultimate result is a gradual increase in bacterial death rates. Lower temperatures generally favor prolonged survival of fecal coliforms in natural systems (Canale et al., 1973; Easton et al., 2000; Gerba, 1975). Reddy et al. (1981) observed that die-off rates in bacteria, and probably viruses, were approximately doubled with each 10°C rise in temperature between 5°C and 30°C. Other studies have, however, reported limited dependence of bacteria survival/death rates on temperature. Auer and Niehaus (1992)
observed no direct correlation (that is a scatter in temperature-death relationship plots) between temperature and death rate of fecal coliform in field and laboratory studies and attributed such scatter to the dependence of nutrient utilization and variability in natural systems on temperature. Moeller and Calkins (1980), as cited by Auer and Niehaus (1992), observed no temperature-related differences in the death rate coefficient among five temperature groups: \(<10, 10-14, 15-19, 20-24\) and \(>24^\circ C\).

Solic and Krstulovic (1985) in studying the separate and combined effects of solar radiation, temperature, and salinity, observed increase in temperature and salinity to be more detrimental to fecal coliform survival in the presence of sunlight, suggesting a possible synergistic action of sunlight with temperature or salinity. Increasing salinity was progressively more detrimental to fecal coliform survival at lower range values (7-15 ppt) than at higher ranges (15-40 ppt). The dissolved oxygen concentration in a waste stream has also been identified as a key variable by which the effects of irradiance may be manifested. Curtis et al. (1992) found light to have no direct impact on the numbers of culturable organisms in the absence of oxygen. In the same study, dissolved oxygen concentrations below 8mg/L did not significantly impact fecal coliform survival in the absence of light, resulting in what they called: an ecological play-off between the amount of light and the amount of oxygen (that is to say that a synergism existed between light and oxygen).

The detrimental effects of these external factors (particularly high pH and high salt concentrations) on survival is even more pronounce if there is damage to the physical structure of the organism (Curtis et al., 1992). *Escherichia coli* survivability was observed by Filip et al. (1988) under simulated saturated soil conditions to be as high as 100 days at 10°C. Reddy et al. (1981) conducted a review of bacterial survival in soil systems and found average die-off rate constants of fecal coliforms to be 1.14 d\(^{-1}\). Other documented die-off rate constants for bacteria in groundwater are reported in Table 1.3.
Table 1. Fecal coliform and *Escherichia coli* decay coefficients determined from laboratory studies

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Die-off rate (day⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.32</td>
<td>a Keswick et al., 1982</td>
</tr>
<tr>
<td></td>
<td>0.92</td>
<td>a Reddy et al, 1981</td>
</tr>
<tr>
<td></td>
<td>0.36</td>
<td>a McFeters and Stuart, 1974</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>a Bitton et al., 1983</td>
</tr>
<tr>
<td>Fecal coliform</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.53</td>
<td>a Reddy et al., 1981</td>
</tr>
<tr>
<td></td>
<td>0.50 – 4.57</td>
<td>Auer and Niehaus, 1992</td>
</tr>
</tbody>
</table>

* – adapted from Gerba and Bitton (1984)

1.3.2 Filtration

Filtration is a phenomenon by which the transport of a microorganism in the subsurface is limited due to heterogeneity in pore size distribution (Gerba, 1975). The impact of this interconnectivity on bacterial transport is factored into a filtration mechanism referred to as suffusion. Filtration is recognized as a primary mechanism by which bacteria are removed within the subsurface of the MUS (Richardson, 2002), as has been documented in other literature relating to subsurface bacterial transport (Corapcioğlu and Haridas, 1984; Bitton and Harvey, 1992). The grain size of the porous media under investigation significantly impacts the degree to which microorganisms are retained (Gannon et al, 1991; Fontes et al, 1991; Gerba and Bitton, 1984). For suffusion to significantly limit bacterial transport, geometrical suffusion security \( \eta_{SG} \) must be greater than 1.5 (Matthess and Pekdeger, 1985):

\[
\eta_{SG} = \frac{d_m}{F_s d_k} > 1.5
\]  

where \( d_m \) = diameter of microorganism

\( F_s \) = empirical transit factor for suffusion (typically 0.6 is used for most systems, representing the heterogeneity of the porous media)

\( d_k \) = hydraulic equivalent diameter of pore canals = 0.2\( d_{10} \) or \( \sqrt[6]{U(e)} d_{17} \cdot 0.455 \)

\( U \) = uniformity coefficient \( (d_{60} / d_{10}) \)
\[ e = \text{void ratio} \]

1.3.3 Straining

Straining is recognized as an important mechanism by which bacterial transport through soil columns could be limited (Lance, 1984). For straining to be significant, the ratio of the suspended particle diameter to the soil grain diameter must be small (≤ 0.05) (Corapcioglu and Haridas, 1985). Because the rate of bacterial removal by straining is inversely proportional to the grain size of soils, bacteria strained at the soil surface promote the retention of yet finer particles (Bitton and Harvey, 1992). The formation of dendrites as a result of the gradual accumulation of bacteria on soil surfaces increases the effects of bacterial straining. As more bacteria clusters are formed, increased straining effect results in a higher retention of bacteria until bacterial clusters become large enough to break off (Corapcioglu and Haridas, 1985; 1984).

1.3.4 Adsorption

Adsorption of bacterial cells onto soil surfaces is known to be a contributory factor to the effectiveness of bacterial retention in a soil matrix (Gerba and Bitton, 1984). However, soils in their natural setting have a finite surface area for attachment of bacterial cells and therefore a finite adsorption capacity. Yee et al. (1999) rigorously tested the extent and reversibility of adsorption kinetics/equilibrium in the laboratory using two minerals; corundum and quartz, and concluded that the initial adsorption reaction was fully reversible and hence an equilibrium process. Adsorption of microbial particles is known to rely on several factors including: 1) the physical and chemical structure of the absorbate (bacteria) and absorbent (soil), 2) pH of the solution, 3) the characteristics of flow, and 4) the degree of saturation (Corapcioglu and Haridas, 1984). The presence of cations, clay minerals, and low concentrations of soluble organics also contribute to adsorption of bacteria in soils due to their small size, surface charge and an effectively large surface area (Bitton and Harvey, 1992; McDowell-Boyer et al., 1986; Gerba and Bitton, 1984; Hagedorn, 1983). The adsorption of microbial particles onto solid surfaces
increases with the reduction of pH below 8.0, and with the addition of cations, especially in the divalent species.

Wastewater injection may create a dynamic environment that favors bacterial desorption due to changes in pore water chemistry, subsequently affecting system performance (Watson Jr. and Rusch, 2002). Groundwater with a high salt content increases adsorption due to double layer compression (Corapcioglu and Haridas, 1985; 1984; Bouwer, 1984). This increase in adsorption is further piqued by an increase in the ionic strength of a solution, which enhances the affinity of bacteria for soil substrates by increasing the availability of ions in solution. The resulting change in ionic composition contributes to the formation of bridges between the charged sites on the soil surface and the bacterial cell, eventually decreasing the thickness of the double layer (Fontes et al, 1991). It is well documented that the suspension of bacterial cells in low ionic strength (LIS) solutions could substantially increase their transport in porous media (Redman et al., 2004; Logan and Rogers, 2000; Gannon et al. 1991).

Because bacterial surfaces in most natural environments are negatively charged like most other colloids, bacteria tend to form stable colloidal suspensions (Bitton and Harvey, 1992; Marshall, 1986). These negatively charged biocolloids result in interaction with soil particles (sand, silt, clay minerals, metal oxides). The process of sorption, which describes the binding between bacterial cells and solid surfaces (Daniels, 1972; Marshall, 1971), exerts a major influence on the transport of microorganisms through porous media (Harvey and Garabedian, 1991; Yates and Yates, 1988; Gerba et al., 1991; Tan et al., 1994; Lindqvist et al., 1994). When soil surfaces are not smooth, bacteria tend to accumulate first in crevices that are protected from shear stresses. Consequently, later deposition shields/protects the bacteria deep inside the biofilm from being detached. However, if the biofilms emerge from the crevices at a later time (dependent on groundwater flow), detachment rates tend to approach those for smooth surfaces (Rittman and McCarty, 2001).
Because quantification of these sorptive losses may be difficult in the field (Lin et al., 2003), batch studies have been used to efficiently determine these losses in order to employ corrective factors to measurements made. Several models (adsorption isotherms) have been developed to describe the adsorption of bacterial cells to soil particles. An adsorption isotherm describes the relation between the amount or concentration of adsorbate that accumulates on the adsorbent and the equilibrium concentration of the dissolved adsorbate (Droste, 1997). The most commonly used model, the Freundlich isotherm, describes the relationship between the adsorbed and suspended bacterial concentrations and is expressed in its empirical form as:

$$S = k_f C^{1/m}$$

$$\log S = \log k_f + m^{-1} \log C$$

where $S$ is the bacterial concentration at the surface of soil grains, $C$ is the suspended phase bacterial concentration, $k_f$ and $m$ ($m>1$) are constants that describe the sorption capacity and adsorption intensity respectively. The adsorption of viruses and bacteria takes place rapidly (2 and 24 hours, respectively), and the continuous adsorption-desorption reactions cause a retardation of the microorganisms with respect to the surrounding groundwater (Matthess and Pekdeger, 1985).

The retardation of microorganisms is described by the retardation factor $R_d$, which is the quotient of the mean pore water velocity $u$ to the mean transport velocity of microorganisms, $v_m$:

$$R_d = \frac{u}{v_m} = 1 + K_d \left( \frac{\rho_b}{n_e} \right)$$

where $K_d$ = empirical distribution coefficient

$\rho_b$ = bulk density

$n_e$ = effective porosity

The empirical distribution coefficient $K_d$, which gives a measure of the extent of adsorption, can be determined by batch tests in the laboratory as detailed above. It effectively describes the adsorption of bacteria onto solid surfaces and will be equal to the coefficient of the Freundlich or
Langmuir isotherm in dilute natural systems. Subsequent determination of the retardation factor can be done if the bulk density and effective porosity of the aquifer material is known. Retardation factors of between 1 and 2 have been found for bacteria (*E. coli* and *Serratia marcescens*) in field experiments. Mathess et al., (1988) reported a retardation factor of 10 for bacterial populations traveling through porous aquifers. Even higher retardation factors (as high as 500) can be expected for loamy soils with high cation concentrations (Mathess and Pekdeger, 1985).

1.3.5 Advective-Dispersive Transport

Advection, a major mechanism of bacterial transport refers to the transport of contaminants at the same speed as the mean pore water velocity (*u*) (Anderson, 1984):

\[ u = \frac{KI}{n} \]  

(1.5)

where K is the hydraulic conductivity, I is the head gradient, and ne is the effective porosity. The effective porosity represents the portion of the pore spaces that effectively conduct fluids through porous media. Under fully saturated conditions, the effective porosity is assumed equal to the total porosity (Charbeneau, 2000). Determination of groundwater velocity is typically done by use of soluble conservative tracers such as chloride, bromide and other conservative dyes (fluorescein) that supposedly travel at the same speed as groundwater (Yates and Yates, 1991).

Advective forces are largely responsible for bacterial migration during the active injection phase.

The presence of large-scale heterogeneities is believed to be primarily responsible for dispersion on a macroscopic scale (Anderson, 1984). Dispersion causes mixing of the injected wastewater with uncontaminated groundwater and hence serves as a mechanism for dilution (Anderson, 1984). This becomes important in quantifying microbial transport because it may cause contaminants to arrive at discharge points (such as in a monitoring well or a stream) prior to the arrival time calculated from the average groundwater velocity. Dispersion is a time-dependent process whose magnitude increases with the degree of heterogeneity/anisotropy in
porous media. Such movements in the subsurface may be attributable to bulk fluid movement in both longitudinal and transverse directions. Transverse mechanical dispersion (fluid movements in directions perpendicular to longitudinal bulk movements) is largely responsible for horizontal plume movement in the MUS (Watson Jr., 2000).

Bacterial transport in both column and field-scale experiments can be described by a one dimensional advective-dispersive transport equation (with first-order decay) as:

$$\frac{dC}{dt} = \left(a_L u\right) \frac{d^2 C}{dx^2} - u \frac{dC}{dx} - kC$$

(1.6)

where $C$ = bacterial concentration; $u$ = pore water velocity; $a_L$ = longitudinal dispersivity; and $k$ = rate of bacterial deposition onto soil grains, which a measure of the retardation factor. The applicability of analytical solutions when it comes to the importance of dispersion in column experiments is dependent on the chosen boundary conditions (Logan and Unice, 2000). In distinguishing between the relative importance of advection and dispersion in subsurface transport, the following relation could be used:

$$Pe = \frac{uL}{D}$$

(1.7)

where

- $Pe$ = Peclet number of longitudinal dispersion
- $u$ = pore water velocity, $m \cdot s^{-1}$
- $L$ = characteristic length L, (m)
- $D$ = coefficient of axial dispersion, $(m^2 \cdot s^{-1})$

The dimensionless Peclet Number is used as a measure of the dispersion tendency: the smaller the value of $Pe$, the greater the extent of dispersion (Roberts et al., 1985). If the Peclet number is significantly greater than 1, advection is the dominant factor in mass transport. The reverse however holds for a Peclet number significantly less than 1, with dispersion dominating as the main transport mechanism (Metcalf and Eddy, 2003).
are related to the fact that field studies of flow through porous media are usually conducted on a macroscopic level rather than a microscopic level. A better scaling parameter for the Peclet number is with use of the mean grain size diameter (Charbeneau, 2000; Fetter, 1999). A general model that incorporates the effects of mechanical dispersion and diffusion is given by Charbeneau (2000):

\[ D = \tau D_m + a_L v \]  \hspace{1cm} (1.8)

where \( D \) is the coefficient of hydrodynamic dispersion, \( \tau \) is the tortuosity, \( D_m \) is the molecular diffusion, and \( a_L v \) represents the mechanical dispersion. In investigating the role of hydrodynamic dispersion on bacterial transport, Logan and Unice (2000) reported hydrodynamic dispersion to be negligible under typical boundary conditions employed in short laboratory column experiments (~10 cm in length). However, hydrodynamic dispersion on a whole is known to increase with distance, in proportion to the length scale of an experiment. Laboratory dispersivity values measured are typically in the range from 0.01 to 0.1 cm dependent on the order of the pore size (Charbeneau, 2000). Neglecting dispersion in a column experiment could translate into a larger error in the prediction of a field-scale travel distance and collision efficiency computations (Logan and Unice, 2000).

1.3.6 Other Contributory Mechanisms

The existence of concentration gradients in natural systems causes bacteria to move toward a richer food supply. Such movements are termed chemotaxis, a phenomenon that simulates/represents the systematic movement of bacteria (Corapcioglu and Haridas, 1984; Corapcioglu and Haridas, 1985; Bitton and Harvey, 1992). In fact, the injection and resting phases of the MUS present typical scenarios under which bacterial movement by this mechanism is possible. The injection/introduction of wastewater results in the supply of nutrients (in the form of organic matter transformed into carbohydrates and proteins (Metcalf and Eddy, 2003). This nutrient supply may cause bacteria to move in preferred directions, as allowed by the
interconnectivity of pores in the subsurface. Once injection has ceased, concentration gradients are likely to change, causing bacteria to move in yet a different direction. In so doing bacteria appear to relate their present position to the attractant to which they were exposed, leading to a transport phenomenon referred to as tumbling (Corapcioglu and Haridas, 1984). Substances that act as attractants for bacteria include simple molecules such as sugars (glucose, galactose, ribose) and amino acids (serine, aspartic acid). The direct proportionality between the average particle flux and the concentration gradient in the subsurface could result in diffusion by Brownian motion.

Bacterial cells, like other colloids, to some degree rely on Brownian motion for their mobility. This phenomenon results from the tendency of bacteria to collide with each other and with soil sediments, in response to changes in thermal gradients (Corapcioglu and Haridas 1984). The path of the bacterial cell becomes erratic as a result of continual bombardment from other molecules. The longevity of this impact and the involvement of a large number of particles can cause the development of a pressure gradient and a resultant movement (discharge) of bacteria in preferred directions. The mass discharge of bacteria by Brownian motion can be described by the expression (Corapcioglu and Haridas, 1984):

\[ J = -D_B \theta \Delta C \]  \hspace{1cm} (1.9)

where \( D_B \) is the diffusion coefficient of the suspended bacteria, estimated by the Stokes-Einstein equation:

\[ D_B = \frac{k_B T}{3 \pi \mu_w d} \]  \hspace{1cm} (1.10)

where \( k_B \) is the Botlzmann constant; \( T \) is the absolute temperature, \( \mu_w \) is the groundwater viscosity, and \( d \) is the diameter of the suspended bacterial cell.

The importance of deposition of bacterial cells on mineral surfaces was recognized by Knapp et al. (1997) when they investigated the transport of bacterial cells in iron-oxyhydroxide grain coatings. The tendency of bacteria to concentrate/accumulate at solid surfaces of particles
rather than in uniformly dispersed form may have important consequences for their survival. Just as adsorption of natural organic matter on clays protect them from rapid decomposition; bacteria-clay agglomerates may give some protection to the organism, and even perhaps some nutrition. This nutrient supplement may be as a result of exchangeable cations within the agglomerate that may be useful for some physiological functions within the organism (Mortland, 1985). The presence of organic and iron oxide coatings increases retention of bacteria on sand surfaces (Lawrence and Hendry, 1996; Mills et al., 1994).

1.4 Research Objectives

The MUS evaluated in this document is part of 10 years of ongoing research into viable and cost-effective alternatives to conventional wastewater treatment systems for coastal areas. Over the 10-year period of system evaluation, extensive work on the effectiveness of the system in the removal of fecal pathogens and nutrients have proven the system’s effectiveness in treating fecal pathogens to effluent standards when site hydrogeological conditions permitted.

The current system was evaluated under near-freshwater conditions to determine the impact on wastewater migration and the effectiveness of bacteria retention. In the approach adopted in this document, *Escherichia coli* is also monitored, being a facultative gram-negative member of the coliform family. *Escherichia coli* survivability is not significantly affected in marine environments unlike some coliform bacteria, and are therefore more reflective of pathogens (Watson Jr. and Rusch, 2002). The objectives of this research were: 1) to investigate the extent of wastewater migration and bacterial retention capacity under the near freshwater conditions within the MUS, 2) to determine the retardation potential of the field media in limiting bacterial transport, 3) to quantify possible inactivation rates of bacteria in the MUS, and 4) to develop governing equations and the associated removal rate constants describing microbial transport.
2.1 Introduction

The Marshland Upwelling System (MUS) was developed as a total wastewater treatment system that addresses problems associated with the use of septic systems in coastal areas (Watson Jr. and Rusch, 2002; Watson Jr., 2000; Streamlau, 1994). The MUS consists of the following components: 1) a primary collection/distribution tank, 2) an injection pump and programmable timer, 3) the subsurface soil matrix that acts as the receptacle and treatment media for the untreated waste, 4) an injection well, and 5) a monitoring well for regulatory purposes. The main advantage of the MUS over conventional decentralized wastewater treatment systems is its ability to operate in anaerobic, saturated soil conditions. The system uses a progressive cavity pump (high pressure, low flow) to intermittently inject settled wastewater from the primary collection/distribution tank (settling chamber) deep into the saline substrata marsh environment (Figure 2.1).

Figure 2.1 The MUS consists of primary and secondary injection wells, and one monitoring well positioned at the most ideal depth for contaminant removal.
The density difference between the injected wastewater and the subsurface saline pore water causes an upward transport of the wastewater plume during which process bacteria are retained/removed by the soil matrix.

Intermittent injection results in two distinct phases of wastewater migration: an injection phase and a resting phase. During the injection phase, wastewater is directed radially from the base of the injection well by pump-driven forces. Displacement of bacteria in this phase is primarily controlled by the injection flow rate (Richardson, 2002). Once injection has ceased, the wastewater plume is subject to buoyancy forces, tidal movements, and advective forces resulting from natural groundwater flow. Native groundwater with low salinity regimes (near freshwater conditions) promotes lateral transport of the wastewater plume due to reduced buoyancy forces.

The survival of allochthonic bacteria introduced into the MUS during active wastewater injection depends on factors such as temperature, salinity, pH, nutrient availability, and dissolved oxygen concentration. High temperatures generally result in high bacterial death rates. Lower temperatures favor prolonged survival of fecal coliforms in natural systems (Easton et al., 2000; Gerba, 1975; Canale et al., 1973). Soil moisture and availability of organic matter can indirectly influence survival rates by regulating the growth of antagonistic organisms. The nature of the soil matrix, and the factors influencing survival rates result in several mechanisms of bacterial removal in the subsurface including straining or filtration at pore constrictions, sedimentation in the pores, and adsorption. Possible interaction among these mechanisms of bacterial transport may act to make them operationally indistinguishable (Gannon et al., 1991).

Wastewater injection into the subsurface results in the accumulation of bacteria immediately surrounding the injection point, leading to an increase in biofilm formation processes. The eventual increase in bacteria density enhances the potential for substrate, electron acceptor, or nutrient limitation (Taylor and Jaffe, 1990). Bacterial removal by straining is enhanced as the deposited cells promote the removal of yet finer particles by reducing the dimensions of pore constrictions. Straining occurs within pores that are smaller than the limiting
dimension of the bacterial cell (Scholl et al., 1990). The rate of bacterial removal by straining is inversely proportional to the grain size of soil (Bitton and Harvey, 1992). While different soil properties such as particle size, cation-exchange capacity, and clay content influence the retention capacity of soils, the resistance of bacteria to these environmental factors varies among different species and strains. Filtration processes are believed to be largely responsible for bacterial removal, and is mainly attributable to heterogeneity in pore sizes (Gerba, 1975). Filtration has been identified as a primary mechanism by which bacteria are removed within the MUS, with a limited contribution of adsorption (Richardson, 2002). The heterogeneity in pore sizes may result in bacterial removal through a filtration mechanism referred to as suffusion. For suffusion to significantly limit bacterial transport, geometrical suffusion security $\eta_{SG}$ must be greater than 1.5 (Matthess and Pekdeger, 1985):

$$\eta_{SG} = \frac{d_m}{F_s d_k} > 1.5$$  \hspace{1cm} (2.1)

where $d_m$ = diameter of microorganism

$F_s$ = empirical transit factor for suffusion (typically 0.6 is used for most systems, representing the heterogeneity of the porous media)

$d_k$ = hydraulic equivalent diameter of pore canals = $0.2d_{10}$ or $\sqrt[3]{U} \cdot 0.455$

$U$ = uniformity coefficient ($d_{60}/d_{10}$)

$e$ = void ratio

In general, larger diameter bacterial cells experience greater interactions (collisions) with the media thus limiting their transport (Lawrence and Hendry, 1996). The overall impact of this interaction on bacterial transport is factored into a term known as retardation (Yates and Yates, 1991). Retardation causes a delay in the transport of bacteria in relation to the overall travel time of pore water. The retardation of microorganisms is described by the retardation factor $R_d$, which
is the quotient of the mean pore water velocity $u$ to the mean transport velocity of microorganisms, $v_m$:

$$R_d = \frac{u}{v_m} = 1 + K_d \left( \frac{\rho_b}{n_e} \right)$$  \hspace{1cm} (2.2)

where $K_d$ = empirical distribution coefficient

$\rho_b$ = bulk density

$n_e$ = effective porosity

The empirical distribution coefficient ($K_d$) describes the adsorption of bacteria onto solid surfaces and is equal to the coefficient of the Freundlich or Langmuir isotherm in dilute natural systems. The retardation factor can be determined if the bulk density ($\rho_b$) and effective porosity ($n_e$) of the aquifer are known. However, the difficulties associated with field-scale determination of the retardation factor necessitate the need to concurrently run laboratory experiments. For instance, the bacterial retardation factor can be determined by co-injecting a conservative tracer with bacteria into a one-dimensional flow column packed with soil. Knowing the column dimensions, injection flowrate into the column, and the pore volume, the retardation factor can be determined by monitoring effluent concentrations of the bacteria and conservative tracer over time.

Laboratory experiments were performed to provide a better understanding of bacterial transport and survival rates within the field-scale MUS. Soil media and wastewater from the Bayou Segnette, Louisiana site were used for the laboratory studies. Many similar studies have not used media from field sites (Bitton and Harvey, 1992), thus limiting the applicability of the results to real systems. The objectives of the study were to: 1) quantify the impact of natural die-off on bacterial removal within the MUS, 2) determine the extent of bacterial retardation in a MUS using continuous input laboratory columns packed with sandy loam soils, and 3) evaluate the overall implications of such laboratory-scale determinations on field-scale subsurface
transport. The flow conditions examined placed emphasis on the injection phase of the MUS since a greater penetration of bacteria was expected.

2.2 Materials and Methodology

The methodology adopted in studying the transport limitations is presented in two separate sections. Firstly, the experimental protocol for the die-off study is enumerated, followed by the procedure adopted for the one-dimensional laboratory column experiment aimed at studying the bacterial retardation factor during active wastewater injection. Field conditions were simulated as much as possible to enable a direct application of the pertinent parameters to field-scale transport. Medium strength wastewater (with respect to fecal coliforms) was used for both studies, and a sandy loam soil was used for the retardation study. These media were collected from the Bayou Segnette, Louisiana experimental site.

2.2.1 Bacterial Decay Rates

The predominant factors that may influence bacterial die-off rates in the MUS are the effects of temperature, pH, dissolved oxygen and pore water salinity of the media. Laboratory experiments were executed under conditions similar to those observed in the field for the following parameters; temperature, dissolved oxygen, pH and salinity. The rate loss of fecal coliform bacteria was assumed to be proportional to the bacterial concentration as shown by first-order kinetics (Thomann and Mueller, 1987):

\[
R = \frac{dC}{dt} = -kC
\]  

(2.3)

where \( R \) = rate of change in fecal coliform bacteria concentration \( \left( MPN \cdot 100 mL^{-1} \cdot d^{-1} \right) \)

\( C \) = fecal coliform bacteria concentration \( \left( MPN \cdot 100 mL^{-1} \right) \)

\( k \) = overall first-order loss coefficient \( \left( d^{-1} \right) \)

\( t \) = time (d)
A 2x3 factorial experimental design was used to investigate the impact of temperature and salinity on the first-order loss rate coefficient. The experiments were performed at two different temperatures (20°C and 25°C) and three different salinities (0 ppt, 5 ppt, and 10 ppt), which mimic salinity concentrations at the study site. The temperatures were representative of mean values recorded over different field-scale evaluation periods. The studies were performed in duplicate for each temperature/salinity combination. All experiments were performed using two-liter borosilicate glass bottles. A constant temperature was maintained in the respective experiments by placing the two-liter borosilicate glass bottles in a water bath heated to a specific temperature. The water bath was covered with black visquine to reduce the impact of irradiance, and to minimize temperature fluctuations.

Medium strength wastewater (with respect to fecal coliform concentration) from the Bayou Segnette site (3.3 x10^3 – 23.7 x 10^3 MPN·100mL⁻¹), and autoclaved, synthetic seawater (Crystal Sea™, Marine Enterprises International) were used for all experiments. A series of wastewater-saltwater mixtures were prepared until the desired salinities (measured using a refractometer) were obtained. The borosilicate bottles were filled with the prepared mixtures and samples were collected for measurement of initial fecal coliform concentrations using the multiple tube fermentation technique (most probable number method; Procedure 9221E) (APHA, 1998). The bottles were then placed in a water bath pre-set to a specific temperature (20 or 25°C). With dissolved oxygen (DO) concentrations near zero in the subsurface environment of the MUS, a DO of zero (or near zero) was maintained throughout the study by spurring samples with nitrogen gas at the start of the experiment and after each sampling event. The spurred samples were immediately capped to ensure very minimal aeration occurred.

All sample bottles were vigorously shaken before sampling to reduce the effects of sedimentation on survival rates (Auer and Niehaus, 1992). Samples were collected at 12-hour intervals for the initial period of study. This was increased consistently up to 24-hour intervals when die-off rates did not change significantly in subsequent sampling events (approximately
from day 3). The samples were insulated from irradiance by wrapping the sample bottles with aluminum foil. The samples were immediately analyzed for fecal coliforms. *Insitu* parameters such as pH, temperature, DO, and salinity were monitored on each sampling event. The duration of the experiments ranged from 7-8 days.

Experimental results were fit to the theoretical die-off model defined in equation 2.2. SAS version 8.0 was used to perform covariance analyses on the decay rate coefficients to determine whether significant differences existed. Results for different temperature and salinity combinations were also compared to determine the statistical significance. All statistical evaluations were performed at a 95% confidence level.

### 2.2.2 One-Dimensional Laboratory Column Characterization

A continuous injection experiment was performed in triplicate to determine the degree to which microorganisms were retarded in relation to the overall transport velocity during the injection phase of the MUS. The dimensions of the 1D column were designed such that flow within, occurred in one direction (neglecting transverse and vertical dispersions). The column was constructed using a 5.1 cm diameter (I.D.) clear polyvinyl chloride (PVC) pipe at a length (bed depth) of 30 cm (Figure 2.2).

Hand-augered composite soil core borings from the Bayou Segnette experimental were used in all experiments. The soils were dried at room temperature and ground for characterization and packing into the columns. Media properties were determined using standard protocol (ASTM 1995) (Table 2.1). Sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422) were performed to identify the relative percentages of sandy, silty and clayey soils. The USDA textural classification system was used to classify the soils as sandy loams, based on the percent sand, silt, and clay (Oweis and Khera, 1998). To better simulate background conditions present at the site, a background salinity of approximately 10 ppt was maintained in the column. Media saturation was achieved by gradually pouring the soil into columns filled with an equivalent pore volume of synthetic saline solution (Crystal Sea™, Marine Enterprises International).
Prior to wastewater injection, the saturated column was flushed with an additional pore volume (~312 mL) of synthetic saline water (~10 ppt) using a variable-flow peristaltic pump (Cole Palmer, Inc.; Model # U-07518-10). Background samples were collected for fecal coliform analyses after the column had been flushed. This was to determine if the soil media had an...
inherent bacterial concentration prior to wastewater injection. Approximately two liters (six pore volumes) of prefiltered wastewater was then injected into the column at a flowrate of approximately 6.2±0.3 mL/min resulting in one pore volume injected every 50 minutes. To help decrease solids loading to the column and to reduce the likelihood of clogging the saturated media, care was taken to ensure that the introduced wastewater contained a negligible amount of solids by passing through GF/D membrane filters (2.7 µm pore size) prior to injection into the 1D column. The wastewater (< 1 ppt salinity) served as a conservative tracer during injection into the saturated saline media. The soil media was replaced at the end of each trial to eliminate the residual effects of bacterial concentrations from preceding wastewater injection cycles on subsequent trials. Effluent samples were collected every 10 to 20 minutes for salinity, conductivity, temperature and fecal coliforms. All salinity, conductivity and temperature measurements were made using a sensION5™ conductivity probe (Hach Inc.; Model # U-19604-00) with a detection limit of 0.1 ppt. The total time for each continuous injection experiment was approximately 5 hours. The target temperature was 20°C.

Conservative tracers allow for the determination of a retardation factor for microbial transport, a process that involves a comparison of the time of arrival of the peak concentration of the tracer (wastewater) to that of the microorganism that had been co-injected with the tracer into the aquifer (Bitton and Harvey, 1992). In the approach adopted in this paper, the injected wastewater was assumed to conservatively travel through the soil media, and the retardation factor for the injected microbes was determined relative to this conservative transport. The bacterial retardation factor for each trial was determined using the moment of analysis method in which all parameters were represented in dimensionless form. The mean retention time was determined for the respective breakthrough curves by dividing the first by the zeroth moments of the breakthrough curves (Equation 2.4) (Levenspiel, 1999):
The retardation factor is described by the ratio of the mean retention times for the fecal coliform and wastewater breakthrough curves (Equation 2.5).

\[ RF = \frac{T_{FC}}{T_{WW}} \]  

(2.5)

where \( T \) is the mean dimensionless detention time, \( C \) is the dimensionless incremental bacterial concentration, \( t' \) is the dimensionless time at each incremental bacterial concentration, and RF is the retardation factor.

2.3 Results and Discussion

2.3.1 Bacterial Decay Rates

Log-normal plots were developed for each temperature/salinity combination to calculate a decay rate (Figure 2.3). Inactivation rates and the statistical trends identified from the various concentrations are presented in Table 2.2. Mean pH values for all experiments ranged from 7.56 to 7.81, and were not significantly different (p>0.05). Dissolved oxygen concentrations in all experiments never exceeded 1 mg/L (0.51± 0.12 mg/L). Fecal coliform decay rate constants ranged from 0.57 to 1.03 d\(^{-1}\). Water temperatures measured for the 25\(^\circ\)C study did not vary greater than 1\(^\circ\)C (25.3 ± 0.47\(^\circ\)C). Slightly higher temperature variations (>1\(^\circ\)C) were recorded for the 20\(^\circ\)C study (20.3 ± 1.54\(^\circ\)C). In general, less than 10 % of fecal bacteria remained after 3 days suggesting that die-off proceeded at a rapid rate for the initial period after which the process slowed down. This may explain why constant decay rates have been used in the past for indicators of fecal pollution, based on the assumption that a 90% reduction was enough to reduce the risk of infection (Easton et al., 2000).
Figure 2.3. Fecal coliform decay curves for (a, b, c) the 20°C, and (e, f, g) the 25°C experiments. Salinity concentrations studied for the different removal curves are: 0 ppt (a & e), 5 ppt (b & f), and 10 ppt (c & g). Each data point represents geometric mean fecal coliform concentration for duplicate analysis performed for different temperature and salinity combinations.
Table 2.2 Laboratory determined inactivation rates and statistical trends identified for fecal coliform degradation studies

<table>
<thead>
<tr>
<th>Experimental Parameter</th>
<th>Temp (°C)</th>
<th>Influent bacterial conc. (MPN/100 mL)</th>
<th>Sal. (ppt)</th>
<th>pH</th>
<th>Decay rate constant (d⁻¹)</th>
<th>p-value</th>
<th>T₉₀</th>
<th>T₉₉</th>
<th>T₉₉₉</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 [20.3]</td>
<td>4748</td>
<td>0</td>
<td>7.76 ± 0.24ᵃ (n=24)</td>
<td>0.65ᵇ &lt;0.0001</td>
<td>3.55</td>
<td>7.10</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4919</td>
<td>5</td>
<td>7.69 ± 0.19ᵃ (n=24)</td>
<td>0.65ᵇ &lt;.0001</td>
<td>3.52</td>
<td>7.04</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3283</td>
<td>10</td>
<td>7.81 ± 0.12ᵃ (n=24)</td>
<td>0.57ᵇ &lt;.0001</td>
<td>4.06</td>
<td>10.1</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 [25.3]</td>
<td>23664</td>
<td>0</td>
<td>7.56 ± 0.24ᵃ (n=22)</td>
<td>0.70ᶜ &lt;.0001</td>
<td>3.30</td>
<td>6.59</td>
<td>9.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5258</td>
<td>5</td>
<td>7.67 ± 0.11ᵃ (n=22)</td>
<td>1.03ᶜ &lt;.0001</td>
<td>2.23</td>
<td>4.46</td>
<td>6.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4141</td>
<td>10</td>
<td>7.73 ± 0.07ᵃ (n=22)</td>
<td>0.74ᶜ &lt;.0001</td>
<td>3.13</td>
<td>6.25</td>
<td>9.38</td>
<td></td>
</tr>
</tbody>
</table>

[ ] mean experimental temperatures recorded; ᵃ recorded values are not significantly different; ᵇ,c decay rates are not significantly different within each group; p-value < 0.05 indicates that the trend observed for the corresponding decay curves is significant at 95% confidence interval.

Additional parameters of interest are estimates of the time required for 90 percent die-off (T₉₀), 99 percent die-off (T₉₉), and 99.9 percent die-off (T₉₉₉). These percentages correspond to 1-log, 2-log, and 3-log removals, respectively. Fecal coliform population reduction based on the time estimates predicted by the above percentiles has been well documented (Easton et al., 1999; Mancini and Ridgewood, 1978). From a broader perspective, T₉₀ is used to represent inactivation rates in the development of inactivation models (Canteras et al., 1995). The inactivation rates obtained indicate that fecal coliforms die-off quicker for the 25°C experiment, with the 5 ppt salinity being most detrimental to their survival.

Regrowth of bacteria is evident in some data sets. As was typical of the 20°C/0ppt combination, there was a period of rapid die-off from day 0 to day 4, after which bacteria regrowth occurred (Figure 2.4a). The plot represents the net/effective fecal coliform decay rate, since it factors the effect of bacterial regrowth. Excluding the regrowth phase yields an inactivation time of 1.68 days for a 1-log reduction of influent concentrations. This time is two times faster than that predicted using the net/effective decay coefficient (3.55 days).
Figure 2.4 Fecal coliform regrowth was evident in the 20°C/0ppt, 20°C/5ppt, 25°C/0ppt, and 25°C/10ppt combinations. Effective decay rate is represented in curves a, c, e, and g. Curves b, d, f and h exclude the regrowth phase. Each data point represents geometric mean fecal coliform concentration for duplicate analysis performed for different temperature and salinity combinations.

\[ y = 1100e^{-0.6483x} \]
\[ R^2 = 0.3291 \]

\[ y = 1200e^{-0.6543x} \]
\[ R^2 = 0.3136 \]

\[ y = 2624e^{-0.6983x} \]
\[ R^2 = 0.6634 \]

\[ y = 1380e^{-0.7365x} \]
\[ R^2 = 0.7815 \]

\[ y = 4700e^{-1.7692x} \]
\[ R^2 = 0.7489 \]

\[ y = 4000e^{-2.2267x} \]
\[ R^2 = 0.8358 \]

\[ y = 1200e^{-0.6483x} \]
\[ R^2 = 0.3291 \]

\[ y = 1100e^{-0.6483x} \]
\[ R^2 = 0.3291 \]

\[ y = 5000e^{-1.1217x} \]
\[ R^2 = 0.7486 \]
Excluding the regrowth phase for the 20°C/5ppt combination yielded an inactivation time of 1.26 days, which is three times that predicted using the corresponding net decay coefficient (Figure 2.4). Inactivation times of 2.18 and 2.05 days were recorded for the 25°C/0ppt and 25°C/10ppt combinations respectively. The net/effective decay rate coefficients predicted 3.30 and 3.13 days respectively for these combinations (Table 2.3). While recognizing that the effect of temperature on bacterial regrowth may be manifested in some of the observations made, the effect of salinity and initial fecal coliform concentrations on regrowth rates was not clearly defined. Liu (2002) found no significant impacts of initial fecal coliform concentration on regrowth.

Regression analyses on the die-off rate constants (slope of the regression line) showed all trends to be significant (p<0.0001). The analysis of covariance method was used to contrast the different salinity and temperature combinations studied, and to compare the overall decay rate constants for the two different temperatures. Overall, fecal coliform decay rate coefficients recorded within a specific experimental temperature did not differ significantly from each other (p-value ranged from 0.45-0.95). Inactivation rates for the 25°C experiment were significantly higher than the 20°C experiment (p=0.0041). Studies performed on the inactivation of fecal coliforms in estuarine waters showed inactivation rates to be significantly and inversely related to temperature (Burkhardt III et al., 2000). Sarikaya and Saatci (1995) also observed bacterial decay rate in the dark to be dependent on temperature variations.

Using the mixed procedure, different temperature and salinity combinations were tested to determine if specific combinations favored fecal coliform decay (Table 2.3). The highest temperature/salinity combination (25°C/10 ppt) was not significantly different from the lowest combination (20°C/0 ppt) (p=0.135). This suggests that even though increased temperature was detrimental to fecal coliform survival, increased salinity concentrations did not necessarily cause higher inactivation rates. While the 25°C/5ppt and 20°C/5ppt were significantly different
(p=0.0044), the 25°C/10ppt and 20°C/10ppt were not (p=0.3726), suggesting that different temperature and salinity combinations may result in differing inactivation rates.

Table 2.3 Statistical comparisons of different temperature and salinity combinations to determine possible synergism among parameters studied

<table>
<thead>
<tr>
<th>Contrasted temperatures (°C)</th>
<th>Contrasted salinity concentrations (ppt)</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 vs 25</td>
<td>0 vs 0</td>
<td>0.1943</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>5 vs 5</td>
<td>0.0044</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>10 vs 10</td>
<td>0.3726</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>0 vs 10</td>
<td>0.1350</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>10 vs 0</td>
<td>0.4893</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>10 vs 5</td>
<td>0.0257</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>5 vs 10</td>
<td>0.1211</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>0 vs 5</td>
<td>0.0052</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>5 vs 0</td>
<td>0.1758</td>
</tr>
<tr>
<td>20 vs 20</td>
<td>0 vs 5</td>
<td>0.9495</td>
</tr>
<tr>
<td>20 vs 20</td>
<td>0 vs 10</td>
<td>0.4978</td>
</tr>
<tr>
<td>20 vs 20</td>
<td>5 vs 10</td>
<td>0.4588</td>
</tr>
<tr>
<td>25 vs 25</td>
<td>0 vs 5</td>
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</tr>
<tr>
<td>25 vs 25</td>
<td>0 vs 10</td>
<td>0.6238</td>
</tr>
<tr>
<td>25 vs 25</td>
<td>5 vs 10</td>
<td>0.5541</td>
</tr>
</tbody>
</table>

Note: respective contrasted salinities match-up with the temperature combinations. i.e. 0 vs 0 indicate a comparison of 0 ppt at 20°C and 0 ppt at 25°C.

A multiple linear regression model was tested to determine the dependence of the fecal coliform decay rates on temperature and salinity. The complete model tested was of the form (Neter and Wasserman, 1974):

\[ Y_{ij} = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 TS + \Sigma_{ij} e \]  

where  

- \( T \) = temperature (°C)  
- \( S \) = salinity (ppt)  
- \( Y_{ij} \) = predicted fecal coliform decay rate coefficient for a given temperature i, and salinity j  
- \( \Sigma_{ij} \) = error function  
- \( \beta_0, \beta_1, \beta_2, \beta_3 \) = model parameters

Results of the statistical test indicated that fecal coliform decay rate constants were not dependent on temperature (p=0.1215) and salinity (p=0.6599). Additionally, there was no interaction
between temperature and salinity (p=0.7629). Therefore, the mean response given by the model takes the form \( Y_i = \beta_0 \), indicating that decay rate coefficients cannot be predicted using the statistical model.

The overall mean fecal coliform decay coefficient (0.72±0.16 day\(^{-1}\)) from the experiments performed is close in magnitude to that determined by Richardson (2002) (0.52±0.20 day\(^{-1}\)). While dissolved oxygen (DO) concentration levels for experimental results presented in this document were well below 1 mg/L (~mean of 0.5 mg/L), Richardson (2002) did not regulate DO levels. Therefore, the difference in decay coefficients cannot be explained based on the DI levels monitored. However, salinities studied by Richardson (2002) were orders of magnitude higher (up to 35 ppt) at similar temperatures, and could be a determinant factor for the variation in decay rate coefficients.

The inactivation times reported in other literature range from several minutes to days. Canteras et al. (1995) obtained 33.6 and 116.8 minutes for \textit{in situ} experiments performed in the summer and winter months respectively. Easton et al. (1999) and Wilson and Noonan (1985) recorded inactivation times for fecal coliforms within the range 2.7 - 6.2 days. The inactivation times determined in this paper follow similar trends observed by Reddy et al. (1981). The die-off rate of bacteria is doubled, after a 10\(^\circ\)C rise in temperature. However, the 5 ppt die-off study recorded inactivation times more than double the predictions made by Reddy et al. (1981). Irrespective of the wide use of the model described by equation 2.2, other models incorporate an initial growth/survival phase of bacteria. Bacteria introduced into the subsurface may survive for a period of 1-7 days, after which elimination may be approximated by an exponential function used to estimate the order of magnitude of the number of bacteria at any time (Mathess and Pekdeger, 1985).

Seasonal variations in rainfall intensity and system loading rates may regulate the external factors affecting fecal coliform survival rates within the MUS. For instance, higher ambient temperatures in the summer may result in higher fecal coliform inactivation rates. Other
considerations during such periods may be: that predation and competition among the microorganisms is increased as a result of the increased microbial activity, causing significant reduction in fecal coliform populations. Increased camp usage during the summer months may introduce variations in organic, solids and bacterial loading rates into the system. This has important consequences for bacterial survival as high bacterial populations influence nutrient availability. Biofilm accumulation and the formation of bacteria-clay agglomerates may offer some protection to residing bacteria, and even perhaps some nutrition (Mortland, 1985). Changes in background salinity concentrations as a result of infiltration of rainfall during certain periods of the year may cause varying die-off rates of injected fecal coliform bacteria. Intermittent wastewater injection into the subsurface may create a dynamic environment that favors the supply of nutrients to residing bacteria. If such impacts were significant, longer survival times would be expected under field conditions.

2.3.2 Bacterial Retardation Studies

Fecal coliform breakthrough curves \((\frac{C}{C_0})\) for the three trials are presented in Figure 2.5. In this figure, the normalized effluent concentration is plotted against the number of pore volumes passed through the column. Fecal coliform retardation factors obtained ranged from 4.7 to 7.7 \((6.1 \pm 1.5)\) (Table 2.4). Observed fecal coliform breakthrough was delayed in all experiments resulting in significant retardation. Initially, the column effluent was free of bacterial cells. Bacterial breakthrough was not achieved until after approximately 0.5 pore volumes had left the bed. For all replicate trials, water temperatures within the column did not vary greater than 1°C, with mean temperatures of 20.2±0.34, 19.7±0.69 and 21.7±0.29°C recorded for trials one, two and three respectively.
Figure 2.5 Combined experimental breakthrough curves

Table 2.4 Laboratory repacked soil columns were used to determine the retardation factor of fecal coliforms

<table>
<thead>
<tr>
<th>Experimental Trial</th>
<th>Injection Flowrate (mL/min)</th>
<th>Retardation Factor, $R_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>6.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Trial 2</td>
<td>6.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Trial 3</td>
<td>6.1</td>
<td>4.7</td>
</tr>
</tbody>
</table>

It is important to recognize the trends existing in the experimental breakthrough curves, and the order of magnitude retardation factors obtained. This trend may be a random occurrence, or an experimental artifact attributable to the media packing in the columns. Variations in the column packing may influence bacterial migration due to existent weak spots in the column. Biofilm formed by successive wastewater injection decreases pore sizes, and may encourage preferential flow paths within certain portions of the column. These pathways, if present, will favor wastewater transport, resulting in isolated instances of significant fecal coliform detection during system initiation (typical of trial 3). At the same time, impermeable zones may trap
significant colonies of microorganisms that may later be released by successive wastewater injection schemes. This may explain the slight variations in the fecal coliform retardation factors obtained. It is plausible that attachment of bacteria onto the soil particles were favored by the ionic potential of the soil and bacterial cells, since pore water of high ionic strength enhances the attachment affinity of bacterial cells to media grains (Fontes et al., 1990; Scholl et al., 1990). Slight differences in the method of wastewater distribution into the columns may also explain these variations (Ausland et al., 2002).

It is also evident from the plots that the magnitude of $C/C_0$ for the wastewater or fecal coliform breakthrough curves never reached one (the maximum possible value). One reason for this observation was because of the inherent salinity concentrations associated with the packing material (~0.2ppt) and the injected wastewater (~0.4ppt). Consequently, salinity concentrations never reached zero for all trials (0.8-1.0 ppt as the lower bound value), even with close to 8 pore volumes flushed. This has important consequences for bacterial transport and may lead to yet higher magnitudes of bacterial retardation factors. The injected fecal coliform populations were not fully recovered because of entrapment in the columns. Richardson (2002) obtained fecal coliform retardation factors ranging from 2.59 to 3.56 (3.07±0.49) using clean sand ($d_{50}=0.37$ mm) packed in a one-dimensional laboratory column. Comparing the mean grain size diameter and clay content suggest that pore size distribution and the surface charge of clay relative to sand impacted bacterial retardation rates. Additionally, the attachment rate can be attributed to the high organic content of the bed (11.6±2.5). Mathess and Pekdeger (1985) reported retardation factors as high as 500 for loamy soils.

While the variability in packing may significantly influence the extent of bacterial penetration, it should be recognized that natural systems are rarely, if ever, homogeneous in all directions. Repacked soil columns are generally more effective at retaining bacteria than intact natural soils (Smith et al., 1985). The nature and structural integrity of the packing media may in fact account for the heterogeneity encountered in natural systems. Pore constrictions are further
reduced if packing is efficiently done, causing bacteria to move within smaller pores where they are more susceptible to being adsorbed. The effect of pore size constrictions on bacterial transport in the columns was estimated using the geometric suffusion security (equation 2.1) developed by Mathess and Pekdeger (1985). Bacterial cell diameters were assumed to be within the range 0.2-5µm. Estimates using the largest possible size of bacterial cells suggest that suffusion enhanced filtration processes in the columns. Bolster et al. (1999) used the geometric suffusion security to determine the importance of straining in intact sand columns used for the investigation of the spatial distribution of deposited bacteria. Mean grain diameter for the packing media was 0.74 mm.

The salinity of the pore fluid percolating through porous media affects the hydraulic conductivity, particularly in materials containing trace amounts of swelling clays (montmorillonite, illite, etc.) (Mehnert and Jennings, 1985). Therefore, with media salinity concentrations within the range 10.7-12.3 ppt for the three trials, any variations in mineral content will cause changes in the hydraulic conductivity of the bed, and subsequently, the bacterial travel rates. The retardation factor is also dependent on the bulk density, effective porosity and the adsorption isotherm for the packing media (equation 2.2). An estimate of the adsorption coefficient was made using a mean retardation factor of 6.1, an effective porosity of 0.509, and a bulk density of 0.8g/cm$^3$. The bulk density was estimated using laboratory-determined porosity and particle density of 0.509 and 1.59g/cm$^3$ respectively. The effective porosity was assumed equal to the total porosity under fully saturated conditions (Charbeneau, 2000). Substituting these parameters in equation 2.2 yielded a $K_d$ of 3.2 L/kg.

2.4 Overall Implications on Field-Scale Transport

The overall implications of studying bacterial transport in the MUS are pertinent in the determination of the efficiency of the system for bacterial removal. Fecal coliform decay rate constants ranged from 0.57–0.65d$^{-1}$ and 0.70–1.03d$^{-1}$ for the 20°C and 25°C experiments respectively. Actual field-scale mortality rates of 0.18-0.81d$^{-1}$ and 0.17–0.44d$^{-1}$ were determined
for fecal and allochthonous bacteria respectively in natural aquatic ecosystems with freshwater conditions (Menon et al., 2003). To investigate the impact of these decay rates in field scale operations, the half-life of fecal bacteria was compared with a typical mean hydraulic retention time obtained in a MUS installed in Scatlake soils. The highest decay rate coefficient determined from the laboratory experiments ($1.03 \text{ d}^{-1}$) was used in computing the bacterial half-life (0.67 days). Comparing this to a mean hydraulic retention time of 49.2 days (Richardson et al., 2004), indicates that decay of fecal coliforms could contribute to removal rates in a MUS.

Temperature variations associated with seasonal changes will cause changes in overall decay rates within the system. Removal may also be attributed to a combination of different retention properties of the soil matrix, such as, through natural filtration processes and adsorption. The 90% loss rate of fecal coliforms during the first three days of the die-off studies indicated that fecal coliforms initially underwent a rapid decay rate followed by a much slower die-off process. Active wastewater injection during system operation may act as a source of nutrient supply for residing bacteria, resulting in longer survival times.

The migration of bacteria under the force of the injection pump during active wastewater injection results in greater bacterial penetration. Mimicking this transport scenario in the continuous input experiment implies that the mean bacterial retardation factor determined represents a lower bound value for actual field transport. Increased impact of dispersion, tidal movements, and lower advective forces during the resting phase may result in a higher retardation factor. Therefore, fecal coliform retardation factors will be highly variable. Additional variability may be associated with the heterogeneity of the soil matrix. A closer estimate of the retardation factor can be obtained by comparing results from the continuous input experiments with results from insitu experiments, which can be determined by injecting a conservative dye/tracer relative to which bacterial concentrations are monitored in select monitoring wells.
2.5 Conclusions

Fecal coliform survival rates/decay coefficients at the two temperatures studied (20°C and 25°C) were significantly different with the higher temperature being more detrimental to fecal coliform survival. Increase in salinity concentrations was not detrimental to fecal coliform survival. Rapid die-off of fecal coliform occurred in the initial periods ($T_{90}$ of between 2.2 and 4.0 days). This die-off rate slows down as time progresses, with instances of bacteria regrowth. Approximately twice as much time was needed for a 2-log removal, with a 3-log removal requiring three times the inactivation times. To better protect public health, a more conservative approach will be to model fecal coliform using a two-step model that accounts for possible regrowth through the supply of nutrients to residing bacteria as wastewater is intermittently injected.

Investigations done using the field media provided a good basis for evaluating the extent of bacterial retardation. A mean bacterial retardation factor of 6.1 was determined to limit transport under laboratory-simulated conditions. The mean bacterial retardation rate is 2 orders of magnitude bigger than that obtained by Richardson (2002) using sand. Clay mineral contents, a high porosity, and the presence of pore constrictions impacted movement of microbes in the laboratory repacked columns. The structure and distribution of pore sizes enhanced bacterial straining, increasing the likelihood of filtration processes in the column ($\theta_{SG}>1.5$). Actual limitations in field-scale transport may be higher due to media heterogeneities, and the impacts of rainfall on pore size constrictions.
Chapter Three: Retention and Removal of Fecal Coliforms in a Marshland Upwelling System Operated Under Near Freshwater Conditions

3.1 Introduction

According to the U.S. Census Bureau (1999), approximately 22 percent of the estimated 102 million occupied homes in the United States are served by onsite systems. Monitoring of these systems has, however, revealed that approximately fifteen billion liters of wastewater are released daily (USEPA, 2003); making groundwater contamination a likely occurrence. Of these onsite treatment systems, nearly all, regardless of daily wastewater flow rate or strength, use septic systems (USEPA, 2002). Septic systems are composed of two individual units, the septic tank and the absorption field. The septic tank serves as the means of primary solids removal and typically operates under anaerobic conditions (Hagedorn, 1984). Settleable solids, oils, greases, and floating debris in the raw wastewater are efficiently removed by 60 - 80% (Boyer and Rock, 1992; Baumann et al., 1978).

The absorption field treats the nutrients and pathogens present in the effluent from the septic tank (BTNEP, 1999). Bacterial transport through absorption fields is controlled by the porosity and the degree of saturation of the soil. Incipient rainfall generally lowers the ionic strength of the pore fluids, thus promoting bacterial transport through soils (Logan and Rogers, 2000; Bitton and Harvey, 1992; Gannon et al., 1991; Corapcioglu and Haridas, 1985; Gerba and Bitton, 1984), a phenomenon which is best explained by a reduction in the electrostatic forces of attraction for the negatively charged bacterial cells (existing as colloidal suspensions). However, implementation of septic systems in coastal areas can adversely affect system operation and potentially lead to the release of untreated wastes. Many of the systems currently in use do not provide the level of treatment necessary to adequately protect public health and surface and groundwater quality (USEPA, 2000). The 1995 shellfish register ranks individual wastewater treatments systems among the top five pollution sources contributing to harvest limitations in shellfish growing waters (NOAA, 1997). On a national scale, more than half of the existing
systems are over 30 years old, and at least 10% of all systems record various degrees of failure each year (USEPA, 2003).

System failure can occur as a result of a high water table, poor soil conditions, and/or influent flow exceeding the adsorptive capacity of the soil (USEPA, 1999). A 1999 survey of onsite wastewater treatment systems in the Barataria-Terrebonne estuarine system lists limited uplands, high water tables, minimal elevation and clay soils as limitations in the applicability of traditional onsite wastewater treatment systems, such as septic systems (BTNEP, 1999). In 1989-90, 13 out of 26 waterborne outbreaks in the US were due to contaminated groundwater with viruses being the main etiologic agents (MMWR, 1992), and malfunctioning onsite sewage treatment systems were identified as the main cause of this mishap. Overflow from septic tanks have been estimated to be responsible for 42% of the outbreaks and 71% of the illness caused by using untreated groundwater in non-municipal systems (Craun, 1981). It is evident that improved operation and proper management of onsite/decentralized systems is essential if the nation’s water quality goals and health needs are to be realized.

The Marshland Upwelling System was developed as an alternative treatment method in response to problems associated with use of septic systems in coastal dwellings (Watson Jr. and Rusch, 2002; Watson Jr. and Rusch, 2001; Watson Jr., 2000, Streamlau, 1994). The MUS consists of a collection/distribution tank, an injection pump connected to a programmable timer, the subsurface soil matrix that acts as the receptacle and treatment media for the injected wastewater, an injection well, and a monitoring well for regulatory purposes. The main advantage of the MUS over conventional onsite systems is its ability to operate in saturated, anaerobic soil conditions. The system uses a progressive cavity pump (high pressure, low flow) to intermittently inject settled, domestic wastewater (black and gray) into the marsh subsurface strata via a shallow injection well (Figure 3.1). The intermittent injection allows for dissipation of pressure that accumulates during active wastewater injection. The salinity regime of the groundwater in coastal areas makes it unusable as a potable water source. Effective system operation is dependent on the
natural filtration properties of the soil, the injection flowrate and frequency, and the saline groundwater.

Figure 3.1 Schematic of the Marshland Upwelling System showing the primary and secondary injection wells, and a monitoring well for monitoring the wastewater plume. During an injection cycle, wastewater is directed radially from the base of the injection well by pump-driven forces. Displacement of bacteria in this phase is primarily controlled by the injection flow rate (Richardson, 2002). Advective forces dominate wastewater transport during this phase. Once injection has ceased, the wastewater plume is subject to buoyancy forces derived from the density difference between the injected wastewater and the native saline groundwater. Native groundwater of low salinity regimes (near freshwater conditions) promote increased lateral transport of the wastewater plume due to reduced buoyancy forces. Dispersive forces are expected to increase correspondingly, resulting in reduced penetration rates for the injected microorganisms. Lower ionic strength characteristics of the plume cause greater bacterial travel distances. The tendency for bacteria to be removed through adsorptive processes is further reduced as the thickness of the double-layer increases (Corapcioglu and Haridas, 1985; 1984).
Previous studies performed at sites with highly saline groundwater (up to 35ppt) found the system to be effective for the retention of fecal pathogens from settled, raw wastewater (Richardson and Rusch, 2004; Watson Jr. and Rusch, 2002; Watson Jr. and Rusch, 2001).

The MUS evaluated in this document was installed in a floatation marsh along the Bayou Segnette Waterway (Louisiana), a site characterized by native groundwater of low salinity regime. The objectives of this study were to: 1) evaluate the extent to which near-freshwater conditions impact bacterial retention in the subsurface environment of the MUS, 2) determine loading rates (i.e. hydraulic, bacterial, and solids) that provide the highest efficiency of removal for these floating marsh conditions, and 3) determine removal rate constants and the distances of travel for which bacteria is effectively removed.

3.2 Materials and Methods

3.2.1 Field Site Description

Two MUS systems are in operation along the Bayou Segnette Waterway, Louisiana, which is south of Westwego and Northwest of the Town of Jean Lafitte. Bayou Segnette is a 2.3 square kilometer, bald cypress swamp, and a U.S. Army Corps of Engineers maintained waterway that is 19.6 kilometers long from the head of the natural bayou at Westwego to the end of the man-made reach at Bayou Villars, which intersects with the Gulf Intracoastal Waterway near Lafitte. The Bayou Segnette State Park is located in the Jefferson Parish and is part of the Barataria-Terrebonne Estuarine System in the Mississippi Deltaic Plain. There are approximately 150 camps along the Bayou Segnette Waterway. Those camps that have a treatment system are using the three-cell segmented holding tank system followed by a chlorine contact chamber approved by the Louisiana Department of Health and Hospitals (LDHH). Most of the camps, however, discharge their waste untreated into the marsh, which eventually washes into the Bayou.

Subsurface salinity concentrations range from 0 (near surface) to 12 ppt at 7.6 m depth. Surface water bodies in close vicinity to the sites have salinity concentrations ranging from 0 to 3ppt. Surface water discharges into the Bayou Segnette waterway require adherence to total fecal
coli concentrations for primary contact, which is a geometric mean concentration of 200 colonies/100mL. Full body contact recreational waters are required to have geometric mean E. coli concentration not exceeding 126 E. coli/100mL, with a 95% confidence limit (USEPA, 1986). However, the National Shellfish Sanitation Program (NSSP) standard of 14 MPN/100mL for total fecal coliforms was conformed to in the determination of effluent requirements for the system (USFDA, 2000). This standard was selected to ensure that system effluent conformed to requirements for shellfish growing waters.

### 3.2.2 System Description

Two systems were evaluated: a single-camp system and a four-camp cluster system. The single-camp system was installed on the east side of the bayou, and has two injection wells: a primary injection well at a depth of 4.3 m and a secondary injection well (designed to inject in the event of clogging of the primary well) at a depth of 6.1 m (Figure 3.2).

![Diagram](image-url)

Figure 3.2 The MUS study site consists of two systems: one serving a cluster of four camps which is used on a week-end basis and another system serving a single camp, which is occupied all year round. Positioning of the two camps with respect to the surrounding water bodies is as shown.
The single camp system is occupied permanently, providing a continual source of wastewater. The generated wastewater (black and gray) gravity drains into a primary collection/distribution tank and is injected into the marsh via a progressive cavity pump (high pressure, low flow) controlled by a programmable timer and a float switch (Figure 3.3). The timer is programmed to inject wastewater at predetermined time intervals. A float switch prevents the pump from operating if the water level from the primary tank is below a set minimum level.

![Diagram of the MUS](image)

**Figure 3.3** The MUS is equipped with a progressive cavity pump that triggers when the water level in the primary collection/distribution tank reaches a set minimum level. The process schematic of the MUS showing the plumbing works, flow and pressure monitoring devices are as shown.

The two injection wells installed for the single camp system are connected to each other via a pressure relief valve. Rapid pressure build-up as a result of clogging of the primary injection well during active wastewater injection will trigger a pressure relief valve installed at the top of the primary well. This will send wastewater down the marsh via the secondary injection well. Thirty-eight monitoring wells are positioned at varying depths and horizontal distances from the injection well (Figure 3.4).
Figure 3.4 The single camp system of the MUS consists of primary and secondary injection wells surrounded by thirty-eight monitoring wells at depths of 2.7 m, 3.9 m, 4.3 m, 4.6 m, 6.1 m, and 7.6 m. Also shown is the walkway by which the site is accessed.

Detailed schematics and design of the MUS injection and monitoring wells are outlined in (Watson Jr., 2000). The nomenclature used for well identification is based on the positioning of the monitoring wells as one moves radially outwards from the injection well, and the depth to which the well was sunk. Thirty-eight monitoring wells at depths of 2.7 m, 3.9 m, 4.3 m, 4.6 m, 6.1 m, and 7.6 m were installed on the east side to provide a means of monitoring the wastewater plume as it is intermittently injected into the subsurface. The innermost ring of wells is located at a horizontal distance of approximately 0.9 m from the injection well, increasing successively to
monitoring wells positioned radially at 1.5 m, 3.0 m, and 4.6 m. The outermost ring of monitoring wells installed is positioned radially at a horizontal distance of approximately 6.1 m from the injection well. This distance was selected in response to expected lateral dispersion of the injected wastewater over time.

The camps tied into the cluster camp system are used intermittently throughout the year, with the summer months being the period of most usage. Each of the four camps is tied into a 114-liter lift station, which pumps into a primary collection/distribution tank from where it is injected down the injection wells through the same process as for the single camp system (Figure 3.3). The system is located on the west side of the bayou and consists of two injection wells positioned at the same depth (6.3 m) (Figure 3.2). Both injection wells are open for wastewater injection during an injection cycle. The use of two separate injection wells was to accommodate the high wastewater generation potential expected from the cluster of four camps. Excessive pressure build-up in any injection well during active wastewater injection will cause wastewater to flow into the injection well with a lower pressure, allowing for a gradual dissipation. Surrounding the injection wells are rings of monitoring wells placed at varying depths and horizontal distances from the injection well (Figure 3.5).

3.2.3 Soil Matrix Characterization

Soil characteristics such as high porosity and permeability can result in the rapid migration of organic/inorganic contaminants and microbial organisms to the saturated zone (USEPA, 1999). The Bayou Segnette soils are identified as black muck organic soils that flow easily between the fingers when squeezed, leaving a small residue in the hand. They form a part of the Kenner series, which are commonly near Allemandes, Barbary, and Larose soils. The Kenner series consists of level, very poorly drained and poorly drained, rapidly permeable and very rapidly permeable organic soils. Soils of the Kenner series are ponded or flooded for the most part of the year, unless drained (USDA, 1983).
Figure 3.5. The cluster camp system of the MUS consists of primary and secondary injection wells surrounded by thirty-two monitoring wells at depths of 0.6 m, 1.5 m, 2.7 m, 3.1 m, and 3.9 m. Also shown is the walkway by which the site is accessed.

Soil characterization for hand-augered composite soil borings for the Bayou Segnette study sites were performed in conformance to the standard protocol (ASTM, 1995). Sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422) were performed to identify the relative percentages of sandy, silty, and clayey soils. The fraction of organic content in soil borings was also determined (ASTM D2974). The subsurface stratum at both sites is a flotation marsh consisting of plants and organic matter up to a depth of approximately 0.6 m, underlain by a dark, humic, unconsolidated material. This material floats on top of a compact stratum that extends from an approximate depth of 3.0 m (Table 3.1). Higher clay contents were recorded for...
the compact material. Generally, soils at the cluster camp system have higher clay content even though percent sand content is lower than for the single camp system.

Table 3.1 - Selected soil properties at various depths for the Bayou Segnette site, LA.

<table>
<thead>
<tr>
<th>Property</th>
<th>Units</th>
<th>Depth Interval (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.6 - 2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7 - 4.6</td>
</tr>
<tr>
<td>Sand content</td>
<td>%</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>78.9</td>
</tr>
<tr>
<td>Silt</td>
<td>%</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>14.8</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.3</td>
</tr>
<tr>
<td>Median grain size diameter (d&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>Mm</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
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<tr>
<td>Uniformity Coefficient (d&lt;sub&gt;60&lt;/sub&gt;/d&lt;sub&gt;10&lt;/sub&gt;)</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Fraction of organic content (f&lt;sub&gt;oc&lt;/sub&gt;)</td>
<td>%</td>
<td>35.7±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.6±2.5</td>
</tr>
</tbody>
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**Cluster Camp System**

<table>
<thead>
<tr>
<th>Property</th>
<th>Units</th>
<th>Depth Interval (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>0.6 - 2.7</td>
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<td></td>
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<td></td>
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<tr>
<td>Silt</td>
<td>%</td>
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<td>10.9</td>
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<tr>
<td>Clay</td>
<td>%</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Median grain size diameter (d&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>Mm</td>
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<td></td>
<td></td>
<td>0.25</td>
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<tr>
<td>Uniformity Coefficient (d&lt;sub&gt;60&lt;/sub&gt;/d&lt;sub&gt;10&lt;/sub&gt;)</td>
<td></td>
<td>54</td>
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<td></td>
<td></td>
<td>170</td>
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<tr>
<td>Fraction of organic content (f&lt;sub&gt;oc&lt;/sub&gt;)</td>
<td>%</td>
<td>25.5±3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.8±1.1</td>
</tr>
</tbody>
</table>

Note: 0 – 0.6 m = ‘plant root layer’

3.2.4 System Evaluation

Prior to system initiation, background samples were collected from sixteen monitoring wells (4.6, 6.1, and 7.6 m depths) installed at the single camp system and from a surface water body in close vicinity to the site. The surface water samples were grabbed from a common sampling point located in the middle of the bayou (approximately 20 m away from the single camp dockside). These samples were collected at approximately 0.3 m below the water surface.

In addition, a sample was collected from the Bayou Segnette boat launch. All samples were analyzed for *insitu* water quality parameters such as pH, salinity, and temperature. Additionally, all surface water samples were analyzed for dissolved oxygen concentration. The samples collected were immediately preserved on ice, and transported to the LSU water quality laboratory, where they were analyzed for CBOD₅, nutrients, fecal coliforms and *E. coli*.  

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60
The single camp was operated for 1.25 years (December 2002 to March 2004), over which a total of three studies were performed. The first study; a high flow/low temperature study (HFLT), defined by high flowrates and under winter conditions (where microbial activity was at a minimum) was performed to determine an upper limit design flowrate for which effective bacterial retention was achieved in an unacclimated soil matrix. Flowrates of 0.95 L/min, and 1.9 L/min were also evaluated at an injection frequency of 15 minutes every hour in two separate studies. Settled, raw wastewater was injected via the secondary injection well (6.1 m) during the HFLT study. However, the 0.95 and 1.9 L/min studies used a 4.3 m injection well for wastewater injection.

A biweekly sampling scheme was set up at the single camp system to monitor the wastewater plume developed after system operation had commenced. Select monitoring wells were sampled on each sampling event and analyzed for fecal coliforms and *E. coli* in accordance with standard methods (9222D, 9221D and 9221F). Analyses of fecal coliform and *E. coli* concentrations were done by the following methods: the membrane filtration technique [mFC (Standard Methods 9222D) and mTEC *E. coli* enumeration method (USEPA, 1985)], and the most probable number (MPN) method - (Procedures 9221D and 9221F) (APHA, 1998). These different analytical procedures correspond to two distinct scenarios under which the system was evaluated. The change in analytical procedure from mFC to MPN was necessitated by the inconsistencies in fecal coliform data, depicted as ‘false positives’. Broth (media) preparation for the MPN method followed Standard Methods (9221B and 9221E). A sample was grabbed from the primary collection/distribution tank (influent) on every sampling event and analyzed for the following parameters: solids (total suspended solids 2540-D and volatile suspended solids 2540-E), dissolved oxygen, CBOD₅ (5210-B), nutrients (orthophosphate (4500-P E), total phosphorus (4500 P), ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, and total Kjeldhal nitrogen), fecal coliforms and *E. coli*. Both filtered and unfiltered portions of the influent samples were used for
CBOD₃, TAN, TKN and TP analyses. Redox measurements were taken from two monitoring wells placed at 2.7 m and 4.6 m depths. All redox measurements were made in triplicate.

The frequency of sampling for the cluster camp system was lower than that for the single camp system because of limitations in wastewater generation, as well as problems of dilution of the primary collection/distribution tank. Flooding of the marsh area as a result of excessive amounts of rainfall resulted in infiltration of the surface water into the lift stations from where the water was pumped into the primary tank. This resulted in significant dilution of the wastewater, and the system was left for a brief period (two weeks) to reacclimatize. Investigation of the reason for infiltration revealed defects in construction of the lift stations to be responsible for such occurrences. This was subsequently fixed and the system returned to normal acclimated conditions with increased microbial activity. Consequently, there were periods of no active wastewater injection. The method of sample collection, preservation and analysis were however the same as outlined for the single camp system.

Specific monitoring well depths monitored for the cluster camp system were 0.6 m, 1.5 m, 2.7 m, 3.1 m, and 3.9 m. The system follows a similar placement of wells from the injection wells as for the single camp. System operation started in December 2002 and run for a total of 9 months. Two separate studies were run: 1) a HFLT study defined under the same conditions as for the single camp system, and 2) a 1.9 L/min study at an injection frequency of 15 minutes every hour.

All but the fecal coliform and *E. coli* data are presented by arithmetic means ± standard error averaged over the number of sampling events (n) recorded for a particular study. The bacteria data are presented with respect to well depth and vector distance from the point of injection using the geometric mean ± standard error. Background data for fecal coliforms and *E. coli* are presented using geometric mean ± standard deviation. All other parameters are presented by arithmetic mean ± standard deviation. A detection limit of 2 coliform units was used in instances where fecal coliform concentrations were zero. Hydraulic loading rates into the system
were computed for the respective studies using the cumulative volume of wastewater injected over specific time periods. The computed loading rates were then used in combination with bacterial concentrations in the system influent to estimate bacterial loading rates. Similar computations were made for solids loading into the system.

Surface water samples, representing background conditions for the system, were compared with fecal coliform concentrations in the shallowest monitoring wells via t-tests. Regression analyses were performed on removal rate coefficients to determine if any significant difference existed among the different flowrates evaluated (SAS version 8.0). All statistical evaluations were performed at $a = 0.05$. The data were fit to a probability density function used to calculate the probability of exceeding discharge limits. Microsoft Excel 2000 was used to analyze fecal coliform removal with respect to vector distance of travel from the injection point. The resulting equations were used to compute distances required for reduction of influent concentrations to meet NSSP standards. Contour plots of bacterial distribution with depth of monitoring well were developed and superimposed on salinity contours using Sigma Plot 8.0.

3.3 Results and Discussion

The different injection schemes employed were used to develop theoretical loading rates based on the assumption that adequate wastewater was available in the primary collection/distribution tank on each injection cycle (Table 3.2). Also reported is the time frame over which the respective injection schemes were employed.

Results of fecal coliform analyses are reported in two separate units of measurement: colonies/100 mL as applied to the membrane filtration method (mFC) and MPN/100 mL as determined by the multiple tube fermentation technique (most probable number method). The membrane filtration method, which was used for bacterial analyses during the HFLT study, often resulted in $E. coli$ counts exceeding fecal coliform counts (Figure 3.6).
Table 3.2 Estimates for the theoretical loading rates and the injection flowrate and frequency employed for both systems over the entire course of study

<table>
<thead>
<tr>
<th>System Evaluated</th>
<th>Study Period (mm/dd/yy)</th>
<th>Elapsed Time (days)</th>
<th>Injection Flowrate (L/min)</th>
<th>Injection Frequency</th>
<th>Theoretical Hydraulic Loading Rate (L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCS</td>
<td>12/01/02 - 03/10/03</td>
<td>90</td>
<td>HFLT</td>
<td>15 min every hour</td>
<td>1980°</td>
</tr>
<tr>
<td></td>
<td>03/10/03 - 11/03/03</td>
<td>238</td>
<td>0.95</td>
<td>15 min every hour</td>
<td>348</td>
</tr>
<tr>
<td></td>
<td>11/03/03 - 03/22/04</td>
<td>140</td>
<td>1.9</td>
<td>15 min every hour</td>
<td>680</td>
</tr>
<tr>
<td>CCS</td>
<td>12/10/02 – 03/10/03</td>
<td>81</td>
<td>HFLT</td>
<td>15 min every hour</td>
<td>1980°</td>
</tr>
<tr>
<td></td>
<td>03/10/03 – 11/17/03</td>
<td>378</td>
<td>1.9</td>
<td>15 min every hour</td>
<td>680</td>
</tr>
</tbody>
</table>

HFLT = High flow low temperature study, typified by excessively high flowrates; ° Assumes adequate volume of wastewater in primary distribution tank to trigger injection

An approximate flowrate of 5.5L/min was used in computations

Figure 3.6 Incidence of *Escherichia coli* counts significantly exceeding fecal coliform counts for samples run over an 8-week period.

The graph compares the bacterial measurements recorded for four sampling events (over an 8-week period) where *E. coli* concentrations were orders of magnitude higher. This led to an investigation of the possible causes, and subsequently, a switch in methods to the most probable number method (MPN). The switch in methods eliminated the occurrence of ‘false positives’.
Similar reports of fecal coliform false positives were reported for studies performed on the distribution of fecal coliforms in small tidal creeks (Esham and Sizemore, 1998). Counts obtained by the mTEC method were consistently higher than mFC counts for all salinity concentrations (0, 10-14, and 23-26 ppt) evaluated. The limitations associated with \textit{E. coli} detectability has been attributed to the existence of other species with similar characteristics (USFDA, 2002). \textit{Citrobacter, Klebsiella, and enterobacter} are similar to \textit{E. coli} in phenotypic characteristics, and may not be easily distinguished.

Alonso et al. (1998) investigated the problems related to the recovery of \textit{E. coli} and attributed some inconsistencies in bacterial concentrations to the incubation temperature. A study on the fecal coliform population in Louisiana oysters showed a seasonal variation in fecal coliform population (specifically related to ambient temperature variations) (Paille, et al., 1987). Baudisova (1997) found significantly high elevations in \textit{E. coli} counts in the summer months, and observed that elevated temperatures during the summer months helped in the development of non-fecal bacteria. Jin et al. (2004) observed a weak correlation between fecal coliform and \textit{E. coli} counts in sediment and surface water samples under brackish water conditions.

3.3.1 Single Camp System

3.3.1.1 Evaluation of Background Conditions

Results of background analyses performed for surface and subsurface water samples collected for the single camp system are presented in Table 3.3a. The subsurface water quality parameters reported here are for monitoring wells for which analyses were done prior to system initiation, and the surface water quality represents conditions by the single camp system.

Mean temperatures for the surface water were computed for the entire study period (December, 2002-March, 2004), while those for the subsurface samples were averaged over the period September-October, 2002. The high levels recorded for the subsurface water quality conditions are indicative of the indiscriminate wastewater disposal practices of some camp dwellers that commonly discharge wastewater into the marsh. Samples from the Bayou Segnette
boat launch generally contained higher fecal coliform concentrations than background surface water conditions (Table 3.3b). Approximately 84% of the boat launch samples exceeded the 14MPN/100mL NSSP standard. Background surface water samples exceeded this standard 17% of the time.

Table 3.3a Background data measured for the single camp marshland upwelling system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>N</th>
<th>Surface water (Mean ± SD)</th>
<th>(Mean ± S.D) Subsurface water&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6 m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.1 m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.6 m</td>
</tr>
<tr>
<td>Temp</td>
<td>ºC</td>
<td>36</td>
<td>23 ± 8.0</td>
<td>21 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21± 1.0 (n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21± 2.0 (n=5)</td>
</tr>
<tr>
<td>pH</td>
<td>S.U.</td>
<td>35</td>
<td>7.2 ± 0.52</td>
<td>6.6 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.3 (n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.7 (n=5)</td>
</tr>
<tr>
<td>DO</td>
<td>mg/L</td>
<td>33</td>
<td>7.6 ± 2.2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Salinity</td>
<td>ppt</td>
<td>31</td>
<td>1.2 ± 1.5</td>
<td>7.3±2.0 (n=16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.6±2.0 (n=14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12± 2.0 (n=10)</td>
</tr>
<tr>
<td>TKN&lt;sup&gt;f&lt;/sup&gt;</td>
<td>mg/L-N</td>
<td>22</td>
<td>3.1 ± 2.5</td>
<td>32±3.1 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43±29 (n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67±28 (n=5)</td>
</tr>
<tr>
<td>TAN&lt;sup&gt;f&lt;/sup&gt;</td>
<td>mg/L-N</td>
<td>22</td>
<td>0.062 ± 0.18</td>
<td>6.9±0.55 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14±2.9 (n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19± 1.1 (n=5)</td>
</tr>
<tr>
<td>Nitrite&lt;sup&gt;f&lt;/sup&gt;</td>
<td>mg/L-N</td>
<td>22</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Nitrate</td>
<td>mg/L-N</td>
<td>22</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Fecal Coliform&lt;sup&gt;a&lt;/sup&gt;</td>
<td>col./100 mL</td>
<td>5</td>
<td>44 ± 117</td>
<td>2.3± 0.7 (n=16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 ± 24 (n=12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42 ± 124 (n=10)</td>
</tr>
<tr>
<td>E. coli&lt;sup&gt;a&lt;/sup&gt;</td>
<td>col./100 mL</td>
<td>5</td>
<td>68 ± 164</td>
<td>4.9± 7.0 (n=16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14 ± 27 (n=12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42 ± 124 (n=10)</td>
</tr>
<tr>
<td>Fecal Coliform&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MPN/100 mL</td>
<td>24</td>
<td>39 ± 159</td>
<td>NA</td>
</tr>
<tr>
<td>E. coli&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MPN/100 mL</td>
<td>24</td>
<td>17 ± 40</td>
<td>NA</td>
</tr>
<tr>
<td>CBOD&lt;sub&gt;F&lt;sup&gt;f&lt;/sub&gt;&lt;/sub&gt;</td>
<td>mg/L</td>
<td>24</td>
<td>1.0 ± 0.14</td>
<td>4.3± 0.32 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3± 0.13 (n=12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.1± 0.77 (n=8)</td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td>mg/L-P</td>
<td>34</td>
<td>0.18 ± 0.53</td>
<td>0.114 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.073 (n=7)</td>
</tr>
<tr>
<td>Ortho-Phosphate&lt;sup&gt;f&lt;/sup&gt;</td>
<td>mg/L-P</td>
<td>31</td>
<td>0.059 ± 0.047</td>
<td>0.005 (n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003 (n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.029 (n=5)</td>
</tr>
</tbody>
</table>

<sup>*NA</sup> = Not analyzed; BDL – Below Detection Limit (< 0.05 mg/L – N for nitrate, and <0.02 mg/L-N for nitrite); <sup>a</sup> (geometric means ± SD); <sup>b</sup> = water quality parameters representative of conditions at single camp system, values reported as mean± SD; <sup>f</sup> = reported data is for analysis performed on filtered samples, TAN – total ammonia nitrogen, TKN-total kjeldhal nitrogen.

Table 3.3b Geometric mean (±SE) for fecal coliforms and <i>E. coli</i> measured for the boat launch surface water samples, Bayou Segnette, LA

<table>
<thead>
<tr>
<th></th>
<th>Fecal coliforms</th>
<th>E. coli (MPN/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>50 ± 21 (colonies/100mL)</td>
</tr>
<tr>
<td>Boat Launch</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>96 ± 527 (MPN/100mL)</td>
</tr>
</tbody>
</table>

<sup>*NA</sup> = Not analyzed
3.3.1.2 Fecal Coliform Removal

Fecal coliform removal rates were first evaluated for the entire study period followed by an evaluation of the respective flow regimes. A total of 130,987 L of settled, raw wastewater was injected into the marsh subsurface over the entire course of study (Figure 3.7). The cumulative volume of wastewater injected yielded approximately 20 and 9 pore volume exchanges respectively through the 4.6 m monitoring wells positioned at 0.9 m and 1.5 m radial distances from the injection point. Computations for the number of pore volumes flushed through the system were made based on laboratory-estimated porosity of the field media (soil) and the volume of wastewater injected over different time scales (Figure 3.8).

A porosity of 0.509 determined for soil borings collected from 2.7 – 4.6 m was used. This porosity was assumed to be applicable over the depth of the soil profile. The upward transport of wastewater due to buoyancy forces was assumed to be within a vertical cylinder that extends to the depth of the monitoring well. The radius of this cylinder is represented by the radial distance

Figure 3.7 Cumulative volume of wastewater injected over the course of the single camp MUS study. The different studies undertaken are as delineated.

\[ y = 244.63x \]

\[ R^2 = 0.9633 \]
from the injection point to the monitoring well under evaluation. The number of pore volumes flushed for a monitoring well depth is dependent on the radial distance from the injection point. Thus for the same monitoring well depth, different pore volumes are flushed at different radial distances from the injection point.

![Graph showing number of pore volumes flushed over time](image)

Figure 3.8 Close to 40 pore volumes of subsurface media were flushed at 2.7 m depth within 0.9 m radius of the injection well. Lower pore volumes were flushed at distances further outward from the injection point.

The derived equations can be used to predict the number of pore volumes flushed at different times if the same flowrates were employed for a MUS with similar soil characteristics. For instance, after an elapsed time of 500 days (representing the complete period of system evaluation), approximately 33 pore volumes are flushed for 2.7 m wells positioned at a 0.9 m radial distance.

Influent wastewater parameters characterized for the entire study period are comparable with typical concentrations encountered in high strength wastewater (Metcalf and Eddy, 2003) (Table 3.4). Geometric mean influent fecal coliform and *E. coli* concentrations were found to be 92,510±489,614 (n=18) and 52,067±208,976 MPN/100mL (n=18), respectively, for the entire
study exclusive of the HFLT study. The HFLT study is reported separately because of difference in the units of measurement.

Table 3.4 The influent wastewater parameters measured for the single camp system are compared with typical values encountered in medium to high strength wastewater

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Results (Mean ± S.E)</th>
<th>Typical Wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Filtered</td>
<td>Unfiltered</td>
</tr>
<tr>
<td>Temp(a)</td>
<td>°C</td>
<td>NA</td>
<td>24 ± 6.53 (n=23)</td>
</tr>
<tr>
<td>pH(b)</td>
<td>S.U.</td>
<td>NA</td>
<td>7.32 ± 1.33 (n=22)</td>
</tr>
<tr>
<td>DO(b)</td>
<td>mg/L</td>
<td>NA</td>
<td>1.78 ± 1.59 (n=19)</td>
</tr>
<tr>
<td>TKN(a)</td>
<td>mg/L-N</td>
<td>101 ± 24.1(n=22)</td>
<td>106 ± 27 (n=20)</td>
</tr>
<tr>
<td>TAN(a)</td>
<td>mg/L-N</td>
<td>89 ± 23.7 (n=22)</td>
<td>84± 33 (n=20)</td>
</tr>
<tr>
<td>Nitrite(a)</td>
<td>mg/L-N</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Nitrate(a)</td>
<td>mg/L-N</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>FC(b)</td>
<td>col/100 mL</td>
<td>NA</td>
<td>277,330 ± 490,901 (n=5)</td>
</tr>
<tr>
<td>EC(b)</td>
<td>col/100 mL</td>
<td>NA</td>
<td>333,074 ± 594,460 (n=5)</td>
</tr>
<tr>
<td>FC(b)</td>
<td>MPN/100 mL</td>
<td>NA</td>
<td>92,510 ± 489,614 (n=18)</td>
</tr>
<tr>
<td>EC(b)</td>
<td>MPN/100 mL</td>
<td>NA</td>
<td>52,067 ± 208,976 (n=18)</td>
</tr>
<tr>
<td>CBOD(5)</td>
<td>mg/L</td>
<td>214 ± 13.7 (n=25)</td>
<td>--</td>
</tr>
<tr>
<td>TSS(a)</td>
<td>mg/L</td>
<td>NA</td>
<td>184 ± 40 (n=18)</td>
</tr>
<tr>
<td>VSS(a)</td>
<td>mg/L</td>
<td>NA</td>
<td>129 ± 24 (n=18)</td>
</tr>
<tr>
<td>TP(a)</td>
<td>mg/L-P</td>
<td>12.0 ± 3.42 (n=18)</td>
<td>13.8 ± 3.39 (n=16)</td>
</tr>
<tr>
<td>OP(a)</td>
<td>mg/L-P</td>
<td>11.1 ± 2.80 (n=23)</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(a\)(Mean ± SD); \(b\)(Geometric mean ± SD); \(c\)Metcalf & Eddy, 2003, NA = Not analyzed; BDL– Below Detection Limit (< 0.05 mg/L – N for nitrate, and <0.02 mg/L for nitrite); TAN – total ammonia nitrogen, TKN(total kjeldhal nitrogen.

Variations in the influent concentrations for the HFLT study are even more pronounced [277,330±490,901 (n=5); 333,074±594,460 (n=5) colonies/100mL for fecal coliforms and \(E. coli\) respectively]. While this variability in influent concentrations may be because of the frequency of camp usage and the sources contributing to the waste USEPA (2002), the single camp was permanently occupied over the entire study period by the same people. It is therefore likely that this variation resulted from the different analytical methods used.

The three separate injection schemes (HFLT, 0.95L/min, and 1.9 L/min) were analyzed for the extent of bacterial retention/removal with respect to monitoring well depth and vector distance from the point of injection. A total of 260 samples were retrieved from different monitoring wells and analyzed for presence of fecal coliforms. 180 samples (62 %) out of this
number returned positive for fecal coliforms, with *E. coli* constituting 77.2% (139 positives) of this number. Greater than 90% of bacterial concentrations recorded for the respective studies were less than the corresponding mean influent values (Table 3.5).

Table 3.5 Geometric mean (±SE) bacterial distributions for specific flowrates presented by depth of monitoring wells installed at the single camp system

<table>
<thead>
<tr>
<th>Sampling Point</th>
<th>Fecal coliform concentration (MPN/100mL)</th>
<th>E. coli concentration (MPN/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HFLT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95 L/min</td>
</tr>
<tr>
<td>Influent&lt;sup&gt;b&lt;/sup&gt;</td>
<td>277,330±490,901</td>
<td>71,408±423,625</td>
</tr>
<tr>
<td>2.7 m</td>
<td>NA</td>
<td>4±2</td>
</tr>
<tr>
<td>3.9 m</td>
<td>NA</td>
<td>3±0.9</td>
</tr>
<tr>
<td>4.3 m</td>
<td>NA</td>
<td>21±159</td>
</tr>
<tr>
<td>4.6 m</td>
<td>245±91</td>
<td>311±724</td>
</tr>
<tr>
<td>6.1 m</td>
<td>3±1</td>
<td>6±23</td>
</tr>
<tr>
<td>7.6 m</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Surface water</td>
<td>44±117</td>
<td>45±166</td>
</tr>
<tr>
<td>Boat Launch</td>
<td>50±21</td>
<td>96±527</td>
</tr>
</tbody>
</table>

<sup>a</sup>Fecal coliform/*E. coli* concentrations presented in units of colonies/100mL; <sup>b</sup>(geometric mean±SD); NA – Not analyzed.

Using the shallowest monitoring wells (2.7 m depth) as the system effluent, statistical tests were performed to determine if effluent bacterial concentrations exceeded background conditions. The background data used for this comparison were for the surface water body in close vicinity to the site (Table 3.3a). This water body was monitored over the entire period of system operation. Effluent fecal coliform values were significantly less (p<0.0001) than background concentrations.

Bacterial concentrations in the single camp system influent for the respective studies represent wastewater of medium to high strength (Table 3.6). Effluent to influent ratios were computed using geometric mean bacterial concentrations from the system influent and the shallowest monitoring well as recorded for specific flowrates. Overall, very low effluent fecal coliform concentrations (4.0±7.6 presented as geometric mean±standard error) were recorded, representing greater than 99% reduction of mean influent concentrations. Ran et al., (2004)
demonstrated a 95% removal of fecal coliforms for settled, domestic primary effluent applied to a constructed wetland. An effluent to influent ratio of $9.1 \times 10^2$ was observed in the same study. Decamp and Warren (2000) achieved 96.6 – 98.9% *E. coli* removals in pilot-scale subsurface flow systems. Williams et al. (1995) used decimal reduction distance (distance required for a ten-fold reduction of mean influent concentrations) to predict removal rates. Similarly, Decamp and Warren (2000), used DRD to predict *E. coli* removal kinetics based on an exponential decrease in concentrations with horizontal distance along a subsurface flow wetland. The effluent to influent fecal coliform concentration ratios predicted by Williams et al. (1995) ranged $7.4 \times 10^2$-1100 x $10^5$. Higher ratios in the range 2600 x$10^5$- 8130 x $10^5$ were obtained by Gerba et al. (1999) in evaluations performed on subsurface wetlands. The ratios recorded for the MUS (2 x $10^5$- 88 x $10^5$) proved the system’s effectiveness in providing an effluent of acceptable bacterial quality.

Table 3.6 Effluent to Influent ratios and variability in bacterial concentrations recorded for specific flowrates

<table>
<thead>
<tr>
<th>Injection Flowrate</th>
<th>Range of Influent Bacterial Concentrations (x10^3)</th>
<th>Effluent to Influent ratios (x10^-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fecal coliform (MPN/100mL)</td>
<td><em>E. coli</em> (MPN/100mL)</td>
</tr>
<tr>
<td>HFLT$^a$</td>
<td>32 – 1,116</td>
<td>50 – 1,366</td>
</tr>
<tr>
<td>0.9</td>
<td>5.4 – 1,600</td>
<td>2.6 – 920</td>
</tr>
<tr>
<td>1.9</td>
<td>49 - 1,600</td>
<td>17 – 160</td>
</tr>
<tr>
<td>1.9$^b$</td>
<td>14 - 9,600</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$ Effluent values reported for 4.6 m wells; $^b$ Richardson, 2002; NA – Not analyzed; $^{ab}$ unit of measurement is colonies/100mL.

Presenting fecal coliform concentrations with respect to vector distance from the injection point enables a complete evaluation of the degree of bacterial removal. Vector distance (V.D.) is defined as the most direct path of travel from the base of the injection well to a specific monitoring well. First-order fecal coliform removal curves were fitted for specific injection flowrates and frequencies. The removal rate curves give a relation that is useful in the determination of the vector distance required to reduce influent concentrations to below the 14 MPN/100 mL NSSP standard. Overall, fecal coliform and *E. coli* concentrations decreased with respect to vector distance from the injection well (Figures 3.9 and 3.10). Similar linear
regressions relating the number of coliform bacteria counts to the distance along a gravel bed were developed by Williams et al. (1995).

Figure 3.9 – First-order fecal coliform removal with respect to vector distance from the 6.1 m injection well for the a) HFLT study, and from the 4.3 m injection well for the b) 0.95 L/min and c) 1.9 L/min studies respectively. Each data point represents geometric mean ± standard error with respect to vector distance for the respective flowrates evaluated.
Figure 3.10 – First order E. coli removal with respect to vector distance from the 6.1 m injection well for the a) HFLT study and from the 4.3 m injection well for the b) 0.95 L/min and c) 1.9 L/min respectively. Each data point represents geometric mean±standard error with respect to vector distance for the respective flowrates evaluated.
Assuming that the injected wastewater moved back up to the marsh surface along the shortest possible distance of travel (along the injection well stem), a theoretical distance of 6.1 m representing the depth of wastewater injection would be applicable. The HFLT study required 2.4 vector meters of wastewater travel to meet effluent standards. This distance provides a factor of safety for evaluating the system under the design configurations employed. The system proved effective in removing fecal pathogens irrespective of the high loading rates.

The vector distances required for effective bacterial removal for the 0.95 and 1.9 L/min studies were determined to be 2.7 m and 4.7 m respectively. It is evident that a better removal rate was obtained for the HFLT study. However, because the system channelized after a brief period of operation, the HFLT study did not perform efficiently in the long term. The observed removal rates were due to the depth of injection well used (6.1 m for HFLT compared to 4.3 m for all other studies) and the pore sizes at that depth. Bacterial removal by filtration, physical straining and possibly adsorption is enhanced by fine textured soils. Generally, fecal coliform removal in subsurface flow wetlands is dependent on a complex combination of factors (Jillson et al., 2000).

For long-term operations, the 0.95 L/min study was the best operational criteria to meet discharge standards. Physical straining or filtration, and adsorption have been identified as primary mechanisms controlling transport of bacteria through soils (Bitton and Harvey, 1992; Scholl et al., 1990; Corapcioglu and Haridas, 1984; Gerba and Bitton, 1984; Gerba, 1975). Studies on subsurface flow wetlands consisting of gravel bed hydroponics identified adsorption as a possible mechanism of fecal coliform removal (Williams et al., 1995). In the same study, a strong correlation existed between fecal coliform and BOD concentrations, with removal rates associated with fluctuations in temperature and loading rates.

The innermost ring of monitoring wells (1.0 m <V.D. <1.8 m) returned the highest fecal coliform concentrations, with concentrations decreasing further outward. The reduced microbial concentrations further outward from the injection point were expected since increasing horizontal distance from a subsurface distribution/injection point favors higher microbial reductions.
Geometric mean fecal coliform concentrations for these wells ranged from 196 to 594 MPN/100mL representing a 4-log removal of mean influent concentrations. Removals of about 1.5 to 2.1 log were achieved for *E. coli* in subsurface flow wetlands evaluated in dry weather conditions (Green et al., 1997). Lower removal rates were recorded for wet weather flows.

Isolated instances of fecal coliform detections in the outermost ring of 2.7 m wells (V.D.>6.1 m) had concentrations in semblance to the detection limit imposed on the system (2 MPN/100mL), and never exceeded the NSSP standard (Table 3.7). These detections may result from increased lateral transport at the interface between the floating marsh soils and the compact strata underneath. Additionally, the approximate location of the base of the monitoring wells (2.7 m) is at this interface, making such transport paths a possibility. No downward transport of the injected wastewater was observed since only 1 out of 12 monitoring wells located at 6.1 and 7.6 m depths (3.4-3.7 m V.D.) recorded fecal coliform concentrations exceeding the discharge standard.

Table 3.7. Geometric mean (±SE) bacterial concentrations recorded for specific flowrates and presented with respect to vector distance from the single camp system injection well

<table>
<thead>
<tr>
<th>Vector distance (m)</th>
<th>Fecal coliform concentration (MPN/100mL)</th>
<th>E. coli concentration (MPN/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HFLT&lt;sup&gt;b&lt;/sup&gt; 0.95 L/min 1.9 L/min</td>
<td>HFLT&lt;sup&gt;b&lt;/sup&gt; 0.9 L/min 1.9 L/min</td>
</tr>
<tr>
<td>1.0</td>
<td>NA 196 ± 1575 1940 ± 2586 NA</td>
<td>141 ± 1526 1040 ± 591</td>
</tr>
<tr>
<td>1.6</td>
<td>3 ± 0.6 515 ± 1103 5072 ± 3316 10 ± 24</td>
<td>280 ± 449 1789 ± 1898</td>
</tr>
<tr>
<td>1.8</td>
<td>2402 ± 2349 8 ± 5 5 ± 0.5 2326 ± 8355</td>
<td>7 ± 3 4 ± 0.4</td>
</tr>
<tr>
<td>2.2</td>
<td>24 ± 13 NA NA NA</td>
<td>NA NA</td>
</tr>
<tr>
<td>2.4</td>
<td>NA 2 ± 0.5 NA NA</td>
<td>NA NA</td>
</tr>
<tr>
<td>3.0</td>
<td>6 ± 7 2 40 ± 8 5 ± 5 2 ± 1</td>
<td>20 ± 7 2</td>
</tr>
<tr>
<td>3.6</td>
<td>NA 11 ± 28 2866 ± 4917 NA</td>
<td>10 ± 23 1660 ± 2825</td>
</tr>
<tr>
<td>4.6</td>
<td>NA 3 ± 1 NA 2 NA</td>
<td>2 2</td>
</tr>
<tr>
<td>6.3</td>
<td>NA 3 ± 0.8 NA 2 ± 0.2 NA 3 ± 0.4 2 ± 0.2</td>
<td>NA 3 ± 0.4 2 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fecal coliform/*E. coli* concentrations presented in units of colonies/100mL; <sup>b</sup> Vector distance measured from 6.1 m injection well; NA – Not applicable

The only 6.1 m monitoring well that showed positive fecal coliform results (IE-6.1, V.D. =3.6 m) was located alongside the 4.0 m monitoring well (IE-4.3, V.D. = 3 m) exhibiting
significant fecal coliform concentrations. One reason for this observation may be due to the existence of preferential flow paths leading to IE-4.3, which caused wastewater to be transported at rapid rates. Once at this well, movement along the well stem may enhance downward transport further into the 6.1 m well. The monitoring wells placed further outward from the point of injection (V.D. > 3.6 m) did not show concentrations of fecal coliform exceeding the standards for shellfishing. Only 2 out of 39 samples recorded values greater than 14 MPN/100mL. Limited lateral expansion of the wastewater plume over time due to the gradual accumulation of solids enhances filtration rates in the system, making the detection of fecal coliform in such monitoring wells unlikely.

3.3.1.3 Performance Evaluation

The different flowrates evaluated over the course of the study provided a means of assessing the system’s performance for removal of fecal pathogens. *Insitu* measurements, which are generally indicative of wastewater plume movements within the subsurface, demonstrated different trends in movement with depth. Mean *insitu* parameters (salinity, pH, and temperature) were determined by averaging over the number of sampling events within specific flowrates evaluated (Tables 3.8a-c). Average temperatures recorded for the 0.95 and 1.9 L/min studies differed significantly, with relatively high values recorded for the 0.95 L/min study (p < 0.0001). This study also provided a higher bacterial removal rate, indicating that survival rates may be impacted by temperature. However, Jillson et al. (2000) revealed no clear relationship between temperature changes and fecal coliform removal efficiency in a subsurface flow wetland, even though differing removal rates were achieved for the summer and winter months.

Salinities ranged from 3.0 ppt in 2.7 m wells to 12 ppt in 7.6 m wells, suggesting a general increase in salinities with depth. Contradictory results were obtained by Fontenot (2003) in studies performed on a laboratory-scale MUS installation. Richardson (2002) also observed a decrease in salinity concentrations with increased well depth in a MUS installed in Scatlake soils.
### Table 3.8a – Temperature measurements recorded at varying well depths and specific flowrates

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Arithmetic Mean Temperatures for specific flowrates (°C)</th>
<th>HFLT</th>
<th>0.95 L/min</th>
<th>1.9 L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>18 ± 4.4 (n=5)</td>
<td>28 ± 4.8 (n=12)</td>
<td>19 ± 4.7 (n=6)</td>
<td></td>
</tr>
<tr>
<td>7.6 m</td>
<td>20 ± 0.5 (n=2)</td>
<td>22 ± 0.8 (n=5)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>6.1 m</td>
<td>20 ± 0.5 (n=6)</td>
<td>22 ± 0.4 (n=9)</td>
<td>19 (n=1)</td>
<td></td>
</tr>
<tr>
<td>4.6 m</td>
<td>19 ± 0.4 (n=9)</td>
<td>23 ± 0.5 (n=13)</td>
<td>18 ± 0.2 (n=5)</td>
<td></td>
</tr>
<tr>
<td>4.3 m</td>
<td>NA</td>
<td>24 (n=1)</td>
<td>19 ± 0.3 (n=5)</td>
<td></td>
</tr>
<tr>
<td>3.9 m</td>
<td>NA</td>
<td>23 ± 0.2 (n=10)</td>
<td>20 ± 0.3 (n=5)</td>
<td></td>
</tr>
<tr>
<td>2.7 m</td>
<td>NA</td>
<td>23 ± 0.3 (n=9)</td>
<td>18 ± 0.5 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>15 ± 2.7 (n=5)</td>
<td>29 ± 4.9 (n=12)</td>
<td>16 ± 4.0 (n=6)</td>
<td></td>
</tr>
</tbody>
</table>

Note: temperatures recorded for d and e are statistically different; NA – Not analyzed

### Table 3.8b – Salinity measurements recorded at varying well depths and specific flowrates

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Arithmetic Mean Salinity Concentration(ppt)</th>
<th>HFLT</th>
<th>0.95 L/min</th>
<th>1.9 L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>12 ± 0.5 (n=6)</td>
<td>12 ± 0.6 (n=5)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>7.6 m</td>
<td>11 ± 0.6 (n=6)</td>
<td>9 ± 0.6 (n=9)</td>
<td>5.0 ± 0.4 (n=3)</td>
<td></td>
</tr>
<tr>
<td>6.1 m</td>
<td>6 ± 1.1 (n=10)</td>
<td>4 ± 0.6 (n=13)</td>
<td>2.0 ± 0.6 (n=6)</td>
<td></td>
</tr>
<tr>
<td>4.6 m</td>
<td>NA</td>
<td>4 ± 0.4 (n=4)</td>
<td>4.0 ± 0.4 (n=6)</td>
<td></td>
</tr>
<tr>
<td>4.3 m</td>
<td>NA</td>
<td>6 ± 0.6 (n=8)</td>
<td>5.0 ± 0.3 (n=6)</td>
<td></td>
</tr>
<tr>
<td>3.9 m</td>
<td>NA</td>
<td>4 ± 0.7 (n=9)</td>
<td>3.0 ± 0.4 (n=6)</td>
<td></td>
</tr>
<tr>
<td>2.7 m</td>
<td>NA</td>
<td>2 ± 2 (n=6)</td>
<td>0.7 ± 1.3 (n=10)</td>
<td>0.8 ± 0.96 (n=4)</td>
</tr>
<tr>
<td>Background</td>
<td>2 ± 2 (n=6)</td>
<td>0.7 ± 1.3 (n=10)</td>
<td>0.8 ± 0.96 (n=4)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Salinity concentrations for influent are not measured; NA – Not analyzed

### Table 3.8c – pH measurements recorded at varying well depths and specific flowrates

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Arithmetic Mean pH measurements for specific flowrates</th>
<th>HFLT</th>
<th>0.95 L/min</th>
<th>1.9 L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>6.35 ± 2.57 (n=5)</td>
<td>7.42 ± 0.39 (n=12)</td>
<td>8.05 ± 0.70 (n=5)</td>
<td></td>
</tr>
<tr>
<td>7.6 m</td>
<td>6.71 ± 0.04 (n=2)</td>
<td>6.74 ± 0.17 (n=5)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>6.1 m</td>
<td>6.63 ± 0.07 (n=6)</td>
<td>6.67 ± 0.09 (n=9)</td>
<td>7.00 ± 0.02 (n=2)</td>
<td></td>
</tr>
<tr>
<td>4.6 m</td>
<td>6.98 ± 0.12 (n=9)</td>
<td>6.92 ± 0.07 (n=13)</td>
<td>7.00 ± 0.10 (n=5)</td>
<td></td>
</tr>
<tr>
<td>4.3 m</td>
<td>NA</td>
<td>6.54 ± 0.03 (n=4)</td>
<td>7.00 ± 0.03 (n=5)</td>
<td></td>
</tr>
<tr>
<td>3.9 m</td>
<td>NA</td>
<td>6.40 ± 0.10 (n=10)</td>
<td>6.00 ± 0.10 (n=5)</td>
<td></td>
</tr>
<tr>
<td>2.7 m</td>
<td>NA</td>
<td>6.72 ± 0.16 (n=9)</td>
<td>7.00 ± 0.13 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>6.83 ± 0.52 (n=6)</td>
<td>7.29 ± 0.53 (n=12)</td>
<td>7.34 ± 0.50 (n=4)</td>
<td></td>
</tr>
</tbody>
</table>

Note: pH values recorded offered an indication of the presence of wastewater, with typical values in the range 7.0 – 7.4 at 4.6 m depths. Wells placed at this depth provided the most positive fecal coliform detection on all the sampling events undertaken. Deviations from these general observations occurred over the course of the study. In situ measurements (pH and salinity) recorded for the innermost ring of wells [V.D. =1.8 m (A-D 2.7)] suggested the presence of wastewater even though no fecal coliform concentrations were recorded. An insufficiently
buoyed wastewater plume and/or better filtration properties of the subsurface media may explain this occurrence.

Surface/effluent bacterial concentrations were predicted using the removal rate constants derived from fecal coliform removal curves (Table 3.9). Predictions assumed direct transport of the injected wastewater to the marsh surface. However, this is not a likely transport path for actual field-scale conditions. Therefore, the removal rates predicted are conservative estimates since relatively high removals would be expected if the plume were to follow a tortuous path. The high probability of exceeding effluent limits for the HFLT study (42%) compared to the other studies is explained by the depth of the monitoring well used in the computations (4.6m compared to 2.7m for the other studies). More importantly, bacteria are effectively removed after 2.4 vector meters, indicating that the NSSP standard is reached before transport to 4.6 m depth wells.

Table 3.9 Removal rate constants and predicted surface fecal concentrations at specific flowrates

<table>
<thead>
<tr>
<th>Injection Flowrate (L/min)</th>
<th>First Order rate Constant (m$^{-1}$)</th>
<th>Predicted Surface Conc. (MPN/100mL)</th>
<th>Predicted Distance of Travel (m)</th>
<th>Probability of exceeding effluent concentrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fecal coliform &amp; E. coli</td>
<td>Fecal coliform &amp; E. coli</td>
<td>Fecal coliform &amp; E. coli</td>
<td>Fecal coliform &amp; E. coli</td>
</tr>
<tr>
<td>HFLT*</td>
<td>4.0</td>
<td>4.0</td>
<td>5.5 x 10$^{-6}$</td>
<td>6.6 x 10$^{-6}$</td>
</tr>
<tr>
<td>0.95</td>
<td>3.2</td>
<td>3.1</td>
<td>0.085</td>
<td>0.103</td>
</tr>
<tr>
<td>1.9</td>
<td>2.0</td>
<td>1.7</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>1.9$^a$</td>
<td>5.0</td>
<td>NA</td>
<td>0.004</td>
<td>NA</td>
</tr>
<tr>
<td>1.9$^b$</td>
<td>3.5</td>
<td>2.5</td>
<td>0.10</td>
<td>0.05</td>
</tr>
</tbody>
</table>

High flow low temperature study, with predicted bacterial concentrations in units of colonies/100mL;
$^a$ Injection frequency of 30 minutes every 3 hours, Richardson, 2002;  $^b$ Watson Jr. and Rusch, 2001;
$^c$ Probability is based on 4.6m wells (2.1 m V.D.), representing the shallowest well monitored;
$^d$ Probability is based on 2.7 m wells (shallowest well monitored).

Overall, between 2.4 and 4.7 m of travel distance was required for removal of fecal pathogens to NSSP standards, indicating that 41 – 69% of treatment media was theoretically available for treatment. Quinonez-Diaz et al. (2001) recorded at least 99% removal of indicator bacteria originating from an untreated domestic waste after 2 m of penetration in the subsurface. Similar removals were achieved by Arias et al. (2003) in studies performed on the removal of indicator bacteria in a vertical flow constructed wetland system. The high removal rate was
attributed to filtration mechanisms within the wetland resulting in a 1.7 log removal after a 0.8 m penetrating distance. The MUS achieved greater than 99% removal within a 1m travel distance. Fecal coliform removal rate constants determined were 4.0, 3.2, and 2.0 m\(^{-1}\) for the HFLT, 0.95 L/min, and 1.9 L/min studies, respectively. Corresponding \textit{E. coli} removal rate constants were 4.0, 3.1, and 1.7 m\(^{-1}\) respectively. Vector distances required for \textit{E. coli} removal were 2.5, 2.7, and 4.6 m for the HFLT, 0.95 L/min, and 1.9 L/min studies respectively. Regression analyses revealed no difference in filter efficacies for the three operational schemes evaluated (p>0.05). Predicted surface fecal coliform concentrations ranged from 0.085 to 30 MPN/100mL for the 0.95 and 1.9 L/min studies respectively. Predicted surface concentrations for the HFLT study were substantially lower, with surface concentrations of 5.5 x 10\(^{-6}\) and 6.6 x 10\(^{-6}\) colonies/100mL for fecal coliform and \textit{E. coli} respectively. Richardson (2002) and Watson Jr. (2000) predicted surface concentrations of 4.0 x 10\(^{-3}\) and 1.0 x 10\(^{-1}\) cfu/100mL for fecal coliforms.

Bacterial concentrations in the shallowest monitoring wells were used to estimate the probability of exceeding surface discharge limits for the 0.95 and 1.9L/min studies. A surface discharge limit of 14MPN/100mL was used for both fecal coliforms and \textit{E. coli}. Monitoring wells positioned at 0.9 m radial distance from the injection point (4.6m depth; V.D. =2.1m) were used to test the HFLT study. These wells were used because the recorded bacterial concentrations were within the upper and lower bound geometric mean values for the study period. Bacterial concentrations followed a Weibull probability density function:

\[
F(\chi, \beta, \gamma) = 1 - \exp \left( - \frac{\chi}{\beta}^\gamma \right)
\]

(3.1)

where \(\gamma = \) respective shape
\(\beta = \) shape
\(\beta = \) scale

Parameters determined from the Weibull distribution fit (SAS version 8.0) were used to compute respective probabilities (Table 3.10). Overall removal estimates determined for the Bayou
Segnette system compared to that for the Port Fourchon site revealed better performance by the latter in meeting NSSP standards. Yet higher removal rates were achieved at the Grand Bay site revealing the possible impact of subsurface heterogeneities and pore size distribution in limiting bacterial transport.

Table 3.10 Summary of parameters for the Weibull distribution

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Injection Flowrate</th>
<th>HFLT</th>
<th>?</th>
<th>?</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal coliform</td>
<td>HFLT</td>
<td>14 MPN/100mL</td>
<td>1.3</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>14 MPN/100mL</td>
<td>1.5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>14 MPN/100mL</td>
<td>3.6</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>HFLT</td>
<td>14 MPN/100mL</td>
<td>1.3</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>14 MPN/100mL</td>
<td>1.5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>14 MPN/100mL</td>
<td>3.6</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of the 1.9 L/min studies for all three sites revealed that an additional two vector meters and one vector meter were required for the Bayou Segnette system when compared to the Grand Bay and Port Fourchon sites respectively. Richardson (2002) attributed the removal rates at the Grand Bay site to high levels of clay and fine-textured material, which may increase the filtration effects of the subsurface media. Higher ionic strength characteristics of the plume also contributed to the high removals. pH and salinity values recorded for the 1.9 L/min Bayou Segnette study, for instance, suggest lower ionic strength for subsurface conditions, which enhances bacterial transport (Logan and Rogers, 2000; Corapcioglu and Haridas, 1984). Other contributory factors to this difference in removal include: 1) the higher fraction of organic content recorded for the Bayou Segnette soils, which cause increased bacterial survival times (Gerba, 1985), and 2) the higher salt content at the Grand Bay site, increasing adsorption due to double layer compression (Corapcioglu and Haridas, 1985).

The loading rates into the system were normalized with respect to time to enable a comparison of bacterial removal efficacies for the different flowrates investigated (Table 3.11).
Table 3.11 Daily bacterial loading rates for the Influent Wastewater of the MUS study sites

<table>
<thead>
<tr>
<th>Project Site Evaluated</th>
<th>Injection Flowrate (L/min)</th>
<th>Volume of WW injected (L)</th>
<th>Hydraulic Loading Rates (L/d)</th>
<th>Bacterial Loading Rates</th>
<th>Solids Loading rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSSCS</td>
<td>0.95</td>
<td>58709 [341]</td>
<td>0 - 808</td>
<td>NA</td>
<td>24 - 47 [135]</td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>52700 [392]</td>
<td>227 - 558</td>
<td>NA</td>
<td>28 - 57 [42]</td>
</tr>
<tr>
<td>BSCCS</td>
<td>HFLT</td>
<td>7839 [101]</td>
<td>17 - 185</td>
<td>NA</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand Bay</td>
<td>1.9</td>
<td>13215 [199]</td>
<td>0 - 568</td>
<td>NA</td>
<td>53 - 155 [117]</td>
</tr>
<tr>
<td>Port Fourchon</td>
<td>1.9</td>
<td>15132 [66]</td>
<td>1 - 139</td>
<td>NA</td>
<td>2.3 - 8.5 [3.1]</td>
</tr>
</tbody>
</table>

The respective loading rates were computed using the volume of wastewater injected for the respective studies in combination with bacterial and solids concentrations. The range of values reported for the different parameters represent measurements recorded for the biweekly sampling events, statistically analyzed to obtain a range of values (mean±SD).

Mean hydraulic loading rates (HLR) recorded (275-392 L/d) were substantially lower than theoretical loading rates (341-1980L/d) into the system (Table 3.2), with the HFLT study recording the lowest mean HLR. Though this study recorded the highest removal rates, the high flowrates caused system channelization. Braskerud (2003) observed the retention capacity of constructed wetlands to be compromised by increased hydraulic loads. Likewise, the efficiency of fecal coliform removal in gravel beds was reduced due to the application of excessive hydraulic loads (Williams et al., 1995). The hydraulic loading rate and the degree of biofilm development may respectively regulate bacterial removal by straining and adsorption as wastewater is injected (Stevik et al., 2004).

3.3.1.4 Bacterial Migration

Continuous monitoring of insitu parameters (pH, salinity, and temperature) within the Bayou Segnette system helped in quantifying the extent of bacterial retention. Contour plots of bacterial distribution at different depths were developed using Sigma plot 8.0. These plots give an indication of the lateral displacement of wastewater and bacterial migration in the subsurface. The injection point is represented as (0, 0) and all distances are measured in meters. Overall, there appeared to be a reduction in lateral expansion of the wastewater plume over time (Figure 3.11). The gradual and consistent reduction in bacterial concentration with increasing distance from the point of injection can be attributed to filtration effects of the subsurface media. Filtration effects have been associated with the efficient separation of the bacterial and wastewater plumes (Richardson, 2002). Salinity concentrations provided a good indication of the presence of wastewater for wells installed at 4.6 m depth, exhibiting an inverse relation with increasing
bacterial concentrations. High bacterial concentrations were observed for monitoring wells placed within one-meter radius from the injection point.

Figure 3.11 Overall mean wastewater and fecal coliform plumes at depths of (a) 2.7 m and (b) 4.6 m. The injection point is represented as coordinate (0, 0).
Richardson (2002) attributed sporadically high fecal coliform counts measured in outer wells positioned over 6 vector meters from the injection well to surface water contamination and/or the influence of tidal waves on the system. No surface water infiltration was observed at the Bayou Segnette site. The depth of the shallowest monitoring well (2.7 m compared to a depth of 1.5 m for the Grand Bay site) provided a sufficient depth of filtration media (soil) for treatment.

Injection pressures recorded for the HFLT study were ten orders of magnitude higher than those recorded for subsequent studies. Injection pressures reduced from as high as 83 kPa to 7 kPa for the 0.95 L/min study. Abrupt and drastic increases in pressure profiles recorded on initiation of an injection cycle remains steady with occasional spikes during injection, rapidly dissipating at the end of the injection cycle (Figure 3.12).

![Figure 3.12](image)

Figure 3.12. Typical injection profiles for successful pressure assuage due to intermittent injection at the Marshland upwelling system influent, Bayou Segnette, LA. These injection pressures as recorded for the 0.95 L/min study showed the MUS to be self-healing: effectively providing bacterial retention and causing weak zones to seal.

Even though the system acted to assuage such pressure build-up during periods of non-injection, successive injection cycles at flow rates exceeding design capacity may enhance development of weak spots of preferential movement. This was observed for a brief period during
the HFLT study and was successfully ameliorated by reducing the injection flow rate. Consequently, the injection pressures recorded for the 0.95 L/min study showed that the MUS was capable of readjusting itself to seal weak zones in the subsurface if appropriate operational criteria were employed.

The higher removal rates achieved at greater injection depths and high loading rates comes with a trade-off; that the duration of the active injection scheme should be such as to minimize channelization. As was observed in the single camp system, preferential flow paths were developed due to excessively high loading rates, causing wastewater to move rapidly along such paths. The detrimental effects on system performance were successfully ameliorated by switching to a lower injection flow rate. The MUS can be said to be “self-healing” since the conditions reached at the end of the reacclimation period proved efficient in removing fecal pathogens. The percent clay content at higher injection depths and pore sizes contributed this removal. In general, soils with high clay content offer more sites for bacterial adsorption (Bitton and Harvey, 1992; Gerba and Bitton, 1984; and Hagedorn, 1983). Williams et al. (1995) observed the removal of fecal coliforms to strongly correlate with BOD removal, and suggested adsorption to be a possible mechanism of bacterial removal in gravel bed hydroponic subsurface flow wetlands.

Following the HFLT study, inflows into the system were decreased to 0.95 L/min, injecting for 15 minutes every hour. The low injection flowrate employed was to help evaluate the system’s ability to reacclimatize after employing high loading rates. No positive fecal coliform detections were made for depths greater than 4.3 m, indicating an upward transport of the injected wastewater. Isolated detections were however made for the 1.9 L/min study, suggesting transport along the injection well stem. The 1.9 L/min injection flowrate may have caused some shear forces at the interface of the soil media and overall wastewater plume, resulting in desorption of attached bacterial cells. The injection of bacteria into the subsurface has been observed to cause the migration of indigenous bacteria to subsurface observation points.
Studies performed on bacterial detachment rates in aquifers showed a 2-100 fold increase in the number of bacterial strains expected at sampling ports in response to introduced bacterial strains (Johnson et al., 2001).

The 1.9 L/min study required a vector distance of 4.7 m to remove bacteria to NSSP standards. Therefore, the system performed efficiently, even though travel distances were higher than those required for the HFLT and 0.95 L/min studies (Table 3.8). Isolated instances of high bacterial counts in the 4.6 m monitoring wells were observed during 80% of the sampling events. Injection pressures recorded for this period, however, did not suggest excessive pressure build up (ranging from 10 kPa to 35 kPa). It is plausible that biofilm accumulation during the 0.95 L/min study was desorbed from soil sediments in response to the increased injection flowrate. The high influent solids concentrations for the 1.9 L/min study induced higher injection pressures. Continuous monitoring of this flow regime is necessary to completely investigate the extent of bacterial removal.

3.3.2 Cluster Camp System

Bacterial concentrations recorded for the cluster camp system were substantially lower than that for the single camp system. 83,094 L of settled wastewater was injected, substantially lower than expected generation potential of the four camps. The low frequency of camp usage for the cluster camp system did not contribute enough wastewater for this system. In addition, surface water infiltration into a lift station connected to one of the camps led to dilution of the system influent. The infiltrated water was pumped into the primary distribution tank resulting in lower concentrations. Geometric mean influent concentrations for fecal coliforms and *E. coli* represented wastewater of low strength (Table 3.12). Fecal coliform concentrations measured for all 36 monitoring wells ranged between 0-9500 colonies/100mL and 0-16000 MPN/100mL respectively for the HFLT study and the 1.9L/min study. Overall, the path taken by the wastewater could not be determined.
Concerns over the possible entrapment of the injected wastewater within the subsurface resulted in a change in injection depth (from 3.4 - 4 m). However this change did not result in any fecal coliform detection. An evaluation of fecal coliform concentrations in monitoring wells located within 1 m vector distance of either injection well (injection wells # 1 and # 2) showed concentrations ranging from 33-55 MPN/100mL, which are orders of magnitude lower than recorded values for the single camp system (Table 3.6).

Table 3.12 Depth-dependent geometric mean bacterial concentrations for specific flowrates measured at the cluster camp system

<table>
<thead>
<tr>
<th>Sampling Point</th>
<th>Fecal coliform concentration (MPN/100mL)</th>
<th>E. coli concentration (MPN/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HFLT 1.9 L/min</td>
<td>HFLT 1.9 L/min</td>
</tr>
<tr>
<td>Influent</td>
<td>2408 ± 2404</td>
<td>10743 ± 57917</td>
</tr>
<tr>
<td>0.6 m</td>
<td>NA</td>
<td>14</td>
</tr>
<tr>
<td>1.5 m</td>
<td>2</td>
<td>8 ± 7</td>
</tr>
<tr>
<td>2.7 m</td>
<td>2 ± 0.3</td>
<td>7 ± 12</td>
</tr>
<tr>
<td>3.0 m</td>
<td>59 ± 99</td>
<td>43 ± 102</td>
</tr>
<tr>
<td>4.0 m</td>
<td>NA</td>
<td>59 ± 99</td>
</tr>
</tbody>
</table>

NA – Not applicable

Geometric mean fecal coliform concentrations in monitoring wells positioned between 0.6 – 2.7 m depths ranged from 5-14 MPN/100mL. The high of this measured range was likely a result of surface infiltration into a 0.6 m monitoring well. The influx of water during high tides and in the hurricane season possibly may have caused such observations. Fecal coliform removal curves are not plotted because of insufficient data points for this system and possible dilution of the injected wastewater. Bacterial distribution in the system was however analyzed in terms of concentrations measured for the two flowrates evaluated. Concentrations are presented with respect to vector distance from both injection wells (Table 3.13). Fecal coliform concentrations in the immediate surrounding of the injection wells rather had lower concentrations raising concerns about the pathway taken by the wastewater plume.
Table 3.13 Bacterial concentrations presented with respect to vector distance from the injection wells installed at the cluster camp system

<table>
<thead>
<tr>
<th>Origin of Measurement</th>
<th>Vector distance (m)</th>
<th>Fecal coliform concentration (MPN/100mL)</th>
<th>E. coli concentration (MPN/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HFLT 1.9 L/min</td>
<td>HFLT 1.9L/min</td>
</tr>
<tr>
<td>Injection Well # 1</td>
<td>1.0</td>
<td>22 ± 42</td>
<td>33 ± 38</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>NA</td>
<td>5 ± 5</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>NA</td>
<td>7 ± 54</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>2</td>
<td>9 ± 3</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>NA</td>
<td>52 ± 5332</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>NA</td>
<td>40 ± 149</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>NA</td>
<td>164 ± 64</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>NA</td>
<td>64 ± 100</td>
</tr>
<tr>
<td>Injection well # 2</td>
<td>1.0</td>
<td>NA</td>
<td>54 ± 131</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>NA</td>
<td>34 ± 135</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>±</td>
<td>15 ± 52</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>NA</td>
<td>13 ± 71</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>101 ± 347</td>
<td>87 ± 225</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>3</td>
<td>39 ± 37</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>NA</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>NA</td>
<td>2</td>
</tr>
</tbody>
</table>

NA – Not analyzed

3.4 Conclusions

The ability of the MUS to effectively remove fecal bacteria from domestic wastewater of medium-to-high strength is undoubted. Results from the single camp system indicate that the MUS is even more effective for removal of *Escherichia coli* and could serve as a viable alternative to conventional wastewater treatment systems. Under the site-specific conditions existent at the Bayou Segnette site, the effectiveness of bacterial retention is dependent on the depth of injection employed. High bacterial loading rates and flowrates exceeding 5 L/min proved effective for bacterial retention. However, the longevity of this injection scheme was compromised, due to system channelization. The operational criteria employed for the 0.95 L/min study (mean HLR of 341 L/d) offered the best response to bacterial removal, requiring 2.7 vector meters of travel to remove fecal bacteria below NSSP standard.
The near freshwater conditions at the Bayou Segnette site require injection at greater depths compared to that at the Grand Bay site to provide for sufficiently high buoyancy forces for upward transport. In addition, the difference in pore sizes and clay content contributed to bacterial removal, based on vector distance.

Wells within a one-meter radius of the injection well returned the highest fecal concentration representing a 4-log removal of mean influent concentrations. Significant retention was therefore achieved within close vicinity of the injection well. Travel distances of between 2.4 and 4.7 vector meters were required to reduce influent fecal coliform concentrations to below the NSSP standard. Geometric mean influent *E. coli* concentrations declined exponentially with only 2.7 meters required to reduce influent concentrations to below the NSSP standard. Overall first-order rate constants for fecal coliforms ranged between 2.0 and 4.0 m$^{-1}$ while that for *E. coli* ranged between 1.7 and 4.0 m$^{-1}$. Fecal coliform concentrations in the shallowest monitoring wells installed were significantly less than background concentrations, indicating that only 42% of treatment media was theoretically required for treatment. Subsurface heterogeneities appeared to have had greater impacts on bacterial removal. Fresh adsorptive sites during initial phase of system operation enhanced bacterial removal by adsorption.

Wastewater plume development and bacterial migration within the cluster camp MUS was not clearly defined due to problems associated with infrequent camp usage practices and occasional dilution of primary influent. Overall, the path taken by the wastewater plume could not be determined.
Chapter Four: Global Discussion and Conclusions

The contents of this chapter provide an overview of field observations and laboratory simulated analyses of the effectiveness of bacteria retention and survival rates within the MUS. The two main sections of this thesis, evaluate: 1) the implications of fecal coliform laboratory characterization on field-scale transport, and 2) the retention and removal of fecal coliforms in a MUS installed in a floatation marsh with pore water of low salinity regimes.

In studying the impact of natural die-off conditions on bacterial survival, laboratory experiments performed at two distinct temperatures (20°C and 25°C) indicated that the higher temperature was more detrimental to fecal coliform survival (p=0.0041). Fecal coliform decay rate constants ranged from 0.57 to 1.03 d⁻¹. A fraction of the fecal coliform pollution introduced through the intermittent injection of wastewater will likely undergo inactivation, depending on prevalent external factors (salinity concentrations, ambient temperatures and dissolved oxygen concentrations). Introduced fecal coliforms initially undergo rapid die-off (2.2 – 4.0 days) with less than 10% of original population remaining after 3 days. This rapid die-off is followed by a much slower inactivation process (Easton, at al., 2000). The regression model predicts a 3-log fecal coliform removal within 10 days for the 25°C experiment while the 20°C experiments recorded a 3-log removal after approximately two weeks.

The higher likelihood of nutrient availability for allochthonic bacteria in view of the intermittent injection of wastewater suggests that die-of rates in the MUS will be much slower than that predicted by the laboratory studies. No clear relationship existed between increased salinity and fecal coliform inactivation rates. The effect of salinity may be manifested if there was synergism with other external factors such as temperature and dissolved oxygen. However, dissolved oxygen concentrations did not regulate bacterial survival rates. In situ mean pH values (7.3–8.0) recorded for different salinity concentrations did not affect bacterial survival rates. The optimum pH range within which survival rates will be limited lies below 4.0 and above 9.5 (Metcalf and Eddy, 2003).
Continuous injection experiments supported the assertion that bacterial transport is significantly delayed in the presence of clayey sized soils (Bitton and Harvey, 1992; McDowell et al., 1986; Gerba and Bitton, 1984). The presence of clay minerals likely enhanced adsorption processes, offering fresh adsorption sites for bacteria during the initial period of wastewater injection (Corapcioglu and Haridas, 1984). Desorption of microorganisms from successive wastewater injection affected bacterial breakthrough. A mean bacterial retardation factor of 6.1 was determined to limit transport under laboratory-simulated conditions. The mean bacterial retardation rate is 2 orders of magnitude bigger than that obtained by Richardson (2002) using sand.

Bacterial transport through the column was possibly limited by the nature and distribution of pore constrictions, resulting in the magnitude of retardation recorded. While the retardation factor determined herein relates to bacterial transport under the continuous injection phase, an even higher retardation would be expected during the resting phase. Comparatively lower pore water velocities associated with plume movement during the resting phase will largely be responsible for the high retardation factor. Although the injection of pore water of lower ionic strength (such as wastewater) into a saline environment was expected to limit hydraulic conductivity of the laboratory repacked bed (Mehnert and Jennings, 1985), limitations resulting from inherent properties of the bed media will have a greater impact on bacterial retardation. Isolated instances of fecal coliform detection before the injection of one pore volume for trial 3 suggested the possible effect of heterogeneities on field-scale transport of bacteria. Such occurrences may in fact account for isolated instances of fecal coliform detection in some of the shallowest monitoring wells sampled at the Bayou Segnette site.

Because the rate of bacterial removal by straining is inversely proportional to the grain size of soils, bacteria strained at the soil surface promote the retention of yet finer particles (Bitton and Harvey, 1992). Straining, determined from the geometrical suffusion security factor, was deemed likely to limit bacterial transport, though on a much smaller scale. This straining
effect increases in relation to the rate of bacterial accumulation on the soil surface, causing captured particles to eventually behave like a filter. This may lead to biofilm formation at the soil surface. The longevity of this biofilm development process is dependent on surface properties of the soil grains and the nature of the deposition process. Rough surfaces result in initial deposition in the crevices, followed by later depositions that smoothen out the surface. Much lower effects of straining on filtration processes are expected in field-scale transport. Successive injection schemes may cause high enough shear forces that will eventually erode accumulated bacteria at the surface. Consequently detachment rates tend to follow those for smooth surfaces (Rittman and McCarty, 2001).

Field observations on the performance of the MUS in retaining fecal bacteria from settled, domestic wastewater showed the system to be effective under the near-fresh water existent at the Bayou Segnette site. A high flow low/temperature (HFLT) study, a 0.95 L/min study, and a 1.9 L/min study were tested to determine the injection flow rate and frequency most suited for bacterial removal. Even though the HFLT study proved most effective for fecal coliform retention, the hydraulic dysfunction that resulted from excessively high loading rates compromised the longevity of such an operational scheme. The 0.95 L/min flowrate injecting for 15 minutes every hour was most suited for bacterial removal.

In all the different evaluations, a four-log removal of influent fecal coliform concentrations was achieved within one vector meter distance of travel. Overall, subsurface fecal coliform concentrations decreased with increasing distance from the point of injection and represented a first-order decay relationship. Between 2.4 and 4.7 m of travel distance was required to reduce influent fecal coliform concentrations to the 14 MPN/100 NSSP standards, representing 41 – 69 % of treatment media utilized. Even higher removal rates were obtained for *Escherichia coli*. Fecal coliform concentrations in the shallowest wells occasionally showed instances of high concentrations exceeding NSSP standards indicating that media heterogeneities could limit prediction of surface concentrations. Similar findings in the laboratory repacked
columns pointed to preferential flow of microbes along weak spots, a situation likened to subsurface channelization due to high injection flowrates in actual field-scale operations. Predicted surface concentrations never exceeded 30 MPN/100mL.

Under the site-specific conditions existent at the Bayou Segnette site, the effectiveness of bacterial retention is dependent on the depth of injection employed. Higher bacterial removal rates were achieved for the 6.1 m injection depth than the 4.3 m injection depth. The better removal rates offered when injecting at greater depths and at relatively high loading rates comes with a trade-off; that the duration of the active injection scheme should be such as to minimize channelization. While previous MUS suggested *insitu* parameters (pH, salinity, and temperature) to be capable of tracking wastewater plume in the subsurface, the findings of this research indicated pH to be the most reliable indicator of the presence of wastewater. Inherently low salinity concentrations at the Bayou Segnette site did not provide the needed density contrast to enable distinction of the defined plume, based solely on salinity.

Overall removal estimates determined for the Bayou Segnette system compared to that for the Port Fourchon site reveals better performance by the latter in meeting NSSP standards. Yet higher removal rates were achieved at the Grand Bay site, revealing the possible impact of subsurface heterogeneities and pore sizes in limiting bacterial transport. Comparison of the 1.9 L/min studies for all three sites revealed that the Bayou Segnette system required additional travel distances of two vector meters and one vector meter respectively for the Grand Bay and Port Forchon sites. The research findings suggested that the higher ionic strength of the plume within the Grand Bay system aided in bacterial attachment to soil grains in addition to the adsorption sites offered higher clay content. Wastewater transport at the Bayou Segnette site was most impacted by media heterogeneities rather than buoyancy forces acting on the developed wastewater plume.
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Appendix A: Field (Single Camp/Cluster Camp) and Laboratory Soil Properties
Sample Description: Bayou Segnette Single-Camp Sample collected from 0.6-2.7 m

Sieve Analysis

Date: 01/16/2003

Sample Mass (M_s) = 200.0 g

<table>
<thead>
<tr>
<th>Sieve No.</th>
<th>Sieve Opening (mm)</th>
<th>Mass of soil retained on each sieve Wn(g)</th>
<th>Percent of mass retained on each sieve Rn</th>
<th>Cumulative percent retained</th>
<th>Percent finer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2</td>
<td>1.32</td>
<td>0.660826033</td>
<td>0.660826033</td>
<td>99.33917</td>
</tr>
<tr>
<td>40</td>
<td>0.425</td>
<td>72.08</td>
<td>36.08510638</td>
<td>36.74593242</td>
<td>63.25407</td>
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<tr>
<td>80</td>
<td>0.18</td>
<td>53.31</td>
<td>26.68836045</td>
<td>63.43429287</td>
<td>36.56571</td>
</tr>
<tr>
<td>100</td>
<td>0.15</td>
<td>10.44</td>
<td>5.226533166</td>
<td>68.66082603</td>
<td>31.33917</td>
</tr>
<tr>
<td>140</td>
<td>0.106</td>
<td>6.29</td>
<td>3.14893617</td>
<td>71.8097622</td>
<td>28.19024</td>
</tr>
<tr>
<td>200</td>
<td>0.075</td>
<td>16.34</td>
<td>8.180225282</td>
<td>79.98998748</td>
<td>20.01001</td>
</tr>
<tr>
<td>Pan</td>
<td></td>
<td>39.97</td>
<td>20.01001252</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>199.75</td>
<td>100</td>
</tr>
</tbody>
</table>

Hydrometer Analysis

Test date & time            01/22/2003  3.04pm
Hydrometer Type             ASTM 152-H
Dry Weight of Soil (g)       39.97
Conc. of DA (g/l)            4
Vol. Of DA (ml)              125
Temperature of the test      23°C
DA = Dispersing Agent

<table>
<thead>
<tr>
<th>Date &amp; Time</th>
<th>Time Elapsed(min)</th>
<th>Hydrometer Reading(R)</th>
<th>Rcp [R+Rt-Fz]</th>
<th>% Finer</th>
<th>Rcl</th>
<th>L (cm)</th>
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Grain Size Distribution Curve

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<tr>
<th>Grain Size (mm)</th>
<th>% finer by weight(%)</th>
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<tbody>
<tr>
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<td>36.56570713</td>
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<td>0.000952628</td>
<td>1.952440551</td>
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</table>

$D_{10}=0.090\text{mm} \quad \%\text{ sand}=80\%$

$D_{30}=0.128\text{mm} \quad \%\text{ silt}=16.5\%$

$D_{50}=0.26\text{mm} \quad \%\text{ clay}=3.5\%$

$D_{60}=0.38\text{mm}$

$Cu = \frac{D_{60}}{D_{10}} = 4.2$

$Cc = \frac{(D_{30})^2}{(D_{10} \times D_{60})} = 0.479$
Sample Description: Bayou Segnette Single-Camp Sample collected from 2.7 - 4.7 m

Sieve Analysis

Date: 01/16/2003

Sample Mass \( (M_s) = 200.0 \) g

<table>
<thead>
<tr>
<th>Sieve No.</th>
<th>Sieve Opening (mm)</th>
<th>Mass of soil retained on each sieve ( W_n ) (g)</th>
<th>Percent of mass retained on each sieve ( R_n )</th>
<th>Cumulative percent retained</th>
<th>Percent finer</th>
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<tbody>
<tr>
<td>20</td>
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<td>12.11535603</td>
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<tr>
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<td>35.3451266</td>
<td>64.65487</td>
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<tr>
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<td>200</td>
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201.81 100

Hydrometer Analysis

Test date & time: 01/16/2003 3.51pm

Meniscus Correction \( (F_m) \): 1.0

Zero correction \( (F_z) \): + 5.0

Correction \( (F_t) \): 1.15

Correction for SG \( (a) \): 1

Unitless Factor \( (A) \): 0.013

DA = Dispersing Agent
<table>
<thead>
<tr>
<th>Grain Size (mm)</th>
<th>% finer by weight(%)</th>
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</thead>
<tbody>
<tr>
<td>0.85</td>
<td>87.88464</td>
</tr>
<tr>
<td>0.425</td>
<td>76.4085</td>
</tr>
<tr>
<td>0.18</td>
<td>67.44958</td>
</tr>
<tr>
<td>0.15</td>
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<td>0.106</td>
<td>53.08458</td>
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<td>21.09409841</td>
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</table>

D\text{10}=0.0866\text{mm}  \quad \text{sand}=78.9\%
D\text{30}=0.08\text{mm} \quad \% \text{silt}=14.8\%
D\text{50}=0.1\text{mm}  \quad \% \text{clay}=6.3\%
D\text{60}=0.13\text{mm}  
Cu = D_{60}/D_{10} =1.5
Cc = (D_{30})^2/(D_{10} \times D_{60}) = 0.568

Grain Size Distribution Curve
Determination of Percent Organic Composition – Single Camp System

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Crucible Weight (a)</th>
<th>Combined Weight (a+Soil)</th>
<th>Dry Weight of soil</th>
<th>Ash Weight</th>
<th>Final Weight after 550°C</th>
<th>Weight of Organics</th>
<th>% Organic</th>
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<td>101.5251</td>
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ES = East Side (Single Camp System)  
WS = Cluster Camp System

Summary Statistics

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<th>Sample ID</th>
<th>Mean</th>
<th>Stdev</th>
<th>CV</th>
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109
Sample Description: **Bayou Segnette Cluster-Camp Sample** collected from 0.6-2.7 m

**Sieve Analysis**

Date: 01/16/2003

Sample Mass ($M_s$) = 200.0 g

<table>
<thead>
<tr>
<th>Sieve No.</th>
<th>Sieve Opening (mm)</th>
<th>Mass of soil retained on each sieve $W_n$(g)</th>
<th>Percent of mass retained on each sieve $R_n$</th>
<th>Cumulative percent retained</th>
<th>Percent finer</th>
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$\text{Pan} = 199.87$

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<th>$R_{cp}$ (R+Fe-Fz)</th>
<th>% Finer</th>
<th>$R_{cl}$ (cm)</th>
<th>L (cm)</th>
<th>D (mm)</th>
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Hydrometer Analysis

- Test date & time: 01/24/2003 10.58am
- Meniscus Correction ($F_m$): 0.7
- Zero correction ($F_z$): + 4.0
- Temperature correction ($F_t$): 0.65
- Specific Gravity (SG): 2.65
- Unitless Factor (A): 0.0133
- DA = Dispersing Agent
<table>
<thead>
<tr>
<th>Grain Size (mm)</th>
<th>% finer by weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>99.82988943</td>
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<td>0.425</td>
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- $D_{10} = 0.005\text{mm}$ % sand = 73.2%
- $D_{30} = 0.08\text{mm}$ % silt = 16.3
- $D_{50} = 0.2\text{mm}$ % clay = 10.5%
- $D_{60} = 0.27\text{mm}$
- $C_u = D_{60}/D_{10} = 54$
- $C_c = (D_{30})^2/(D_{10} \cdot D_{60}) = 4.74$

Grain Size Distribution Curve
Sample Description: **Bayou Segnette Cluster-Camp Sample** collected from 2.7 - 4.7 m

### Sieve Analysis

**Date:** 01/16/2003

**Sample Mass** (\(M_s\)) = 200.0 g

<table>
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<tr>
<th>Sieve No.</th>
<th>Sieve Opening (mm)</th>
<th>Mass of soil retained on each sieve (W_n)(g)</th>
<th>Percent of mass retained on each sieve (R_n)</th>
<th>Cumulative percent retained</th>
<th>Percent finer</th>
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### Hydrometer Analysis

**Test date & time:** 01/16/2003 3.04pm

- **Meniscus Correction** (\(F_m\)) = 1.0
- **Zero correction** (\(F_z\)) = + 4.0
- **Temperature**
- **Correction(Fₜ)** = 1.15
- **Correction for SG (a)** = 1
- **Temperature of the test** = 24°C

DA = Dispersing Agent

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<th>(R_d) (cm)</th>
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\[ D_{10}=0.002\text{mm} \quad \% \text{ sand}=74.1\% \]
\[ D_{30}=0.1\text{mm} \quad \% \text{ silt }= 10.9 \]
\[ D_{50}=0.25\text{mm} \quad \% \text{ clay}=15\% \]
\[ D_{60}=0.34\text{mm} \]
\[ C_u = \frac{D_{60}}{D_{10}} = 170 \]
\[ C_c = \frac{(D_{30})^2}{(D_{10}\times D_{60})} = 14.7 \]
Sieve Analysis

Sample Description: **Clayey Composite** for 1D Laboratory Studies

Date: 01/16/2004

<table>
<thead>
<tr>
<th>Sieve Opening (mm)</th>
<th>Mass of soil retained on each sieve Wn(g)</th>
<th>Percent of mass retained on each sieve Rn</th>
<th>Cummulative percent retained</th>
<th>Percent finer</th>
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**Hydrometer Analysis**

- Test date & time: 01/16/2004 11.20am
- Hydrometer Type: ASTM 152-H
- Dry Weight of Soil (g): 83.8
- Conc. of DA (g/l): 4
- Vol. of DA (ml): 125
- Temperature of the test: 21°C
- DA = Dispersing Agent

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<th>% Finer</th>
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\[
D_{10} = 0.0015 \text{ mm} \quad \% \text{ sand} = 57.8 \%
\]
\[
D_{30} = 0.07 \text{ mm} \quad \% \text{ silt} = 28.1 \%
\]
\[
D_{50} = 0.085 \text{ mm} \quad \% \text{ clay} = 14 \%
\]
\[
D_{60} = 0.1 \text{ mm}
\]
\[
C_u = \frac{D_{60}}{D_{10}} = 66.7
\]
\[
C_c = \frac{(D_{30})^2}{(D_{10} \cdot D_{60})} = 32.7
\]
Sieve Analysis

Sample Description: **Clayey Composite** for 1D Laboratory Studies (Duplicate Analyses)

Date: 01/16/2004

<table>
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<tr>
<th>Sieve No.</th>
<th>Sieve Opening (mm)</th>
<th>Mass of soil retained on each sieve Wn(g)</th>
<th>Percent of mass retained on each sieve Rn</th>
<th>Cumulative percent retained</th>
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**Hydrometer Analysis**

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<th>% Finer</th>
<th>R_d (R + Fm)</th>
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<th>D (mm)</th>
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Grain Size Distribution Curve

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Grain Size Distribution Curve

- $D_{10} = 0.0035$ mm  % sand = 68.3%
- $D_{50} = 0.075$ mm  % silt = 20.4%
- $D_{60} = 0.1$ mm
- $Cu = D_{60}/D_{10} = 28.57142857$
- $Cc = 16.07142857$
Appendix B: One-Dimensional Retardation Studies
### 1-D Bacterial Retardation Study - Trial 1

**Test Date**: 4/19/2004  
**Conducted By**: B.A  
**Flowrate**: 6.50 mL/min  
**Pore Volume**: ~ 312 ml

#### Date / Time  | Elapsed Time (min) | Pore Volumes | Temp. (°C)  
--- | --- | --- | ---  
4/19/2004 13:40 | 0 | 0.0 | 19.5  
4/19/2004 13:50 | 10 | 0.2 | 20.1  
4/19/2004 14:00 | 20 | 0.4 | 19.9  
4/19/2004 14:10 | 30 | 0.6 | 20.9  
4/19/2004 14:20 | 40 | 0.8 | 19.8  
4/19/2004 14:30 | 50 | 1.0 | 20.3  
4/19/2004 14:40 | 60 | 1.3 | 20.0  
4/19/2004 14:50 | 70 | 1.5 | 19.9  
4/19/2004 15:00 | 80 | 1.7 | 20.0  
4/19/2004 15:10 | 90 | 1.9 | 20.0  
4/19/2004 15:20 | 100 | 2.1 | 20.2  
4/19/2004 15:30 | 110 | 2.3 | 20.2  
4/19/2004 15:40 | 120 | 2.5 | 20.0  
4/19/2004 15:50 | 130 | 2.7 | 20.2  
4/19/2004 16:00 | 140 | 2.9 | 21.0  
4/19/2004 16:10 | 150 | 3.1 | 19.7  
4/19/2004 16:20 | 160 | 3.3 | 20.0  
4/19/2004 16:30 | 170 | 3.5 | 20.6  
4/19/2004 16:40 | 180 | 3.8 | 20.4  
4/19/2004 17:00 | 200 | 4.2 | 20.4  
4/19/2004 17:20 | 220 | 4.6 | 20.3  
4/19/2004 17:40 | 240 | 5.0 | 20.1  
4/19/2004 18:00 | 260 | 5.4 | 20.1  
4/19/2004 18:20 | 280 | 5.8 | 20.1

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\[ T = \frac{C}{0} \int C \left( \frac{C}{C_0} \right) dt \]

\[ RF = \frac{T_{FC}}{T_{ww}} \]

**Trial 1 - 04/19/04**

**Fresh Wastewater Breakthrough**

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**Sum**

\[ 0.934 \quad 0.755 \]

\[ T_{ww} \quad \text{0.808081} \]

\[ RF \quad \text{5.921559} \]

**Bacterial Breakthrough**

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**Sum**

\[ 0.610 \quad 2.920 \]

\[ T_{fc} \quad \text{4.785098} \]
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**Sum** 0.917 0.614

**Rd** 7.667217

### Bacterial Breakthrough

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### 1-D Bacterial Retardation Study - Trial 3

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**Conducted By:** B.A  
**Flowrate:** 6.14 mL/min  
**Pore Volume:** ~ 312 mL

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**Sum** 0.917 0.761

**$T_{ww}$** 0.830117

**$R_d$** 4.747046

### Bacterial Breakthrough

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**Sum** 1.147 4.520

**$T_{fc}$** 3.940601
Appendix C: Fecal Coliform Decay Study
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**Test Date:** 6/26/2003

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Laboratory-Determined Fecal Coliform Decay Rates

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Test Date: 7/14/2003

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Appendix D: Statistical Analyses
SAS Code

• Test for significance of temperature on fecal coliform inactivation rates
• Determination of significance of laboratory-derived decay rate constants

data die_off;
title 'comparison of dieoff slopes';
input FC Temp Sal ET;
logFC=log(FC);
cards;
47.47 20 0 0
1196 20 0 0.5
170 20 0 1
87.5 20 0 1.5
51.1 20 0 2
86.9 20 0 2.5
23.7 20 0 3
12.6 20 0 4
24.9 20 0 5
51.4 20 0 6
29.7 20 0 7
34 20 0 8
4919 20 5 0
765 20 5 0.5
97.6 20 5 1
115 20 5 1.5
74.9 20 5 2
23.9 20 5 2.5
101 20 5 3
32.5 20 5 4
60.2 20 5 5
19.7 20 5 6
14.8 20 5 7
50.3 20 5 8
3283 20 10 0
450 20 10 0.5
386 20 10 1
78 20 10 1.5
66.5 20 10 2
70.2 20 10 2.5
73.4 20 10 3
40.2 20 10 4
40.2 20 10 5
33 20 10 6
9.4 20 10 7
7.9 20 10 8
23664 25 0 0
593 25 0 0.5
2065 25 0 1
364 25 0 1.5
151 25 0 2
748 25 0 2.5
126 25 0 3
156 25 0 4
359 25 0 5
54.7 25 0 6
proc mixed data=die_off;
  class Temp Sal;
  model logFC = Temp Sal Temp*Sal ET (Temp*Sal)/Solution;
  lsmeans Temp Sal Temp*Sal;
  contrast '20 vs 25' ET(Temp*Sal) 1 1 1 -1 -1 -1 -1 -1 -1 -1;
  contrast '20 within 20' ET (Temp*Sal) 1 0 -1 0 0 0 0;
  contrast '20 within 20' ET (Temp*Sal) 0 1 -1 0 0 0 0;
  contrast '20 within 20' ET (Temp*Sal) 1 -1 0 0 0 0 0;
  contrast '25 within 25' ET (Temp*Sal) 0 0 0 1 0 0 -1;
  contrast '25 within 25' ET (Temp*Sal) 0 0 0 1 -1 0 0;
  contrast '25 within 25' ET (Temp*Sal) 0 0 0 1 -1 0 0;
  contrast '20 vs 25' ET(Temp*Sal) 1 0 0 -1 0 0 0;
  contrast '20 vs 25' ET (Temp*Sal) 0 1 0 -1 0 0;
  contrast '20 vs 25' ET (Temp*Sal) 0 0 1 0 0 -1;
  contrast '20 vs 25' ET (Temp*Sal) 0 0 0 0 -1;
  contrast '20 vs 25' ET (Temp*Sal) 0 0 1 -1 0 0;
  contrast '20 vs 25' ET (Temp*Sal) 0 1 0 0 0 0;
  contrast '20 vs 25' ET (Temp*Sal) 1 0 0 0 -1 0;
  contrast '20 vs 25' ET (Temp*Sal) 0 1 0 -1 0 0;
run;
quit;
### The Mixed Procedure

#### Solution for Fixed Effects

#### Standard

| Effect       | Temp  | Sal  | Estimate | Error  | DF  | t Value | Pr > |t| |
|--------------|-------|------|----------|--------|-----|---------|-------|---|
| Intercept    |       |      | 7.2309   | 0.5328 | 57  | 13.57   | <.0001|   |
| Temp         | 20    | 0    | -0.9461  | 0.7362 | 57  | -1.29   | 0.2039|   |
| Temp         | 25    | 0    | 0        | .      | .   | .       | .     |   |
| Sal          | 0     | 0.6422| 0.7535   | 57    | 0.85| 0.3977  |       |   |
| Sal          | 5     | 1.1038| 0.7535   | 57    | 1.46| 0.1485  |       |   |
| Sal          | 10    | 0    | 0        | .      | .   | .       | .     |   |
| Temp*Sal     | 20    | 0    | -0.9620  | 1.0411 | 57  | -0.92   | 0.3594|   |
| Temp*Sal     | 20    | 5    | -1.4257  | 1.0411 | 57  | -1.37   | 0.1762|   |
| Temp*Sal     | 20    | 10   | 0        | .      | .   | .       | .     |   |
| Temp*Sal     | 25    | 0    | 0        | .      | .   | .       | .     |   |
| Temp*Sal     | 25    | 5    | 0        | .      | .   | .       | .     |   |
| Temp*Sal     | 25    | 10   | 0        | .      | .   | .       | .     |   |
| ET(Temp*Sal) | 20    | 0    | -0.4507  | 0.1206 | 57  | -3.74   | 0.0004|   |
| ET(Temp*Sal) | 20    | 5    | -0.4398  | 0.1206 | 57  | -3.65   | 0.0006|   |
| ET(Temp*Sal) | 20    | 10   | -0.5671  | 0.1206 | 57  | -4.70   | <.0001|   |
| ET(Temp*Sal) | 25    | 0    | -0.6983  | 0.1449 | 57  | -4.82   | <.0001|   |
| ET(Temp*Sal) | 25    | 5    | -0.9990  | 0.1449 | 57  | -6.89   | <.0001|   |
| ET(Temp*Sal) | 25    | 10   | -0.7365  | 0.1449 | 57  | -5.08   | <.0001|   |

### The Mixed Procedure

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<td>1</td>
<td>57</td>
<td>2.15</td>
<td>0.1477</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>1</td>
<td>57</td>
<td>1.72</td>
<td>0.1943</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>1</td>
<td>57</td>
<td>8.80</td>
<td>0.0044</td>
</tr>
</tbody>
</table>
The Mixed Procedure

Contrasts

<table>
<thead>
<tr>
<th>Label</th>
<th>Num</th>
<th>Den</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 vs 25</td>
<td>1</td>
<td>57</td>
<td>0.81</td>
<td>0.3726</td>
</tr>
<tr>
<td>20 vs 25</td>
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<td>57</td>
<td>2.30</td>
<td>0.1350</td>
</tr>
<tr>
<td>20 vs 25</td>
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<td>57</td>
<td>0.48</td>
<td>0.4893</td>
</tr>
<tr>
<td>20 vs 25</td>
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<td>57</td>
<td>5.25</td>
<td>0.0257</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>1</td>
<td>57</td>
<td>2.48</td>
<td>0.1211</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>1</td>
<td>57</td>
<td>8.46</td>
<td>0.0052</td>
</tr>
<tr>
<td>20 vs 25</td>
<td>1</td>
<td>57</td>
<td>1.88</td>
<td>0.1758</td>
</tr>
</tbody>
</table>

Least Squares Means

| Effect  | Temp | Sal | Estimate | Error | DF | t Value | Pr > |t| |
|---------|------|-----|----------|-------|----|---------|-------|
| Temp    | 20   | 4.5289 | 0.1759 | 57 | 25.74 | <.0001 |
| Temp    | 25   | 5.2381 | 0.1841 | 57 | 28.46 | <.0001 |
| Sal     | 0    | 5.0958 | 0.2205 | 57 | 23.11 | <.0001 |
| Sal     | 5    | 4.8656 | 0.2205 | 57 | 22.07 | <.0001 |
| Sal     | 10   | 4.6891 | 0.2205 | 57 | 21.27 | <.0001 |

The Mixed Procedure

Least Squares Means

| Effect  | Temp | Sal | Estimate | Error | DF | t Value | Pr > |t| |
|---------|------|-----|----------|-------|----|---------|-------|
| Temp*Sal| 20   | 0   | 4.5347 | 0.3047 | 57 | 14.88 | <.0001 |
| Temp*Sal| 20   | 5   | 4.5671 | 0.3047 | 57 | 14.99 | <.0001 |
| Temp*Sal| 20   | 10  | 4.4850 | 0.3047 | 57 | 14.72 | <.0001 |
| Temp*Sal| 25   | 0   | 5.6569 | 0.3188 | 57 | 17.74 | <.0001 |
| Temp*Sal| 25   | 5   | 5.1641 | 0.3188 | 57 | 16.20 | <.0001 |
| Temp*Sal| 25   | 10  | 4.8933 | 0.3188 | 57 | 15.35 | <.0001 |
SAS Code

- Multiple Regression Analyses of decay rate coefficients for development of statistical model
  - Determine if an interaction existed between temperature and Salinity

```sas
dm 'log;clear;output;clear';
options nodate pageno=1 ls=100 ps=100;
data one;
input y temp sal;
s=sal;
cards;
  .648 20 0
  .654 20 5
  .567 20 10
  .698 25 0
  1.03 25 5
  .737 25 10;

proc mixed;
title 'Check for significance';
class sal;
model y=temp sal s*temp;
run;

proc mixed;
title 'Main Effects model';
class temp sal;
model y=temp sal/solution;
run;

proc reg;
model y=temp sal;
run;
quit;
```

The Mixed Procedure

Type 3 Tests of Fixed Effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>temp</td>
<td>1</td>
<td>1</td>
<td>0.49</td>
<td>0.6115</td>
</tr>
<tr>
<td>sal</td>
<td>2</td>
<td>1</td>
<td>0.99</td>
<td>0.5789</td>
</tr>
<tr>
<td>temp*s</td>
<td>1</td>
<td>1</td>
<td>0.15</td>
<td>0.7629</td>
</tr>
</tbody>
</table>
Main Effects model

The Mixed Procedure

Solution for Fixed Effects

| Effect  | temp | sal | Estimate | Error   | DF | t Value | Pr > |t| |
|---------|------|-----|----------|---------|----|---------|------|---|
| Intercept |     |     | 0.7513   | 0.09519 | 2  | 7.89    | 0.0157 |
| temp     | 20   |     | -0.1987  | 0.09519 | 2  | -2.09   | 0.1722 |
| temp     | 25   |     | 0        |         | .  | .       | .     |
| sal      | 0    |     | 0.02100  | 0.1166  | 2  | 0.18    | 0.8737 |
| sal      | 5    |     | 0.1900   | 0.1166  | 2  | 1.63    | 0.2447 |
| sal      | 10   |     | 0        |         | .  | .       | .     |

Type 3 Tests of Fixed Effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num</th>
<th>Den</th>
<th>DF</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>temp</td>
<td>1</td>
<td>2</td>
<td>4.36</td>
<td>0.1722</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sal</td>
<td>2</td>
<td>2</td>
<td>1.60</td>
<td>0.3851</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Reg Procedure

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2</td>
<td>0.05964</td>
<td>0.02982</td>
<td>1.28</td>
<td>0.3973</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>0.07015</td>
<td>0.02338</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5</td>
<td>0.12979</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Root MSE | 0.15291 | R-Square | 0.4595 |
Dependent Mean | 0.72233 | Adj R-Sq | 0.0992 |
Coeff Var | 21.16909 |

Parameter Estimates

| Variable | DF | Parameter Estimate | Standard Error | t Value | Pr > |t| |
|----------|----|--------------------|----------------|---------|------|---|
| Intercept | 1  | -0.16117           | 0.57044        | -0.28   | 0.7959 |
| temp     | 1  | 0.03973            | 0.02497        | 1.59    | 0.2098 |
| sal      | 1  | -0.00210           | 0.01529        | -0.14   | 0.8995 |
SAS Code

- Determination of normality for background (location 1) and effluent (location 2) datasets
- Determination of normality for log-transformed fecal coliform datasets
- Performance of t-test to determine difference between background and effluent

```sas
data one;
  input location FC;
  logFC=log(FC);
Datalines;
  1 26
  1 330
  1 130
  1 46
  1 13
  1 17
  1 4.5
  1 130
  1 130
  1 70
  1 708
  1 4
  1 22
  1 49
  1 33
  1 23
  1 14
  1 350
  1 2
  1 26
  1 130
  1 33
  1 17
  1 70
  2 2
  2 8.9
  2 2.4
  2 3.9
  2 4.8
  2 2.6
```
; proc univariate data=one normal;
  by location; var FC; var logFC;
run;

proc ttest data=one;
  class location; var logFC;
run;
quit;

SAS Output

Tests for Normality

------------------------------------------ location=1 -----------------------------------
Variable:  FC
  Test                  --Statistic---    -----p Value------
Shapiro-Wilk          W     0.604961    Pr < W     <0.0001

Variable:  logFC
  Test                  --Statistic---    -----p Value------
Shapiro-Wilk          W     0.979543    Pr < W      0.8873

------------------------------------------ location=2 -----------------------------------
Variable:  FC
  Test                  --Statistic---    -----p Value------
Shapiro-Wilk          W       0.8937    Pr < W      0.2532

Variable:  logFC
  Test                  --Statistic---    -----p Value------
Shapiro-Wilk          W     0.964457    Pr < W      0.8513

The TTEST Procedure

T-Tests

| Variable | Method      | Variances  | DF | t Value | Pr > |t| |
|----------|-------------|------------|----|---------|------|---|
| logFC    | Pooled      | Equal      | 30 | 4.43    | 0.0001|
| logFC    | Satterthwaite| Unequal    | 29.7| 6.73    | <.0001|

Equality of Variances

<table>
<thead>
<tr>
<th>Variable</th>
<th>Method</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>logFC</td>
<td>Folded F</td>
<td>23</td>
<td>7</td>
<td>7.98</td>
<td>0.0089</td>
</tr>
</tbody>
</table>
SAS Code

• Determination of normality for temperatures recorded for study 2 (Location 1) and study 3 (Location 2)
• Determination of normality for log-transformed datasets for studies 2 and 3
• Perform t-test to check the closeness of temperatures recorded

data one;
input location FC;
datalines;
1 17.8
1 18.6
1 18.3
1 23.9
1 22.4
1 22.4
1 24.1
1 23.2
1 22.4
1 22.4
1 24
1 23
1 22.3
1 22.2
1 23.2
1 21.9
1 21.8
1 24.2
1 22.8
1 23.8
1 22.4
1 22.4
1 24.8
1 24.8
1 23.3
1 23
1 25.5
1 22.4
1 23.0
1 22.9
1 23.6
1 23.2
1 22.2
1 22.6
1 22.6
1 21.4
1 21.7
1 23.3
1 23.4
1 22.7
1 22.6
1 22.7
1 22.7
1 23.1
1 24.6
1 23.5
proc univariate data=one normal;
by location; var FC;
run;
proc ttest data=one;
class location; var FC;
run;
quit;

SAS Output

Tests for Normality

------------------------------------------------ location=1 ------------------------------------------------

variable: FC

<table>
<thead>
<tr>
<th>Test</th>
<th>--Statistic---</th>
<th>-----p Value------</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shapiro-Wilk</td>
<td>W 0.823348</td>
<td>Pr &lt; W &lt;0.0001</td>
</tr>
</tbody>
</table>

variable: LogFC

<table>
<thead>
<tr>
<th>Test</th>
<th>--Statistic---</th>
<th>-----p Value------</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shapiro-Wilk</td>
<td>W 0.782296</td>
<td>Pr &lt; W &lt;0.0001</td>
</tr>
</tbody>
</table>
variable: FC

Test                Statistic       p Value
Shapiro-Wilk        W  0.964529     Pr < W  0.6116

variable: LogFC

Test                Statistic       p Value
Shapiro-Wilk        W  0.96859      Pr < W  0.7017

The NPAR1WAY Procedure (Non-Parametric Analysis)

Wilcoxon Scores (Rank Sums) for Variable FC
Classified by Variable location

<table>
<thead>
<tr>
<th>location</th>
<th>N</th>
<th>Sum of Scores</th>
<th>Expected Under HO</th>
<th>Std Dev Under HO</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>2067.50</td>
<td>1621.50</td>
<td>75.276000</td>
<td>43.989362</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>278.50</td>
<td>724.50</td>
<td>75.276000</td>
<td>13.261905</td>
</tr>
</tbody>
</table>

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic                278.5000

Normal Approximation
Z                    -5.9182
One-Sided Pr < Z    <.0001
Two-Sided Pr > |Z|   <.0001

t Approximation
One-Sided Pr < |Z|  <.0001
Two-Sided Pr > |Z|  <.0001

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square             35.1040
DF                     1
Pr > Chi-Square        <.0001
**SAS Code**
- Determination of the efficiency of fecal coliform retention for the three injection flowrates evaluated
- Analysis of Covariance method based on the proc mixed procedure

```sas
data filtration_efficacy;
title 'comparison of flowrates';
input VD FC FR;
logFC=log(FC);
cards;
0  277329  5.5
1.52 2.7   5.5
1.78 2402  5.5
2.15 23.8  5.5
3.05 5.5   5.5
0   71408  0.95
0.96 196.4 0.95
1.55 514.6 0.95
1.77 8    0.95
2.38 2.4  0.95
3.05 2.2  0.95
3.55 11   0.95
3.68 2    0.95
4.58 2.6  0.95
6.28 3.1  0.95
0 181357  1.9
0.96 1940  1.9
1.55 5072  1.9
1.77 5    1.9
3.05 40.4 1.9
3.55 2866 1.9
4.58 2    1.9
6.28 2.2  1.9
;
proc mixed data=filtration_efficacy;
classes FR;
model logFC =FR VD(FR)/solution;
contrast 'slopes equal' VD (FR) 1 0 -1;
contrast 'slopes equal' VD (FR) 0 1 -1;
```

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contrast 'slopes equal' VD (FR) 1 -1 0;
lsmeans FR;
run;
quit;

SAS Output

The Mixed Procedure

Contrasts

<table>
<thead>
<tr>
<th>Label</th>
<th>Num</th>
<th>Den</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>slopes equal</td>
<td>1</td>
<td>17</td>
<td>2.25</td>
<td>0.1517</td>
</tr>
<tr>
<td>slopes equal</td>
<td>1</td>
<td>17</td>
<td>1.92</td>
<td>0.1843</td>
</tr>
<tr>
<td>slopes equal</td>
<td>1</td>
<td>17</td>
<td>0.05</td>
<td>0.8330</td>
</tr>
</tbody>
</table>

Least Squares Means

| Effect | FR | Estimate | Error | DF | t Value | Pr > |t| |
|--------|----|----------|-------|----|---------|-------|
| FR     | 0.95 | 3.5168 | 0.9131 | 17 | 3.85 | 0.0013 |
| FR     | 1.9  | 5.6689 | 1.0152 | 17 | 5.58 | <.0001 |
| FR     | 5.5  | 2.3887 | 1.6582 | 17 | 1.44 | 0.1679 |

SAS Code

- Weibull Distribution Fit for fecal coliform for the worst case single camp study

dm "log;clear;out;clear";
data syn st;
title1 'WCS 2nd ring VD(15min/hr) (FC)Weibull Distribution Fit';
title2 'B Addo';
input FC;
cards;
81
4.9
29
26
26;
proc reliability;
distribution Weibull;
probplot FC;
run;
quit;
### SAS Output

#### Weibull Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Error</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV Location</td>
<td>3.5958</td>
<td>0.3508</td>
<td>2.9083</td>
<td>4.2833</td>
</tr>
<tr>
<td>EV Scale</td>
<td>0.7436</td>
<td>0.2586</td>
<td>0.3761</td>
<td>1.4702</td>
</tr>
<tr>
<td>Weibull Scale</td>
<td>36.4448</td>
<td>12.7846</td>
<td>18.3248</td>
<td>72.4824</td>
</tr>
<tr>
<td>Weibull Shape</td>
<td>1.3449</td>
<td>0.4678</td>
<td>0.6802</td>
<td>2.6592</td>
</tr>
</tbody>
</table>

#### Other Weibull Distribution Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>33.4423</td>
</tr>
<tr>
<td>Mode</td>
<td>13.2491</td>
</tr>
<tr>
<td>Median</td>
<td>27.7511</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>25.1282</td>
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</tbody>
</table>

#### Weibull Percentile Estimates

<table>
<thead>
<tr>
<th>Percent</th>
<th>Estimate</th>
<th>Error</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.21435191</td>
<td>0.41306159</td>
<td>0.00490727</td>
<td>9.36298645</td>
</tr>
<tr>
<td>0.2</td>
<td>0.3590227</td>
<td>0.62850319</td>
<td>0.01161484</td>
<td>11.097642</td>
</tr>
<tr>
<td>0.5</td>
<td>0.71039303</td>
<td>1.07852268</td>
<td>0.03623996</td>
<td>13.925462</td>
</tr>
<tr>
<td>1</td>
<td>1.19163697</td>
<td>1.60066571</td>
<td>0.08565801</td>
<td>16.575358</td>
</tr>
<tr>
<td>2</td>
<td>2.00268174</td>
<td>2.3414338</td>
<td>0.20250247</td>
<td>19.805834</td>
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<tr>
<td>5</td>
<td>4.00398957</td>
<td>3.76626518</td>
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</tr>
<tr>
<td>10</td>
<td>6.83819747</td>
<td>5.26064569</td>
<td>1.51394381</td>
<td>30.886843</td>
</tr>
<tr>
<td>20</td>
<td>11.9473499</td>
<td>7.17058112</td>
<td>3.68464769</td>
<td>38.738922</td>
</tr>
<tr>
<td>30</td>
<td>16.9326669</td>
<td>8.52908609</td>
<td>6.30913808</td>
<td>45.444434</td>
</tr>
<tr>
<td>40</td>
<td>22.1166972</td>
<td>9.69241455</td>
<td>9.36892954</td>
<td>52.209624</td>
</tr>
<tr>
<td>50</td>
<td>27.7511378</td>
<td>10.8627531</td>
<td>12.885199</td>
<td>59.768238</td>
</tr>
<tr>
<td>60</td>
<td>34.1511786</td>
<td>12.2478176</td>
<td>16.9096567</td>
<td>68.972606</td>
</tr>
<tr>
<td>70</td>
<td>41.8387954</td>
<td>14.1656475</td>
<td>21.546763</td>
<td>81.241961</td>
</tr>
<tr>
<td>80</td>
<td>51.9170629</td>
<td>17.273976</td>
<td>27.0455734</td>
<td>99.660723</td>
</tr>
<tr>
<td>90</td>
<td>67.7585368</td>
<td>23.6076082</td>
<td>34.2293533</td>
<td>134.131056</td>
</tr>
<tr>
<td>95</td>
<td>82.4030184</td>
<td>30.8515747</td>
<td>39.5598334</td>
<td>171.645249</td>
</tr>
<tr>
<td>99</td>
<td>113.448016</td>
<td>49.5556571</td>
<td>48.192598</td>
<td>267.062846</td>
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<tr>
<td>99.9</td>
<td>153.366586</td>
<td>78.3834395</td>
<td>56.3240634</td>
<td>417.606763</td>
</tr>
</tbody>
</table>

### SAS Code

- Weibull Distribution Fit for fecal coliform for the 0.95 L/min single camp study
dm "log;clear;out;clear";
data syn st;
title1 '0.9L/min (15min/hr) (FC)Weibull Distribution Fit';
title2 'B Addo';
input FC;
cards;
2
9
2.4
2;
proc reliability;
distribution Weibull;
procplot FC;
run;
quit;

SAS Output

Weibull Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV Location</td>
<td>1.6090</td>
<td>0.4099</td>
<td>0.8055 - 2.4125</td>
</tr>
<tr>
<td>EV Scale</td>
<td>0.6678</td>
<td>0.2961</td>
<td>0.2801 - 1.5923</td>
</tr>
<tr>
<td>Weibull Scale</td>
<td>4.9978</td>
<td>2.0488</td>
<td>2.2379 - 11.1616</td>
</tr>
<tr>
<td>Weibull Shape</td>
<td>1.4974</td>
<td>0.6639</td>
<td>0.6280 - 3.5703</td>
</tr>
</tbody>
</table>

Other Weibull Distribution Parameters

<table>
<thead>
<tr>
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<th>Value</th>
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<tr>
<td>Mode</td>
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<tr>
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<td>Standard Deviation</td>
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Weibull Percentile Estimates

<table>
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<th>Estimate</th>
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<th>95% Confidence Limits</th>
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## Weibull Percentile Estimates

<table>
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<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
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### SAS Code

- **Weibull Distribution Fit for fecal coliform for the 1.9 L/min single camp study**

```sas
dm "log;clear;out;clear";
data syn st;
title1 '1.9L/min (15min/hr) (FC)Weibull Distribution Fit';
title2 'B Addo';
input FC;
cards;
3.9
4.8
2.6
6.4
3.7
;
proc reliability;
distribution Weibull;
procplot FC;
run;
quit;
```

### SAS Output
Weibull Parameter Estimates

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<tr>
<th>Parameter</th>
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<th>Error</th>
<th>Lower</th>
<th>Upper</th>
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<tr>
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<td>1.5584</td>
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Other Weibull Distribution Parameters

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Weibull Percentile Estimates

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Weibull Distribution Fit for *Escherichia coli* for the worst case single camp study

**SAS Code**

```sas
dm "log;clear;out;clear";
data syn st;
title1 'WCS 2nd ring VD(15min/hr) (EC)Weibull Distribution Fit';
title2 'B Addo';
input EC;
cards;
76
6.8
50
34
18;
proc reliability;
distribution Weibull;
probplot EC;
run;
quit;
```

**SAS Output**

**Weibull Parameter Estimates**

Asymptotic Normal

<table>
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<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Error</th>
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<th>Upper</th>
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<tr>
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**Other Weibull Distribution Parameters**

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**Weibull Percentile Estimates**

Asymptotic Normal

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148
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SAS Code

- Weibull Distribution Fit for *Escherichia coli* for the 0.95 L/min single camp study

```sas
dm "log;clear;out;clear";
data syn st;
title1 '0.95L/min (15min/hr) (EC) Weibull Distribution Fit';
title2 'B Addo';
input EC;
cards;
2
5.5
2.5
2.0
;
proc reliability;
distribution Weibull;
probplot EC;
run;
quit;
```

SAS Output

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<tr>
<th>Parameter</th>
<th>Estimate</th>
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<th>Lower</th>
<th>Upper</th>
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Other Weibull Distribution Parameters

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## Weibull Percentile Estimates

<table>
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<tr>
<th>Percent</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Limits Lower</th>
<th>95% Confidence Limits Upper</th>
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### SAS Code

- Weibull Distribution Fit for *Escherichia coli* for the 1.9 L/min single camp study

```sas
dm "log;clear;out;clear"

data syn st;
title1 '1.9L/min (15min/hr) (EC)Weibull Distribution Fit';
title2 'B Addo';
input EC;
cards;
3.3
4.8
2.6
3.0
2.8

proc reliability;
distribution Weibull;
procplot EC;
run;
quit;
```

Weibull Parameter Estimates
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0.9L/min (15min/hr) (FC) Weibull Distribution Fit

1.9L/min (15min/hr) (FC) Weibull Distribution Fit
WCS 2nd ring VD(15min/hr) (EC) Weibull Distribution Fit

0.95L/min (15min/hr) (EC) Weibull Distribution Fit

1.9L/min (15min/hr) (EC) Weibull Distribution Fit
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### MUS Temperature Data – Single camp

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156
## MUS Temperature Data – Single camp

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### Notes

- The table above lists the temperature data for a single camp over a period from 4/9/03 to 7/20/03.
- Measurements are recorded at intervals of 0.25 GPM (0.95 L/min).
- NA indicates data not available for that date.
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Note: "clogged" indicates an issue with the temperature measurement.
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166
Appendix F: Field Data for Single Camp System (Fecal Coliforms/Escherichia Coli)
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## Data Summary – Fecal Coliforms/\textit{E. coli} in units of colonies/100mL

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## Data Summary – Fecal Coliforms/E. coli in units of MPN/100mL

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Data Summary – Fecal Coliforms/E. coli in units of MPN/100mL

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178
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**Note:** The table represents the results of the membrane filtration technique for fecal coliform and E. coli. The data includes the sample ID, dilution factor, filtered volume, original sample volume, fecal and E. coli counts, and their respective concentrations in coliforms per 100 mL.
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**Sampling Analysis**

- **Date:** 05/19/2003
- **Time:** Started 8:00am, Ended 11.00pm

**Date Time Started Ended**

- **Sampling Analysis:**
  - **Date:** 05/19/2003
  - **Time:** Started 8:00am, Ended 11.00pm
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Analysis | 06/23/2003 5.00 PM 10.00am
Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

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Sampling
07/07/2003 8:00am 1.10pm
Analysis
07/07/2003 5.00 PM 10.00am
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**Date:** 07/21/2003  
**Time:** 8:00am – 11:00pm

**Date:** 07/21/2003  
**Time:** 5:00 PM – 10:00am
## Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

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### Sampling
- Date: 08/04/2003
- Time: 8:00am - 1:10pm

### Analysis
- Date: 08/04/2003
- Time: 5:00 PM - 10:00am

### Notes
- Date and time of sampling and analysis are provided.
Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

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Analysis 08/22/2003 5.00 PM 10.00am
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- Analysis: 09/08/2003

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## Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

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| BE-15     | 11/03/2003 | 1        | 10                            | 3                          | 2                                   | 7.8             | 4.5                |
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| CE-15     | 11/03/2003 | 1        | 1                             | 1                          | 0                                   | 2               | 0                  |
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| DE-15     | 11/03/2003 | 10       | 1                             | 1                          | 0                                   | 40              | 0                  |
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| EE-15     | 11/03/2003 | 10       | 0                             | 1                          | 0                                   | 0               | 0                  |
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| FE-15     | 11/03/2003 | 1        | 10                            | 0                          | 0                                   | 0               | 0                  |
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| GE-15     | 11/03/2003 | 1        | 10                            | 0                          | 0                                   | 0               | 0                  |
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| HE-15     | 11/03/2003 | 10       | 1                             | 5                          | 5                                   | 2400            | 20                 |
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| IE-14     | 11/03/2003 | 1        | 3                             | 10                         | 0                                   | 0               | 0                  |
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| JE-14     | 11/03/2003 | 1        | 5                             | 10                         | 0                                   | 0               | 0                  |
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| KE-14     | 11/03/2003 | 1        | 5                             | 10                         | 0                                   | 0               | 0                  |
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Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

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- **Time Started:** 8:00am  
- **Time Ended:** 1.00pm

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- **Time Ended:** 11.30pm
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### Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

#### Sample ID | Dilution | LT broth positives | Volume of original sample (ml) | EC/MUG broth gas positives | EC/MUG broth fluorescence positives | Fecal MPN/100 mL | E. coli. MPN/100 mL
---|---|---|---|---|---|---|---
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AE-15 | 100 | 4 | 0.1 | 4 | 4 | 6200 | 4400
| 1000 | 4 | 0.01 | 4 | 2 | 2000 | 2000
| 10000 | 4 | 0.001 | 4 | 4 | 4000 | 4000
BE-15 | 10 | 5 | 1 | 5 | 5 | 700 | 460
| 100 | 4 | 0.1 | 2 | 1 | 200 | 100
| 1000 | 2 | 0.01 | 1 | 1 | 20 | 20
CE-15 | 1 | 5 | 1 | 5 | 5 | >1600 | 33
| 10 | 5 | 0.1 | 5 | 1 | 10 | 10
| 100 | 5 | 0.01 | 5 | 0 | 0 | 0
DE-15 | 1000 | 5 | 0.01 | 5 | 5 | 220000 | 46000
| 10000 | 4 | 0.001 | 4 | 1 | 1000 | 2000
| 100000 | 2 | 0.0001 | 2 | 1 | 100 | 100
EE-15 | 1000 | 5 | 0.01 | 5 | 3 | 220000 | 17000
| 10000 | 4 | 0.001 | 4 | 2 | 1000 | 1000
| 100000 | 2 | 0.0001 | 2 | 1 | 100 | 100
FE-15 | 100 | 5 | 0.1 | 4 | 1 | 3400 | 1100
| 1000 | 4 | 0.01 | 4 | 4 | 1000 | 1000
| 10000 | 0 | 0.001 | 0 | 0 | 0 | 0
GE-15 | 1 | 5 | 10 | 5 | 0 | 1600 | 0
| 10 | 5 | 1 | 5 | 0 | 10 | 10
| 1000 | 5 | 0.1 | 5 | 0 | 100 | 100
HE-15 | 1,000 | 5 | 0.01 | 5 | 4 | 110000 | 26000
| 10,000 | 3 | 0.001 | 3 | 2 | 1000 | 1000
| 100,000 | 1 | 0.0001 | 1 | 1 | 100 | 100
IE-14 | 100 | 5 | 0.1 | 5 | 4 | 17000 | 2200
| 1000 | 4 | 0.01 | 4 | 2 | 1000 | 1000
| 10000 | 1 | 0.0001 | 1 | 0 | 100 | 100
JE-14 | 1 | 0 | 10 | 0 | 0 | 0 | 0
| 10 | 0 | 1 | 1 | 0 | 10 | 10
| 100 | 0 | 0.1 | 0 | 0 | 0 | 0
KE-14 | 1 | 5 | 10 | 1 | 0 | 8.2 | 0
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| 100 | 3 | 0.1 | 1 | 0 | 100 | 100
LE-14 | 1 | 5 | 10 | 5 | 0 | 79 | 0
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| 100 | 2 | 0.1 | 0 | 0 | 0 | 0
IE-20 | 10 | 5 | 1 | 5 | 5 | 16000 | 9200
| 100 | 5 | 0.1 | 5 | 5 | 1000 | 1000

#### Sampling
- Date: 03/01/2003
- Time: 8:00am – 1:00pm

#### Analysis
- Date: 03/01/2003
- Time: 5:00 PM – 11:30pm
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### Fecal Coliform/E. coli – Multiple Tube Fermentation Technique

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MUS Temperature Data - Bayou Segnette Cluster Camp

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### Data Summary – Fecal Coliforms/E. coli Bayou Segnette Cluster Camp

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Note: Bacterial concentrations presented as colonies/100mL
Data Summary – Fecal Coliforms/E. coli Bayou Segnette Cluster Camp

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Note: Bacterial concentrations presented as colonies/100mL.
Data Summary – Fecal Coliforms/E. coli Bayou Segnette Cluster Camp

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Note: bacterial concentrations presented as MPN/100mL.
### Data Summary – Fecal Coliforms/E. coli Bayou Segnette Cluster Camp

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#### Notes:
- Bacterial concentrations presented as MPN/100mL

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Note: bacterial concentrations presented as MPN/100mL
### Data Summary – Fecal Coliforms/E. coli Bayou Segnette Cluster Camp

Note: bacterial concentrations presented as MPN/100mL

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Note: bacterial concentrations presented as MPN/100mL
### Data Summary – Fecal Coliforms/E. coli Bayou Segnette Cluster Camp

Note: bacterial concentrations presented as MPN/100mL

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- **Sampling Date:** 07/21/2003
- **Sampling Time:** 8:35am - 1.30pm

### Analysis Data
- **Analysis Date:** 07/21/2003
- **Analysis Time:** 3.45 pm - 8.20pm
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### Sampling Analysis

- **Date:** 08/22/2003
- **Time:**
  - Sampling: 10:00am - 2:30pm
  - Analysis: 5:00pm - 11:15pm
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Appendix I: Fecal Coliform/Escherichia Coli Contour Plots for Single Camp System
Fecal Coliform and Salinity Contours – Cluster Camp System

12/01/02 - Worst Case Study - 4.57 m Depth

12/16/02 - Worst Case Study - 4.57 m Depth

12/30/02 - Worst Case Study - 4.57 m Depth

01/20/03 - Worst Case Study - 4.57 m Depth

02/03/03 - Worst Case Study - 4.57 m Depth

12/16/02 - Worst case Study - 6.1 m Depth
Escherichia coli and Salinity Contours – Cluster Camp System

12/01/02 - Worst Case Study - 4.57 m Depth

12/16/02 - Worst Case Study - 4.57 m Depth

12/30/02 - Worst Case Study - 4.57 m Depth

01/20/03 - Worst Case Study - 4.57 m Depth

02/03/03 - Worst Case Study - 4.57 m Depth

01/20/03 - Worst Case Study - 6.1 m Depth
06/11/03 - 0.95 L/min Study - 3.96 m Depth

07/07/03 - 0.95 L/min Study - 3.96 m Depth

10/06/03 - 0.95 L/min Study - 3.96 m Depth

11/03/03 - 0.95 L/min Study - 3.96 m Depth

08/04/03 - 0.95 L/min Study - 2.74 m Depth

10/06/03 - 0.95 L/min Study - 2.74 m Depth
11/03/03 - 0.95 L/min Study - 2.74 m Depth

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2/01/03 - 1.9 L/min Study - 4.57 m Depth

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01/12/04 - 1.9 L/min Study - 4.57 m Depth

03/01/04 - 1.9 L/min Study - 4.57 m Depth

03/22/04 - 1.9 L/min Study - 4.57 m Depth
Vita

Benjamin Kojo Addo was born on May 8, 1978, in Akwatia, the eastern region of Ghana. He graduated from his high school, Pope John Secondary School and Junior Seminary in 1996. Following high school he attended the Kwame Nkrumah University of Science and Technology where he earned a bachelor of science degree in geological engineering in March 2002. Kojo has been a full time graduate student at Louisiana State University in the Department of Civil and Environmental Engineering since August 2002 and is presently a candidate for the degree of Master of Science in Civil Engineering.