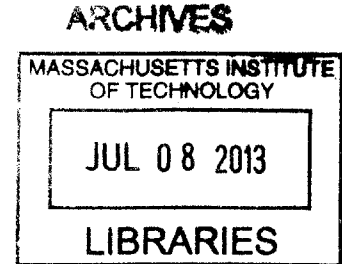


Biofilms as Sources of Fecal Bacteria Contamination in the Stormwater Drainage System in Singapore

by

Tsung Hwa Burkhart

B.S. Civil Engineering
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Submitted to the Department of Civil and Environmental Engineering
in Partial Fulfillment of the Requirements of the Degree of

Master of Engineering
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Signature of Author _____
Tsung Hwa Burkhart
Department of Civil and Environmental Engineering
May 10, 2013

Certified By _____
Peter Shanahan
Senior Lecturer of Civil and Environmental Engineering
Thesis Supervisor

Accepted By _____
Heidi M. Nepf
Chair, Departmental Committee for Graduate Students

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ABSTRACT

A study was performed to examine a possible source of fecal bacteria contamination originating from within the stormwater drainage system in Singapore. The extent of fecal bacteria presence in storm drain biofilms was evaluated as a pathway for fecal bacteria contamination. In the research, biofilms were evaluated as reservoirs for fecal indicator bacteria (FIB), FIB concentrations were measured over time within biofilms and stormwater, and relationships between FIB in biofilms and FIB in stormwater were examined. The concentrations of three bacterial groups (total coliform, *Escherichia coli*, and enterococci) were used as indicators of fecal bacteria contamination. In the Singaporean districts of Choa Chu Kang and Toa Payoh, five locations within the storm drains were monitored once per week each between January 8, 2013 and January 22, 2013. Well-developed biofilms were observed and measured using concrete coupons in the storm drains at Choa Chu Kang Crescent, Verde View, Lorong 6 Toa Payoh, and two points at Lorong 8 Toa Payoh. An initial biofilm growth condition was observed for secondary research at Nanyang Technological University.

The biofilms in the storm drains were observed to be reservoirs for FIB due to measured concentrations of each fecal indicator. The measured FIB concentrations fluctuated over time in the biofilms and the overlying storm drainage waters due to natural processes within the biofilms and the storm drain environments. Greater fluctuations in FIB concentrations in biofilms than in storm drainage waters indicate that the stormwater is more stable and has additional sources of FIB contributing to the contamination. FIB detachment from biofilms is a potential pathway for fecal bacteria contamination of stormwater.

Thesis Supervisor: Peter Shanahan

Title: Senior Lecturer of Civil and Environmental Engineering

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1 Introduction

1.1 Background

This section was written in collaboration with Ndeye Awa Diagne, Margaret Hoff, and Halle Ritter.

1.1.1 Singapore Water Management

Singapore is commended by many organizations, including the World Health Organization, as model for integrated water resources management (Chen et al. 2011). This recognition is not because the small city-state has abundant water. Instead, Singapore lacks sufficient naturally occurring water resources to sustain its population of 4.8 million. Water limitations are serious enough to warrant Singapore's inclusion by the United Nations on its list of water-scarce countries (Ong 2010). Though the country's average annual rainfall of 2,400 mm is above the global average, Singapore's land area is not sufficient to harvest an adequate amount of that precipitation (Tan et al. 2009). Furthermore, the small island has no other natural sources of renewable freshwater, such as large surface water bodies and groundwater. Singapore consumes approximately 1.36 billion liters of water per day (Tortajada 2006), and that demand for water is projected to grow as the population will reach 6.5 million in the next 50 years (Chen et al. 2011). The country's scarce water resources will be further stressed in the future.

Much of Singapore's population has access to adequate water quantity and quality. As Chen et al. (2011) report, 100% of the population has consistent access to water of sufficient quantity to meet their consumption demands. Furthermore, 99.96% or higher of that water supply meets the World Health Organization (WHO) drinking water standard, which is generally considered sufficient to ensure water potability. Similarly, 100% of the population is reported to have access to adequate sanitation (Chen et al. 2011). The country's high performance despite of water scarcity is due to the careful management by Singapore's national water utility, the Public Utilities Board (PUB), of the country's four "National Taps" (Tan et al. 2009). The National Taps are Singapore's four sources of water.

Even though Singapore has an above-average amount of rainfall, the country does not have enough land area to collect most of the rainwater that falls. This spatial limitation has been the target of engineering projects in Singapore's history and has resulted in an intricate network of rainwater collection channels and reservoirs, which are considered the country's first National Tap (Tan et al. 2009). The rainwater collection system provides approximately 50% (Chen et al. 2011) of Singapore's daily water consumption of 1.36 billion liters (Tortajada 2006). Efforts to expand the collection of precipitation are continuing, including progressive rooftop harvesting schemes and a continuous expansion of the reservoir network, with the aim of transforming 90% of Singapore's land area into water catchment. Despite the advanced technology and the government's expansion of rainwater collection systems, physical limitations still necessitate other sources of water to meet the country's needs (Tan et al. 2009).

Singapore's second National Tap is imported water from Johor, Malaysia, making up another 40% of the country's water supply (Chen et al. 2011). Singapore has imported a large percentage of its water since it separated from the Federation of Malaysian States in 1965, but the relationship has often been tense and uncertain in the intervening years. At various times, Malaysia has threatened to discontinue the water supply for political or economic reasons and

agreement on pricing has been a long-standing issue (Chen et al. 2011). There is currently an agreement in place that will provide water to Singapore through 2061 at a price of less than S\$0.01 per 1,000 liters, but further terms are uncertain (Tortajada 2006). Driven by difficult relations with Malaysia, Singapore investigated other international sources for water, including Indonesia, but was deterred by high development costs and the inherent insecurity of relying on other nations for natural resources (Chen et al. 2011). Most recently, Singapore invested significant financial and political resources into careful water resource management, as well as the development of its third and fourth National Taps, desalination of seawater and reuse of wastewater, respectively, with the ultimate goal of national water independence (Tortajada 2006).

The Tuas Desalination Plant, the first large desalination plant in Singapore, cost S\$200 million and opened in 2005 (Chen et al. 2011). Even though desalination technology is rapidly improving, it still has a relatively low capacity and is very energy intensive. Accordingly, the Tuas Desalination Plant has the capacity to produce 113 million liters of water per day (less than 7% of the country's current water demand) at a cost of S\$0.78 per 1,000 liters (Tortajada 2006). This source of water is more than seventy times more expensive than imported water, but was still the lowest cost seawater desalination plant in the world as of 2011 (Chen et al. 2011). High costs and the need for improved technology in desalination have encouraged Singapore to explore water reuse technologies, which typically has lower economic costs than desalination but higher social barriers.

Reuse of highly treated wastewater has been explored as an alternative water source in Singapore since 1972, with the first operational treatment plant built in 2000 (Tortajada 2006). The recycled waste stream and fourth National Tap, locally termed "NEWater," is produced at four facilities across the country and will ultimately account for more than 30% of the national water supply (Chen et al. 2011). The water is treated to a higher level than necessary to meet standards for human consumption, so the majority of NEWater is currently used for industrial water needs rather than potable distribution. Since 2003, a small percentage of the recycled water has been designated for indirect potable use, in which the highly treated effluent is mixed into existing raw water sources (Ching 2010). The percentage of NEWater designated for indirect potable use is expected to rise, but will still remain much lower than industrial usages (Tortajada 2006). The cost of production will likely decrease as the technology evolves, like with desalination, but current reuse treatment costs are already low at approximately S\$0.30 per 1,000 liters, which is less than half the cost of desalination (Tortajada 2006).

Singapore's success in water provision, particularly in the category of water reuse, has largely been attributed to the organization of its water management institution, the PUB. Since 2001, the PUB has managed the entire water cycle within the country, including potable water delivery, sewage, waste treatment, and rainwater collection (Tan et al. 2009). Additionally, the PUB was given general autonomy over its functions, which allowed the agency unilateral authority over all aspects of water governance, such as pricing structures, regulatory frameworks, and enforcement mechanisms (Tortajada 2006). This governing structure reduces water management administrative barriers and improves the efficiency and effectiveness of implementation (Chen et al. 2011). Moreover, the PUB effectively includes the private sector when appropriate and fosters public acceptance and political will through its success (Tortajada 2006).

1.1.2 Singapore Water Quality Concerns

In 2006, the Public Utilities Board (PUB) launched the Active Beautiful Clean (ABC) Waters Programme. The main objective of the ABC Waters Programme is to encourage Singaporeans to be more conscious of water scarcity and the country's water bodies (PUB 2009). The ABC Waters Programme is a strategic initiative to open Singapore's reservoirs and waterways to the public for recreational activities. The recreational activities include kayaking, fishing, barbecuing, and picnicking, which may involve direct contact with the water bodies. However, water quality of the reservoirs and waterways has been a concern for the PUB. In fact, recent studies have observed contamination in the reservoirs and the storm drains feeding them. Urban runoff has been reported to contain high levels of pollutants including suspended solids, nutrients, heavy metals, and pathogenic bacteria (Wang 2012). In order to protect public health, ongoing studies and investigations have been conducted to evaluate the levels of contamination within reservoir catchments and bacteria loading to the reservoirs and waterways (Chua et al. 2010).

1.2 Past Work

Student teams from the Massachusetts Institute of Technology have collected water quality data at several locations across Singapore. In the past, bacterial, physical, and chemical water quality parameters have been analyzed from water samples taken over different timescales (Ekklesia 2011). Due to the observations of fecal indicator bacteria (FIB) presence in Singapore's storm drains, pathways for fecal contamination have been investigated. Doshi (2012) began an investigation of the pathways for underground sewer line leakage into storm drains and discovered the sewer pipe connections between buildings and sewer networks to be prone to damage. As a result, the leakage of sewage at building connections may significantly contribute to fecal contamination in the nearby stormwater. Another study analyzed the temporal and spatial patterns among land use, sewer age, and FIB concentrations (Shin 2012). Shin (2012) found greater concentrations of FIB in the relatively older sewers than the relatively newer sewers, suggesting sewer leakage to be a factor. However, without a diurnal pattern of fecal indicator concentrations, other factors can contribute to the FIB present in stormwater runoff.

1.3 Purpose and Scope

To support the request of the Public Utilities Board of Singapore in evaluating the quality of stormwater runoff, this research has been completed to aid in the understanding of bacterial contamination sources to water in the storm drains. A thorough understanding of contributing sources provides information essential to the eventual development of remedial options for drains and the protection of public health. This research aims to consider an alternative source of FIB in the stormwater runoff: the storm drain itself. Biological activity has been observed to be present in several storm drains in Singapore in the form of biofilm growths (Ekklesia 2012). Such a biofilm is illustrated in Figure 1, in which a concrete coupon (a thin cylinder of concrete) is obscured by some leaves and is surrounded by a biofilm growth. With the presence of sewage in the stormwater, FIB from sewage can come into contact with biofilms. Some FIB in the stormwater may attach to the biofilm surface, leading to in-place survival and growth of bacteria. As with the nature of biofilms, the detachment of clusters of FIB-rich biofilm into the flowing stormwater is inevitable.



Figure 1. Biofilm growth and accumulated leaves around concrete coupon in a storm drain located in the Verde View neighborhood of Singapore on December 28, 2012 (Ekklesia 2012).

1.4 Goals and Objectives

This research provides insight into a previously uninvestigated source of fecal bacteria contamination in Singapore's water conveyance systems. It evaluates the importance of bacteriological contamination that originates from within the stormwater drainage system to the overall fecal contamination problem in Singapore's waters. The goals of this research are fulfilled through the completion of three objectives.

The first objective is to determine if biofilms in the storm drains are indeed reservoirs for FIB. The FIB targeted in the study are total coliform, *Escherichia coli*, and enterococci. This objective is fulfilled through the sampling of existing biofilm growths in several storm drains and analyses for FIB concentrations. Given previous observations (Ekklesia 2012), the hypothesis is that when biofilm exist in a storm drain, there will likely be FIB presence in the biofilm.

The project's second objective is to measure any FIB growth within biofilms. In order to determine growth, the same biofilm colony is sampled over time and the FIB concentrations are measured and compared. It is hypothesized that if there is FIB presence in biofilm, the FIB concentrations in the biofilm will increase over time, given steady stormwater flow in the drain. However, stormwater flow is inherently unsteady due to erratic occurrences of rainstorms in

Singapore, so FIB concentrations in biofilm may not be observed to increase over time within a three-week sampling period.

The final objective is to identify possibilities for FIB detachment from biofilms and subsequent contamination of the overlying stormwater. To accomplish this objective, the stormwater directly downstream of a sampled biofilm is analyzed for FIB concentrations. I hypothesize that by measuring the FIB concentrations in stormwater, I can determine how the FIB concentrations in biofilms are correlated to those stormwater concentrations, if at all.

With the evaluation of a potential source of fecal bacteria contamination in Singapore's water, a better understanding of the country's stormwater quality can be achieved. The results of this study will provide insights into the role biofilms in the stormwater drainage system have in stormwater quality. If biofilms are indeed observed to be reservoirs for fecal bacteria and media for fecal bacteria multiplication and growth, future studies will be necessary in evaluating the magnitude and extent of their contribution to issues concerning fecal bacteria contamination of stormwater.

2 Literature Review

2.1 Water Quality Monitoring with Fecal Contamination Indicators

Pathogens are bacteria, protozoa, and viruses that cause disease in humans. Surface water can become contaminated with pathogens by sewage, wastes from humans and animals, and agricultural runoff entering the water (Alm et al. 2003). To monitor water quality, indicator bacterial levels are observed and the measured concentrations are compared with the concentrations defined in regulations. Though, it is not common practice to monitor for all possible pathogens that can be present in water (Savichtcheva and Okabe 2006; Rowny and Stewart 2012). Fecal indicator bacteria (FIB) for enteric pathogens such as *Escherichia coli* and intestinal enterococci are allochthonous bacteria (Balzer et al. 2010). Allochthonous bacteria are bacteria that originated from an environment a significant distance away from their current location. Allochthonous bacterial levels are typically used to determine the presence and extent of fecal contamination in surface water. The survival of these bacteria in aquatic environments depends on predation by grazers, transfer of plasmids among bacteria, nutrients, salinity, temperature, and sunlight intensity (Barcina et al. 1997). Guidelines from the United States Environmental Protection Agency (U.S. EPA) set FIB to include *E. coli* and *Enterococcus* sp. (USEPA 1986). Tests for total coliform bacteria, *E. coli*, and enterococci are often used to monitor water quality.

2.1.1 Total Coliforms

Total coliform bacteria can be present in the feces of humans and animals, soil, and submerged wood (USEPA 2012). Total coliforms are recommended for testing in drinking water but not in recreational waters.

2.1.2 Fecal Coliforms (*Escherichia coli*)

Fecal coliforms are a subgroup of total coliforms. A species of fecal coliform bacteria used to test recreational waters is *E. coli* (USEPA 2012). *E. coli* is only present in the feces of warm-blooded animals and humans. Low temperatures have been observed to prolong the survival of fecal coliforms within aquatic environments (Vasconcelos and Swartz 1976; Flint 1987; Terzieva and McFeters 1991; Craig et al. 2002a). In freshwater systems, the U.S. EPA standards recommend that *E. coli* levels do not exceed 126 colony-forming units (CFU) per 100 mL.

2.1.3 Fecal Streptococci (Intestinal Enterococci)

Fecal streptococci are present in the digestive tracts of warm-blooded animals and humans (USEPA 2012). A subcategory of fecal streptococci is enterococci. The most relevant species of enterococci from true fecal sources are *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Enterococcus durans*, *Enterococcus casseliflavus*, *Enterococcus gallinarum*, and *Enterococcus raffinosus* (Pinto et al. 1999; Balzer et al. 2010). The most common species of enterococci found in human feces are *Enterococcus faecalis* and *Enterococcus faecium* (Converse et al. 2009). Other species of enterococci may be environmental bacteria. The U.S. EPA standards for recreational waters recommend enterococci densities to not exceed 33 CFU per 100 mL in freshwater and 35 CFU per 100 mL in marine water (USEPA 1986).

2.2 Drawbacks of and Alternatives to Indicator Bacteria

No ideal fecal indicators are known, but the concentration in water of the commonly used FIB positively correlates with instances of human illness. For a fecal indicator to be ideal, it must be detected effectively analytically, be absent in water without pollution, have concentrations that correlate with the contamination amount, and have a longer life than pathogens (USEPA 2001). FIB detection cannot be distinguished between fecal contamination that originates from humans or other animals.

Bacteroides spp. may be a better indicator for fecal pollution because they are present in human feces in high concentrations and are not likely to survive or reproduce in a water environment (Converse et al. 2009). These anaerobic bacteria are found in the intestinal tract of humans and animals. They are able to adapt to the environment and nutrients provided in a human gastrointestinal tract. *Bacteroides* spp. are present in human feces in numbers of 10^{10} cells per gram dry weight of human feces (Salyers 1984). Fecal *Bacteroides* spp. can be quantified using quantitative polymerase chain reaction (QPCR) methods. A QPCR assay is executed by extracting DNA from samples and performing QPCR reactions.

2.3 Fecal Bacteria Presence in Biofilms

Both point sources and non-point sources contribute fecal bacteria to surface water, resulting in fecal bacteria being typically present in both the waters and sediments of marine and freshwater environments (Craig et al. 2002a; Byappanahalli et al. 2003; Balzer et al. 2010). Furthermore, several studies have discovered fecal bacteria to be very persistent in marine and freshwater sediments (Burton et al. 1987; Marino and Gannon 1991; Davies et al. 1995; Craig et al. 2002b, 2004). The sediments are bacterial reservoirs, which thereby create conditions under which bacteria can survive and multiply in aquatic environments.

Bacteria are commonly found in biofilms that form on any surface in a natural water environment (Castonguay et al. 2006). Microorganisms are able to attach to a surface, develop a biofilm colony, and eventually disperse into the water column after growth. Biofilms are structured communities of microorganisms contained by an extrapolymeric substance (Hall-Stoodley and Stoodley 2005). Figure 2 illustrates the three steps of a typical biofilm life cycle.

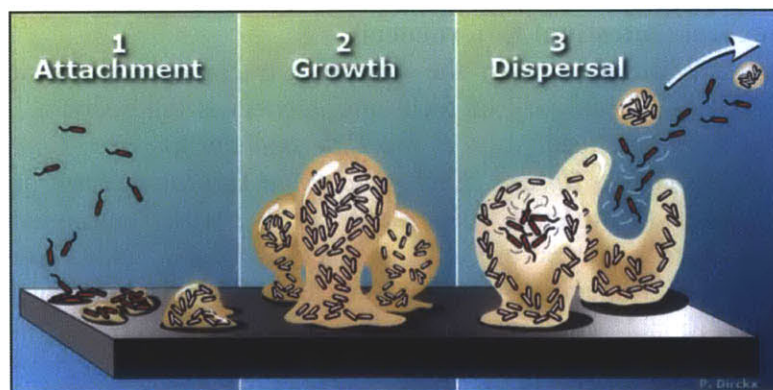


Figure 2. The biofilm life cycle (Cunningham et al. 2010).

Allochthonous microorganisms are the main component of surface water biofilms, but fecal bacteria may become associated with the biofilms if there is a source of fecal pollution (Balzer et al. 2010). The fecal bacteria remain associated with biofilms as the community supplies the bacteria with nutrients and shelter (Elhariry et al. 2012). Epilithic and sediment biofilms have been observed to have higher densities of allochthonous bacterial cells than the overlying water. Organisms that originally form the biofilm are considered to be autochthonous organisms. Autochthonous organisms are the opposite of allochthonous organisms in that they are native to their present environment. There is evidence that the allochthonous *E. coli* has adapted to ensure survival within a biofilm, even with competition from the autochthonous microorganisms.

2.3.1 Sediments of Aquatic Environments

Enteric microorganisms are able to survive in the sediments of aquatic environments because sediments provide nutrients, protection from sunlight, and safeguard against grazers such as protozoa (Alm et al. 2003). The top layers of sediment in a riverbed or beach sand can also be sources of fecal coliforms. Literature focused on storm drain sediments, beach sand, and beach wrack give insight to how these media can harbor fecal bacteria similar to how biofilms can harbor fecal bacteria.

2.3.1.1 Storm Drain Sediments

Storm drains are observed to collect sediments that have been deposited from the conveyed water. The outlet of a storm drain can be the area with the most sedimentation due to the design of the drain (Marino and Gannon 1991). Competition and antagonism among the native microflora and predation by protozoa are factors that determine the survival of the fecal coliforms and fecal streptococci in the sediments. Indicator bacteria populations were observed to remain at high densities in storm drain sediments despite the effects of predation, competition, and antagonism among the microorganism community. The predation by protozoa is a limiting factor for the survival of fecal coliforms and fecal streptococci in storm drain sediments and other aquatic environments, while predation by bacteria is not as important. Fecal coliform and fecal streptococci densities in creek sediments were observed by Marino and Gannon (1991) to remain stable during dry weather without significant external bacterial inputs, implying that the multiplication rates of the fecal coliforms and fecal streptococci must have been equal to the predation rates. High concentrations of human-specific *Bacteroides* in storm drains were concluded to be due to in situ growth, direct contamination, or indirect contamination (Sercu et al. 2009). The studies of storm drain sediments provide insight into the dynamics of fecal bacterial communities within storm drain environments.

2.3.1.2 Beach Sand

Coastal sediments can be reservoirs for pathogens and the associated fecal coliforms (Craig et al. 2002a). The survival and regrowth of fecal indicator bacteria may be possible in beach sediments (Lee et al. 2006). Several studies have measured concentrations of fecal bacteria in beach sands. *E. coli* and enterococci counts were seen to be higher within swash-zone sand sediments to a depth of 20 cm than in the overlying water of beaches on Lake Huron, St. Clair County, Michigan (Alm et al. 2003). At coastal sites in the greater Adelaide metropolitan area of South Australia, the concentration of fecal coliforms in the sediments was higher than in the overlying water (Craig et al. 2002a). *E. coli*, enterococci, and fecal coliform levels were found to be higher in sand than in the shoreline waters of Florida, with greater levels of *E. coli*,

enterococci, and fecal coliform occurring in dry sand than in wet sand (Hartz et al. 2008). A comparison of the generations of fecal bacteria in sand versus water in the Florida study is given in Table 1. Regardless of the temperature and salinity conditions of the sand and water environments, the number of generations of *E. coli* and enterococci produced in the experiments were greater in the sand than in the water.

Table 1. The number of generations of fecal bacteria after 4 days. *Escherichia coli* was kept at 30 °C and enterococci were maintained at 20 °C (Hartz et al. 2008).

Parameter	<i>E. coli</i>		Enterococci	
	Sand	Water	Sand	Water
Temperature (°C)				
20	14	1	9	0
30	18	0	4	0
40	9	0	4	0
Salinity (PSU)				
6	10	0	7	0
13	15	0	4	0
32	18	1	9	0
38.5	15	0	6	0

In situ growth, runoff from land, or filtering of bacteria from tidal water by the sand could cause the numbers of indicator bacteria to be greater in sand than water. High levels of indicator bacteria in beach sand have been observed in areas that have no nearby source of sewage contamination (Bonilla et al. 2007). Microorganisms such as enterococci and *Bacteroidales* human-specific fecal markers were observed to survive for up to 20 days on average in beach sand after sewage deposition (Yamahara et al. 2012). High moisture sands have greater concentrations of fecal indicator bacteria than relatively dry sands. Fecal coliforms in sediments of freshwater and coastal water are often present in concentrations of at least one order of magnitude greater than the concentrations of fecal coliforms in the overlying water (Byappanahalli et al. 2003; Craig et al. 2002; Obiri-Danso and Jones 1999; Valiela et al. 1991). Beach sand performs many of the same functions as biofilms in providing a better habitat for fecal bacteria survival than the overlying waters of aquatic environments.

2.3.1.3 Beach Wrack

Beach wrack is composed of animal remains and detached marine vegetation, such as seaweed or marine algae, which have been washed to the tidelines of beach areas (FMSA 1999). The wrack may be present on dry sand above the coastal water line, on wet sand in the swash zone, or suspended in seawater in the surf zone (Imamura et al. 2011). In a study at Cowell Beach, Santa Cruz, California, FIB were found to be present in wrack, the highest concentrations being in dry wrack and the lowest in surf wrack. At beaches with wrack, FIB may be able to survive for a longer period of time than at beaches without wrack. Wrack can maintain moisture for FIB survival, provide nutrients, and protect against UV radiation from the sun. Storm drains contain debris that can mirror the function beach wrack has on beaches. Clusters of leaves or trash could be the “wrack” of storm drains.

2.3.2 Biofilms in Water Systems

Bacteria are often present in water distribution systems. Measurements of *E. coli*, enterococci, total coliforms, and sulfide-reducing bacteria are used to monitor drinking water quality within these systems (Batte et al. 2006). Bacteria grow in the pipes of water distribution systems and have greater regrowth on rough surfaces and sediments, where biofilms are most likely to be present (Chowdhury 2012). Biofilms in the pipes of water distribution systems may protect pathogenic bacteria from disinfection residuals. Nutrients are also provided to microorganisms by biofilms (Elhariry et al. 2012). Thus, biofilms in distribution systems can be sources of contamination as bacteria detach from the biofilm into the water (Batte et al. 2006). More FIB are present in the water of distribution systems with high residence times, low residual of disinfectant, and high temperatures. Fecal bacteria have particularly been observed to grow within biofilms in the end regions of water distribution systems (Lee and Kim 2003). The literature that describes the potential for biofilms to harbor FIB in water distribution systems implies that this phenomenon is also possible in the stormwater drainage systems of interest to this study.

Coupons are pieces of material that are used to observe biofilm growth. Biofilms in drinking water systems have been sampled by the use of coupons of test materials (Schwartz et al. 1998). Hardened polyethylene, hardened polyvinyl chloride (PVC), copper, and stainless steel are examples of coupon materials that meet the requirements for drinking water systems. Other studies have used cement, cast iron (Sibille et al. 1997), or plastics such as cross-linked polyethylene (PEX), high-density polyethylene (HDPE), and polypropylene (PP) (Manuel et al. 2007). These coupons are immersed in the water for several days to allow a biofilm to develop. The collection of biofilm samples either involves scraping the biofilm from the coupon into a volume of water, or immersing the coupon in a solution and sonicating it to remove the bacteria from the coupon. Biofilm grow on nearly every coupon material, but greater densities have been observed on cast iron, cement, and plastic materials.

Biofilms and sediments are potential sources of bacteria in storm drains. Groups in Southern California have studied biofilms within their storm drainage systems. Storm drains in Southern California were observed to convey fecal bacteria from upstream sources to downstream biofilms (Ferguson et al. 2011). Fecal coliform bacteria and enterococci have specifically regrown in storm drains and street gutters in Newport Beach, California (Skinner et al. 2010). Masses of bacteria in those storm drains have been detected to detach from the biofilm and enter the water column. As fecal bacteria do survive in various aquatic environments according to the literature, the aquatic environment provided by the stormwater drainage system in Singapore is likely supporting fecal bacterial survival and growth.

2.4 Water Contamination of Fecal Bacteria from Environmental Sources

Surface water can be contaminated by runoff pollution that is associated with increased flow rates. A common source of nonpoint source pollution is stormwater runoff (Parker et al. 2010), which often contains elevated concentrations of FIB (Pan and Jones 2012). Contaminant concentrations in stormwater are often represented by the “event mean concentration.” The event mean concentration is a single sample value that is compiled from a set of samples that were taken at different times during a runoff event; it is calculated as the total mass of contaminant (or number of organisms) divided by the total flow. The event mean concentration of FIB is often greater than the water quality criteria set by the U.S. EPA, but varies with the season. The overall

load of FIB in a stream can be due to stormwater runoff when high water flows release the bacteria from sediments (Craig et al. 2002a, 2004; Reeves et al. 2004; Surbeck et al. 2006; Krometis et al. 2007; Balzer et al. 2010; Stumpf et al. 2010). In the summer, the event mean concentration of FIB is relatively greater than in the spring (Pan and Jones 2012). The concentration of FIB in runoff is greater with greater concentration of dissolved organic carbon (Surbeck et al. 2010). Survival of *E. coli* and enterococci is also higher with greater concentrations of dissolved organic carbon and phosphorus.

If a site has low flows and little turbulence, fecal coliforms may have better survival during the winter (Craig et al. 2002a). Low temperatures and low levels of sunlight are characteristics of the winter season. Furthermore, peak concentrations of fecal coliform have been observed during high rainfall periods. The FIB levels in sediments of beaches in Santa Monica Bay, California were observed to peak with a storm (Lee et al. 2006).

3 Experimental Methods, Materials, and Procedures

3.1 Field Work: Collection of Field Data

3.1.1 Sampling Locations

Biofilm and water samples were collected from two districts in Singapore, Toa Payoh and Choa Chu Kang. The districts of Singapore are shown in Figure 3. In the Toa Payoh district, stormwater drainage sites near Lorong 6 Toa Payoh and Lorong 8 Toa Payoh were sampled. Samples were also taken from sites near Choa Chu Kang Crescent and Verde View in the Choa Chu Kang district. All locations were near high-density or low-density residential areas. The sampling locations were selected based on previous observations of bacteria presence in the stormwater and the ease of storm drain access (Shin 2011). A secondary study was done in a storm drain adjacent to Nanyang Technological University (NTU), which is in the Western Water Catchment district. In total there were five sampling points, one sampling point each at Lorong 6 Toa Payoh, Choa Chu Kang Crescent, Verde View, and NTU, and two sampling points at Lorong 8 Toa Payoh. Samples were collected following protocols given by Techniques of Water-Resources Investigations, Book 9 (Myers et al. 2007).

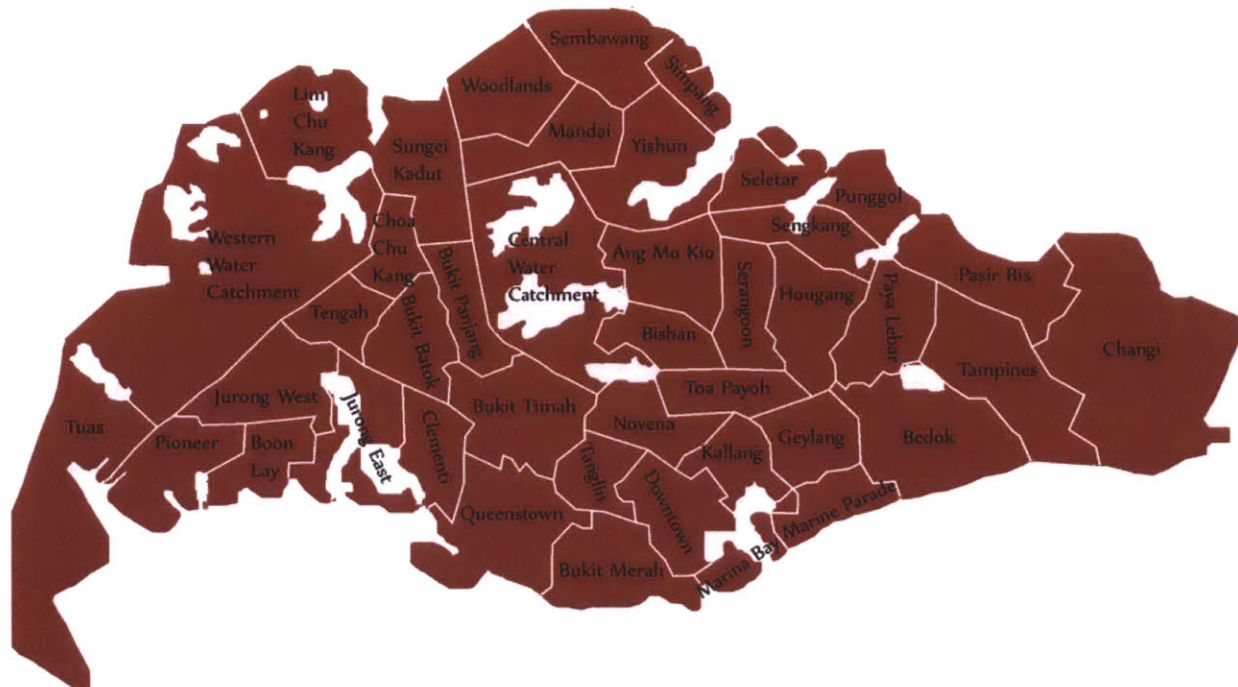


Figure 3. Map of Singapore districts (Property Market SG 2013).

3.1.2 Water Sampling

Water samples were collected from each of the five sampling points. Samples were collected from Lorong 6 Toa Payoh on January 14, 2013 and January 21, 2013. One sample each was collected from Verde View and Choa Chu Kang Crescent on January 16, 2013. The two

sampling points at Lorong 8 Toa Payoh were each sampled once on January 21, 2013. On January 22, 2013, a sample was taken from the Nanyang Technological University (NTU) point. Each sample was collected by gloved hand directly downstream of a concrete coupon in the drain. A 532-mL Whirl-Pak® sampling bag (Nasco, Fort Atkinson, WI, USA) was used to draw a sample of stormwater. Water was poured into the sampling well of an Ultrameter II Model 6PFC water quality meter (Myron L Company, Carlsbad, CA, USA) to measure the water temperature and pH on site. The samples were stored in ice chests at 4 °C at the sampling sites and transported in the ice chests to the refrigerator in the Environment Laboratory II at NTU.

3.1.3 Biofilm and Sediment Sampling

Biofilms and sediments were sampled from each of the five sampling points. Concrete coupons that had been sterilized in an oven and stored in plastic bags were used to measure biofilm growth. The method of using concrete coupons to measure biofilm growth was adapted from the procedure performed by Ferguson et al. (2011).

On December 11, 2012, a single hole to fit a PVC nail was drilled into six 10-cm diameter concrete coupons at the Nanyang Technological University (NTU) Hydraulics Laboratory. Five of the coupons were deployed in drains: one at Lorong 6 Toa Payoh, two at Lorong 8 Toa Payoh (one upstream in a tributary and one downstream in a larger channel), one at Verde View, and one at Choa Chu Kang Crescent. The coupons were attached to the drain surfaces by using a PVC nail hammered through the hole in the coupon into a depression drilled in the drain surface. Cable ties were used to attach the coupon in place. Two coupons were placed in the large drain near the NTU Civil and Environmental Engineering Department buildings on January 16, 2013 but the coupons were washed away by January 17, 2013. Two more coupons were placed in the same drain on January 21, 2013 and remained in place on January 22, 2013.

The concrete coupons were detached from the storm drains by cutting the cable ties and lifting them off of the PVC nails. During sampling, the concrete coupons were held on the rounded edges. Both the top (flat surface facing up) and the bottom (flat surface resting on the storm drain bottom) of each concrete coupon were sampled. The area to be sampled was initially estimated using a marked grid on a transparent sheet, a method that was demonstrated imprecise in the field because of its difficulty. Consequently, a 3-cm² surface area to be wipe-sampled was scribed in the biofilm surface prior to sampling by the use of the edge of a rectangular plastic container. The plastic container was washed with Milli-Q water, pressed into the biofilm surface, and then removed. Sterile cotton swabs were used to wipe samples of biofilms and sediments from the concrete coupon surfaces that were within the scribed area. The used cotton swab was placed in 30 mL of Milli-Q water in an amber glass vial and capped on site. The amber glass vials was washed three times with a 1:10 bleach dilution in the NTU Hydraulics Laboratory and three times with Milli-Q water on site prior to sample collection. Vials were stored in ice chests at 4 °C at the sampling sites and transported in the ice chests to the refrigerator in the Environment Laboratory II at NTU.

On January 8, 2013 at Lorong 8 Toa Payoh, the top of the coupon at the upstream point and the top and bottom of the coupon at the downstream point were sampled by gloved hand using a sterile cotton swab to wipe 1 cm² of the coupon surface. A blank 30-mL sample of Milli-Q water was prepared at Lorong 8 Toa Payoh and stored with the other samples. On January 9, 2013 and January 16, 2013, 3-cm² samples of the top and bottom of the coupons at Verde View

and Choa Chu Kang Crescent were swabbed. On January 14, 2013 and January 21, 2013, 3-cm² samples of the top and bottom of the coupons at Lorong 6 Toa Payoh and Lorong 8 Toa Payoh were collected. The tops of two unused coupons were swabbed in the same manner on January 14, 2013 and January 21, 2013 in the NTU Hydraulics Laboratory. On January 22, 2013 3-cm² samples of the top and bottom of a coupon in a drain near the NTU Civil and Environmental Engineering Department buildings were collected. A 30-mL Milli-Q water sample blank was prepared in the NTU Hydraulics Laboratory on January 22, 2013.

3.2 Laboratory Procedures

3.2.1 Bacteria Analysis in Water

The water samples were undisturbed in the refrigerator for 24 hours or less before bacteria analysis was performed. The most probable number (MPN) method was used to analyze the concentrations of total coliform, *Escherichia coli*, and enterococci in the water samples. In the MPN method, the concentrations of microorganisms present in a sample are estimated by performing a replicate 10-fold dilution series (Sutton 2010). Total coliforms and *E. coli* were quantified using Colilert-18® growth media and Quanti-Tray/2000® sample trays (IDEXX Laboratories, Inc., Westbrook, ME, USA). The procedure for the detection and enumeration of *E. coli* and coliform bacteria is provided in the package inserts for Colilert-18® and Quanti-Tray/2000® (IDEXX 2012a). The procedure involves mixing the contents of a Colilert-18® packet into a sterile vessel containing the sample and dilution water, then emptying the vessel into the Quanti-Tray/2000® sample tray. Enterococci were quantified using Enterolert™ growth media and Quanti-Tray/2000® sample trays (IDEXX Laboratories, Inc., Westbrook, ME, USA). The ASTM-approved procedure for testing for enterococci in water using Enterolert™ is also provided in the package insert (IDEXX, 2012b). The procedure is nearly identical to those for the *E. coli* and total coliform. Dilutions of 1:1, 1:100, and 1:1,000 of the water sample with Milli-Q water were used in order to account for cell counts exceeding 2,419 MPN per 100 mL of water sample, which is the maximum that can be counted with the IDEXX procedure (IDEXX 2011). A volume of 100 mL ± 1 mL of mixed water sample dilution and growth media was poured into a Quanti-Tray/2000® and sealed immediately. The Quanti-Tray/2000® sample trays for total coliform and *E. coli* analysis were incubated at 35 °C. The Quanti-Tray/2000® sample trays for enterococci were incubated at 41 °C. All Quanti-Tray/2000® sample trays were incubated for 24 to 28 hours. After the incubation period, the Quanti-Tray/2000® sample trays were removed for MPN enumeration based on indications of color or fluorescence in accordance with the IDEXX procedure (IDEXX 2011). For total coliform counts, the numbers of yellow wells of the 49 large wells and 48 small wells were each counted. Yellow wells indicate a positive total coliform presence. For *E. coli* and enterococci counts, the numbers of yellow wells that were fluorescent with 365-nm ultraviolet light were counted as positive. The MPN per 100 mL was determined from the positive well counts using the IDEXX conversion table.

3.2.2 Bacteria Analysis in Biofilm

The biofilm and sediment samples (consisting of cotton swabs and 30 mL of Milli-Q in amber vials) were undisturbed in the refrigerator for 24 hours or less before bacteria analysis was performed. To prepare for analysis, the amber vials containing samples were removed from the refrigerator and manually shaken vigorously for 30 seconds to dislodge the biofilm and sediments from the cotton swabs and homogenize the liquid in the vials. The cotton swabs were inspected through the vials to determine if any material was still attached, and if so, were shaken

for an additional 10 seconds. Total coliforms, *E. coli*, and enterococci were analyzed with the same method as the water samples except for the dilutions. For the biofilm samples, the dilutions used were 1:10, 1:1,000, and 1:100,000. The most probable number method was used to analyze the concentrations of total coliform, *E. coli*, and enterococci in the biofilm and sediment samples.

4 Results

4.1 Visual Examination of Biofilms

A large sample of biofilm was collected from the concrete floor of the storm drain near the upstream coupon (C1) at Lorong 8 Toa Payoh on January 21, 2013 and preserved in a freezer. To the naked eye, the sample appeared to consist of fibrous brown-colored material. On April 12, 2013 the sample was examined under a microscope using three methods. Light microscopy was used to examine the general structure of the biofilm, as shown in Figure 4. The biofilm appears to contain a variety of organic matter and long filaments. Figure 5 illustrates a different section of the biofilm, and highlights an algal cell.

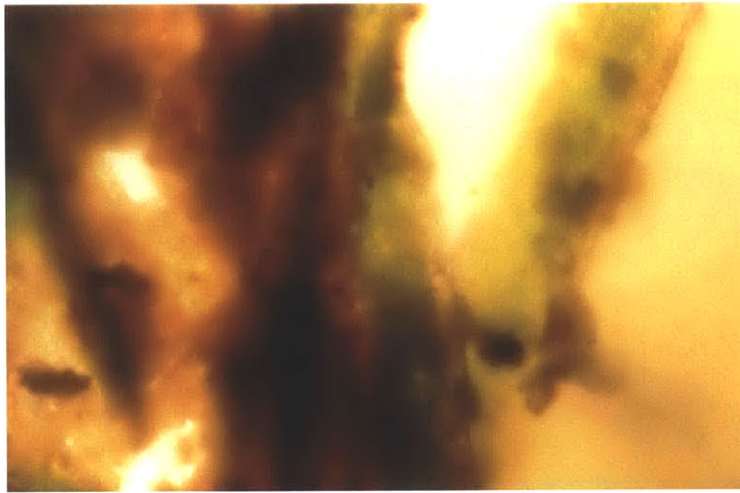


Figure 4. A biofilm specimen collected in a storm drain at Lorong 8 Toa Payoh magnified 400x.

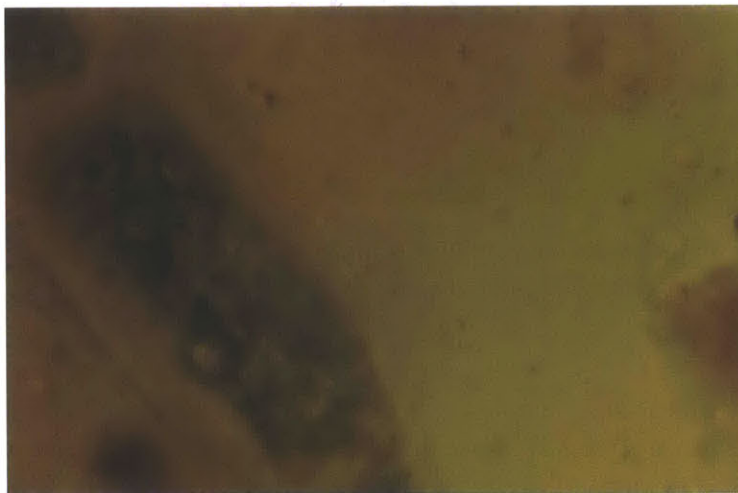


Figure 5. A biofilm algal cell from a storm drain at Lorong 8 Toa Payoh at 400x magnification.

Another method used to examine the biofilm was gram staining of the sample. In a gram-stained sample, the gram-positive microorganisms are blue or violet in color and the gram-negative microorganisms are pink or red in color (ASM 2013). Gram-positive cells, such as enterococci cells, have thick peptidoglycan layers and gram-negative cells, such as total coliform and *Escherichia coli* cells, have thin peptidoglycan layers. In gram staining, the sample that has been air-dried and heat-fixed to a slide is flooded with a crystal violet staining agent. After one minute, the sample is rinsed with tap water and then flooded with Gram's iodine. Again, the sample is washed with tap water but is flooded with a decolorizing agent. After rinsing, safranin is used as a counterstain and the sample is rinsed again. The samples were viewed under a Brightfield microscope. Gram-positive, gram-negative, and few gram-variable cells appeared to be present in the biofilm sample, as indicated in Figure 6 (a). Though, the sample contained more gram-negative cells than any other type. Two algal filaments are shown in Figure 6 (b) and (c).

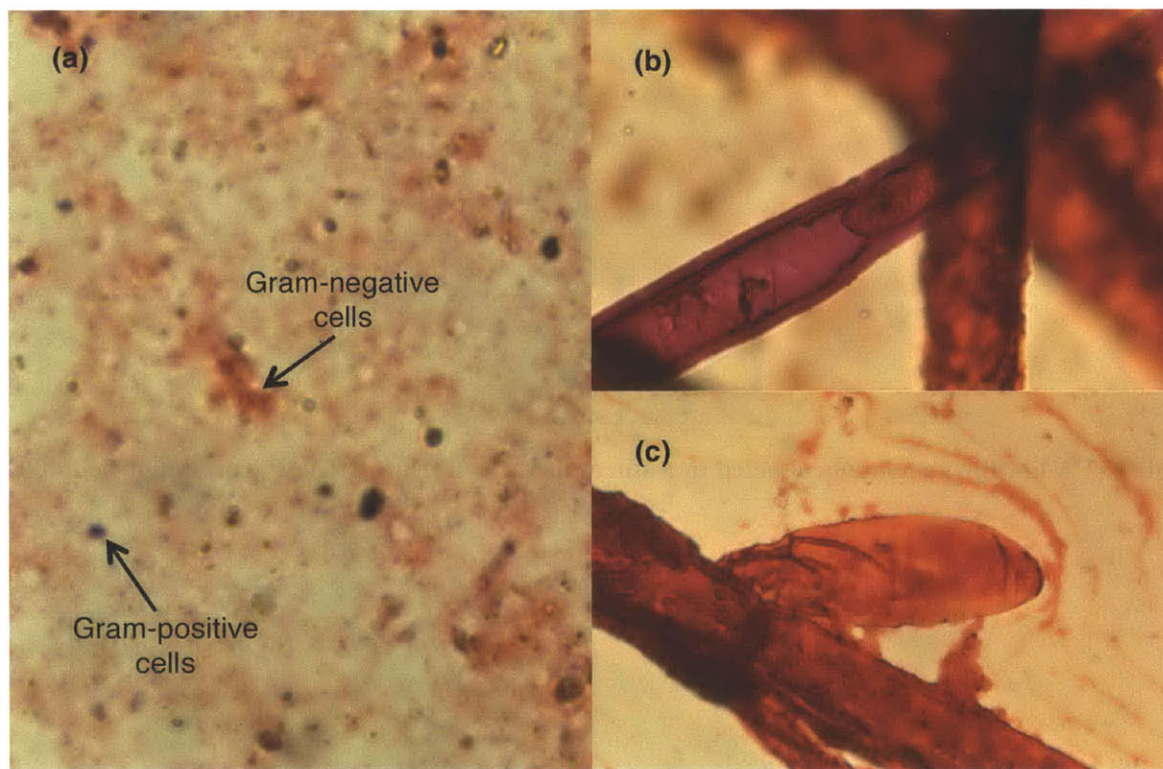


Figure 6. A portion of a gram stained biofilm sample from a storm drain in Lorong 8 Toa Payoh viewed at 1000x in (a) and 400x in (b) and (c).

Fluorescence microscopy was used to examine the sample for cells of bacteria. The sample was stained with a green fluorescent nucleic acid stain. Bacteria will fluoresce green under green fluorescent ultraviolet light (Haberkorn et al. 2011). When exposed to red fluorescent ultraviolet light, chlorophyll fluoresces red. The red fluorescence is shown in Figure 7, while the green fluorescence is illuminated in Figure 8. The fluorescence microscopy showed that different types of microorganisms gather in groups within the biofilm structure. The green

fluorescence photographs indicate clusters of bacteria cells are present within an extracellular polymeric substance, while the red fluorescence photograph highlights clusters of larger photosynthetic cells.

The biofilm specimen collected from Lorong 8 Toa Payoh was highly varied in composition and structure. The biofilm appeared to contain organic matter and photosynthetic materials. Different microorganisms were present among the separate filaments within the biofilm structure, and separate portions of the biofilm structure harbored different colonies of bacterial and algal cells.

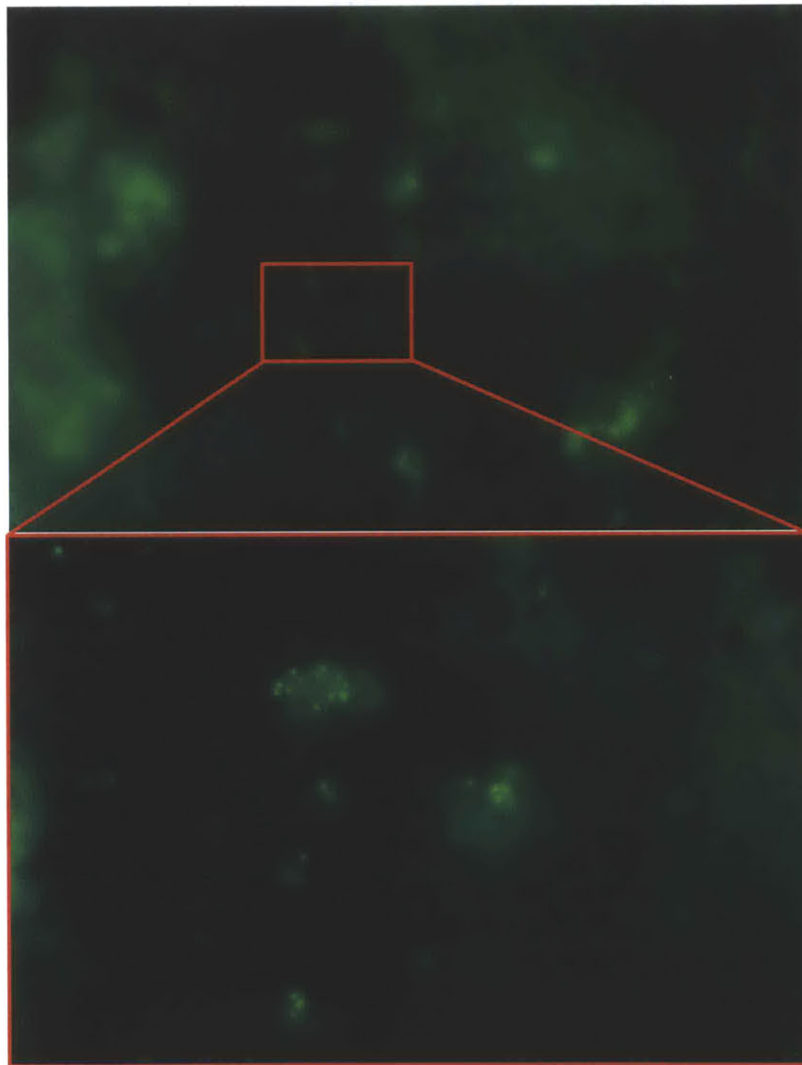


Figure 7. Bacteria cells fluorescing bright green in a biofilm sample from a storm drain in Lorong 8 Toa Payoh at 400x (top) and 1000x (bottom) magnification.

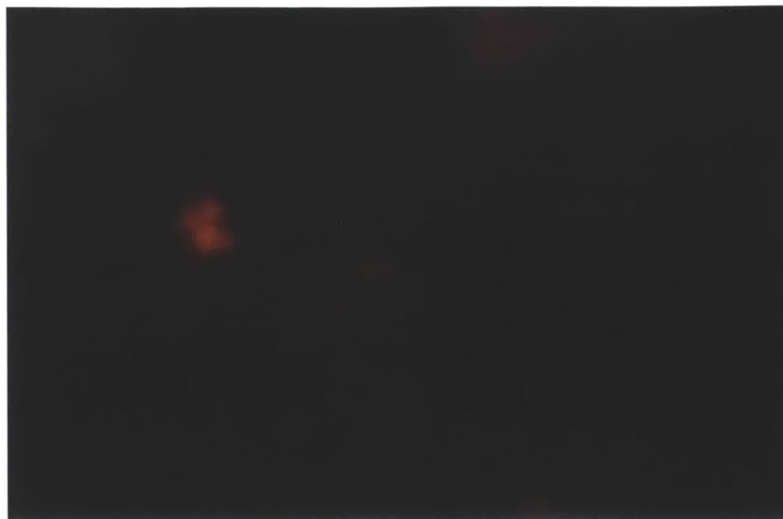


Figure 8. Chlorophyll-containing algal cells fluorescing red in a biofilm sample from a storm drain in Lorong 8 Toa Payoh, viewed at 1000x magnification.

4.2 Fecal Indicator Bacteria in Biofilms

An analysis of the concentrations of fecal indicator bacteria (FIB) was performed on the biofilm samples collected between January 8, 2013 and January 22, 2013 at Choa Chu Kang (CCK) Crescent, Verde View, Lorong 6 Toa Payoh, Lorong 8 Toa Payoh, and Nanyang Technological University (NTU). To determine the MPN per 100 mL values of FIB, Colilert-18® growth media and Quanti-Tray/2000® sample trays (IDEXX Laboratories, Inc., Westbrook, ME, USA) were used to analyze for total coliforms and *E. coli* and Enterolert™ growth media and Quanti-Tray/2000® sample trays (IDEXX Laboratories, Inc., Westbrook, ME, USA) were used to analyze for enterococci. Dilutions of 1:10, 1:1,000, and 1:100,000 were performed on the samples to determine the most representative MPN per 100 mL of FIB.

A total of 25 biofilm samples were collected, along with two wash water controls (C0BLAW and C12BLAW) and two concrete coupon controls (C6BLAC and C11BLAC). The wash water controls were prepared by placing an unused sterile cotton swab into an amber vial filled with 30 mL of water, while the concrete coupon controls were prepared by swabbing an unused concrete coupon. Both controls were analyzed in the same fashion as the biofilm samples, except only a 1:10 dilution was performed.

The FIB concentrations are better expressed in terms of MPN per surface area of concrete coupon surface. Instead of having FIB suspended in a certain volume, the FIB-containing biofilms were attached to the surfaces of the concrete coupons and were sampled within a designated surface area. In order to calculate the MPN per cm² of concrete coupon surface, the MPN per 100 mL values were first multiplied by the volume of water used in the initial rinse of the cotton swab wash (30 mL). It is assumed that the sample of biofilm attached to the cotton swab was negligible in volume compared to the 30 mL of wash water. Finally, the calculated MPN values were divided by the values for the surface areas sampled on the concrete coupons. The calculated concentrations of total coliform, *E. coli*, and enterococci in the biofilm samples are presented in Table 2, and the analysis results for the controls are shown Table 3.

Table 2. Fecal indicator bacteria concentrations in MPN/cm² on concrete coupon surfaces.

Sample Name	Date & Time Sampled	Sampling Location	Area Sampled (cm²)	Total Coliform (MPN/cm²)	<i>E. coli</i> (MPN/cm²)	Enterococci (MPN/cm²)
C1TOP	8 Jan 2013 11:00	Lorong 8 Toa Payoh	1.0	6,990	197	16
C1BOT	8 Jan 2013 11:00	Lorong 8 Toa Payoh	1.0	390,000	7,140	167
C2TOP	8 Jan 2013 11:30	Lorong 8 Toa Payoh	1.0	3,390,000	6,330	321
C3TOP	9 Jan 2013 13:40	Verde View	3.0	7,330	54	4
C3BOT	9 Jan 2013 13:40	Verde View	3.0	341,000	3,180	160
C4TOP	9 Jan 2013 14:45	CCK Crescent	3.0	15,200	613	23
C4BOT	9 Jan 2013 14:45	CCK Crescent	3.0	63,000	4,200	411
C5TOP	14 Jan 2013 11:15	Lorong 6 Toa Payoh	3.0	199,000	4,500	365
C5BOT	14 Jan 2013 11:15	Lorong 6 Toa Payoh	3.0	120,000	1,730	517
C1TOP2	14 Jan 2013 12:50	Lorong 8 Toa Payoh	3.0	2,850	13	9
C1BOT2	14 Jan 2013 12:50	Lorong 8 Toa Payoh	3.0	3,080,000	11,600	1,610
C2TOP2	14 Jan 2013 12:40	Lorong 8 Toa Payoh	3.0	22,800	51	18
C2BOT2	14 Jan 2013 12:40	Lorong 8 Toa Payoh	3.0	14,000	11	<1
C3TOP2	16 Jan 2013 10:18	Verde View	3.0	43,500	60	21
C3BOT2	16 Jan 2013 10:20	Verde View	3.0	420,000	3,150	125
C4TOP2	16 Jan 2013 11:00	CCK Crescent	3.0	105,000	214	30
C4BOT2	16 Jan 2013 11:05	CCK Crescent	3.0	61,300	152	19
C5TOP2	21 Jan 2013 11:20	Lorong 6 Toa Payoh	3.0	259,000	3,180	122
C5BOT2	21 Jan 2013 11:20	Lorong 6 Toa Payoh	3.0	691,000	7,380	69
C1TOP3	21 Jan 2013 12:25	Lorong 8 Toa Payoh	3.0	15,300	40	41
C1BOT3	21 Jan 2013 12:25	Lorong 8 Toa Payoh	3.0	155,000	3,930	517
C2TOP3	21 Jan 2013 12:15	Lorong 8 Toa Payoh	3.0	10,500	62	8
C2BOT3	21 Jan 2013 12:15	Lorong 8 Toa Payoh	3.0	6,200	8	2
C9TOP	22 Jan 2013 10:12	NTU Drain	3.0	44	<1	<1
C9BOT	22 Jan 2013 10:12	NTU Drain	3.0	411	<1	<1

Table 3. Fecal indicator bacteria concentrations in MPN/cm² for experimental controls.

Sample Name	Date & Time Sampled	Sampling Location	Area Sampled (cm ²)	Total Coliform (MPN/cm ²)	<i>E. coli</i> (MPN/cm ²)	Enterococci (MPN/cm ²)
C0BLAW	8 Jan 2013 11:00	Lorong 8 Toa Payoh	-	<1	<1	<1
C6BLAC	14 Jan 2013 15:00	NTU Hydraulics Lab Briefing Room	3.0	488	1	<1
C11BLAC	21 Jan 2013 9:08	NTU Hydraulics Lab Briefing Room	3.0	18	<1	<1
C12BLAW	22 Jan 2013 9:30	NTU Hydraulics Lab Briefing Room	-	<1	<1	<1

Lorong 8 Toa Payoh was selected for a case study because the most samples over time were collected at this location. Two concrete coupons were examined at Lorong 8 Toa Payoh, an upstream coupon (C1), and a downstream coupon (C2), depicted in Figures 9 and 10. Figure 11 illustrates the drain in which C2 is located and the tributary where C1 is located drains into. FIB concentrations on the coupon surfaces were compared over time and organized by each fecal indicator bacterium analyzed: total coliform, *E. coli*, and enterococci, demonstrated in Figures 12 and 13.



Figure 9. Concrete coupon C1 in the tributary at Lorong 8 Toa Payoh with a vertical concrete coupon upstream to block debris.



Figure 10. Coupon C2, located downstream of C1 at Lorong 8 Toa Payoh, with a vertical coupon upstream to block debris.



Figure 11. The main drain at Lorong 8 Toa Payoh, where C1 and C2 are located.

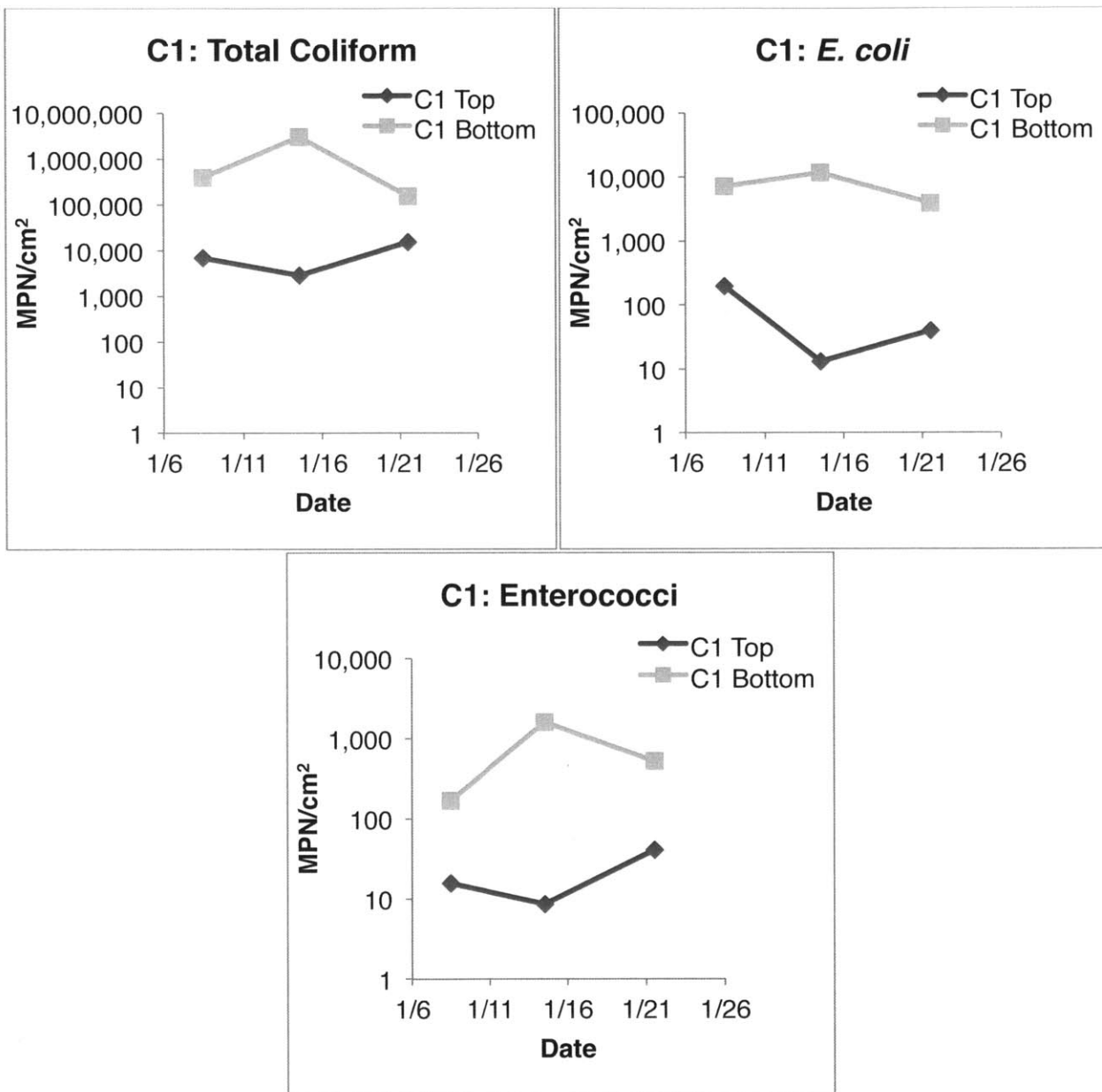


Figure 12. Fecal indicator bacteria concentrations over time on the top and bottom surfaces of the upstream coupon (C1) at Lorong 8 Toa Payoh.

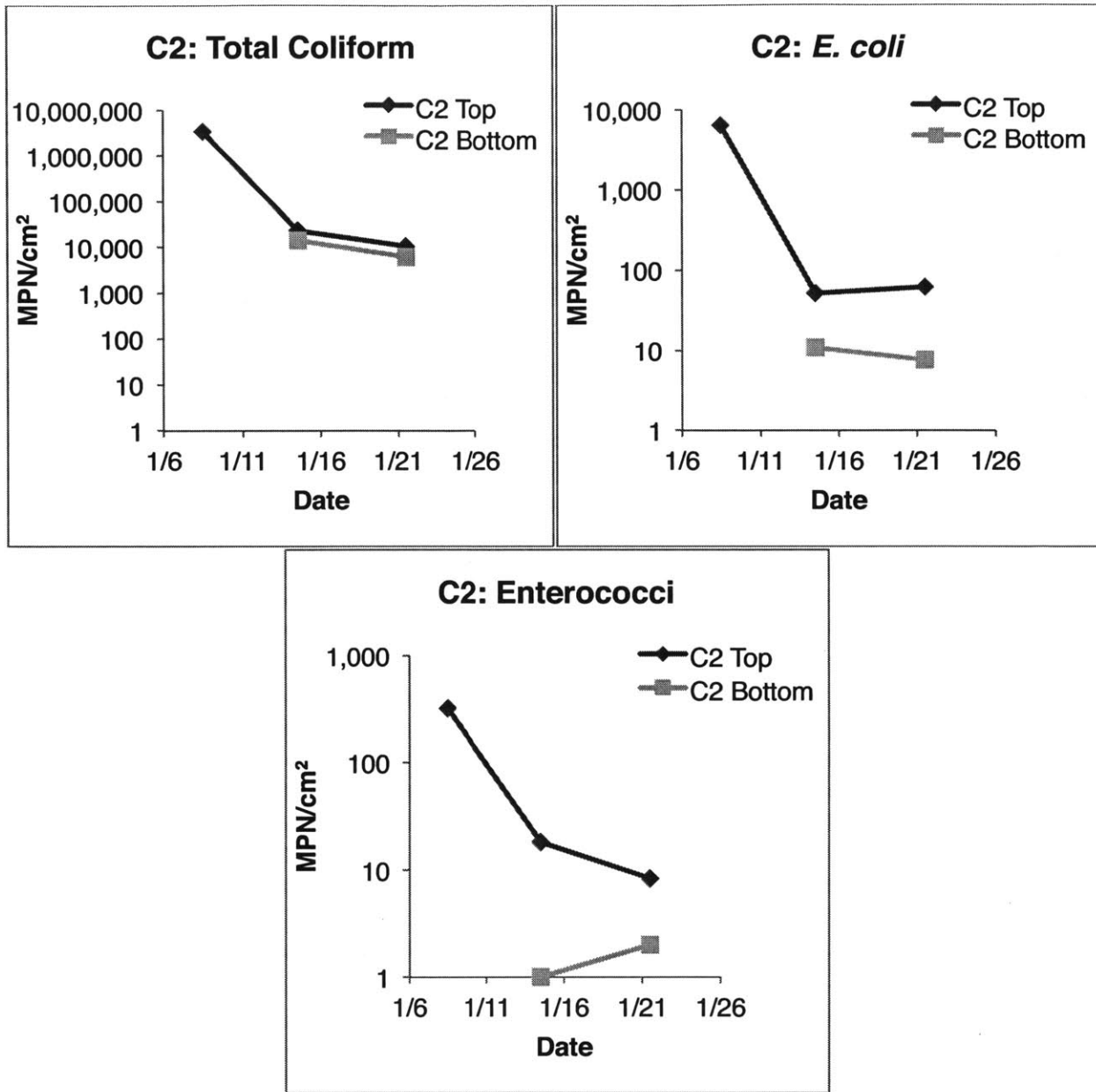


Figure 13. Fecal indicator bacteria concentrations over time on the top and bottom surfaces of the downstream coupon (C2) at Lorong 8 Toa Payoh.

4.3 Fecal Indicator Bacteria in Storm Drainage Waters

Storm drainage waters associated with the studied biofilms were also sampled between January 8, 2013 and January 22, 2013. An analysis of these water samples was performed to determine concentrations of FIB in the stormwater immediately downstream of the concrete coupons in the storm drains. The water samples were analyzed in the laboratory for the same FIB as the biofilm samples: total coliform, *E. coli*, and enterococci. However, the most representative MPN per 100 mL results were derived from 1:1, 1:100, and 1:1,000 dilutions and used without

adjustments, as demonstrated in Table 4. The pH and temperatures of the water samples were measured in the field for 9 out of 11 samples.

Table 4. Fecal indicator bacteria concentrations in MPN/100 mL in storm drainage water.

Sample Name	Date & Time	Sampling Location	pH	Temp. (°C)	Total Coliform (MPN/100 mL)	<i>E. coli</i> (MPN/100 mL)	Enterococci (MPN/100 mL)
C2WAT1	8 Jan 2013 11:48	Lorong 8 Toa Payoh	-	-	11,200,000	253,000	4,080
C1WAT1	8 Jan 2013 12:00	Lorong 8 Toa Payoh	-	-	6,130,000	512,000	15,700
C5WAT1	14 Jan 2013 11:15	Lorong 6 Toa Payoh	7.32	27.1	1,310,000	28,500	3,550
C2WAT2	14 Jan 2013 12:38	Lorong 8 Toa Payoh	7.33	27.8	1,020,000	5,560	3,550
C1WAT2	14 Jan 2013 12:38	Lorong 8 Toa Payoh	8.11	28.2	6,870,000	51,700	15,000
C3WAT1	16 Jan 2013 10:15	Verde View	7.60	27.0	2,760,000	141,000	100
C4WAT1	16 Jan 2013 10:55	CCK Crescent	8.17	26.9	1,270,000	48,800	2,990
C5WAT2	21 Jan 2013 11:10	Lorong 6 Toa Payoh	7.49	26.7	809,000	16,600	1,733
C2WAT3	21 Jan 2013 12:12	Lorong 8 Toa Payoh	7.39	20.3	155,000	3,930	517
C1WAT3	21 Jan 2013 12:23	Lorong 8 Toa Payoh	8.13	27.5	441,000	3,110	2,880
C9WAT1	22 Jan 2013 10:09	NTU Drain	7.25	25.0	13,800	921	1,710

Lorong 8 Toa Payoh is revisited as a case study for the concentrations of FIB in storm drainage water associated with the upstream coupon (C1) and downstream coupon (C2). Figures 14 and 15 compare total coliform, *E. coli*, and enterococci MPN/100 mL observed over time.

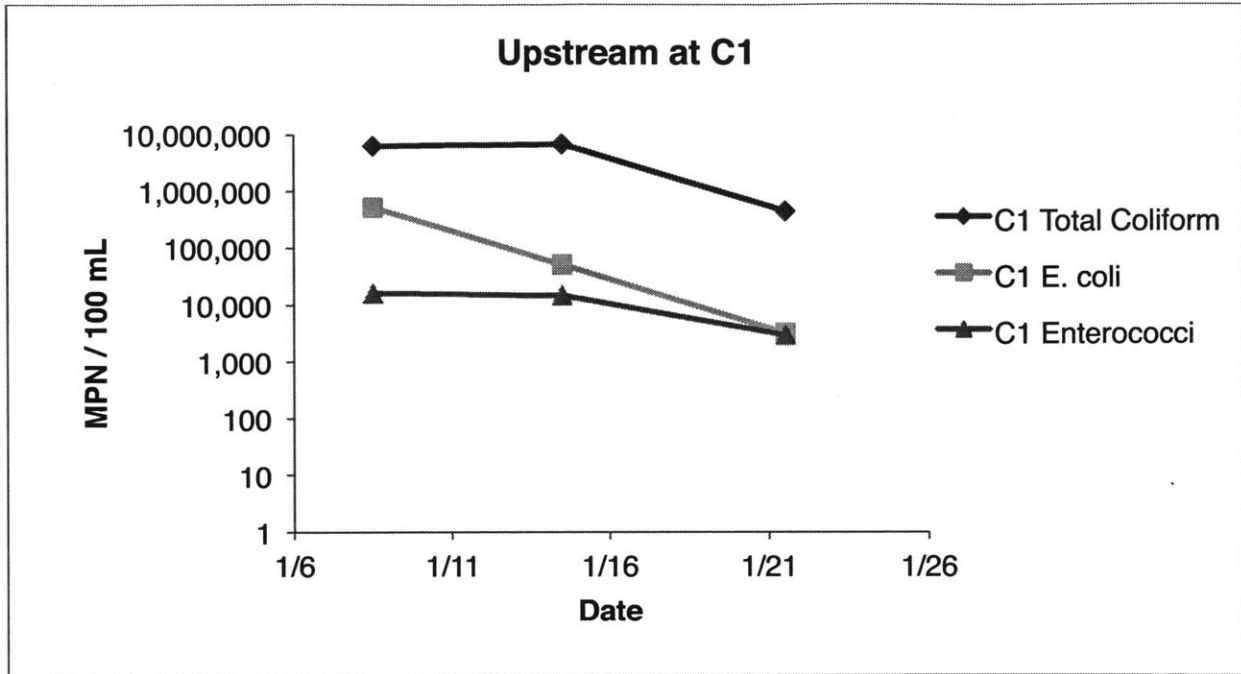


Figure 14. Fecal indicator bacteria concentrations over time in the stormwater immediately downstream of the upstream coupon (C1) at Lorong 8 Toa Payoh.

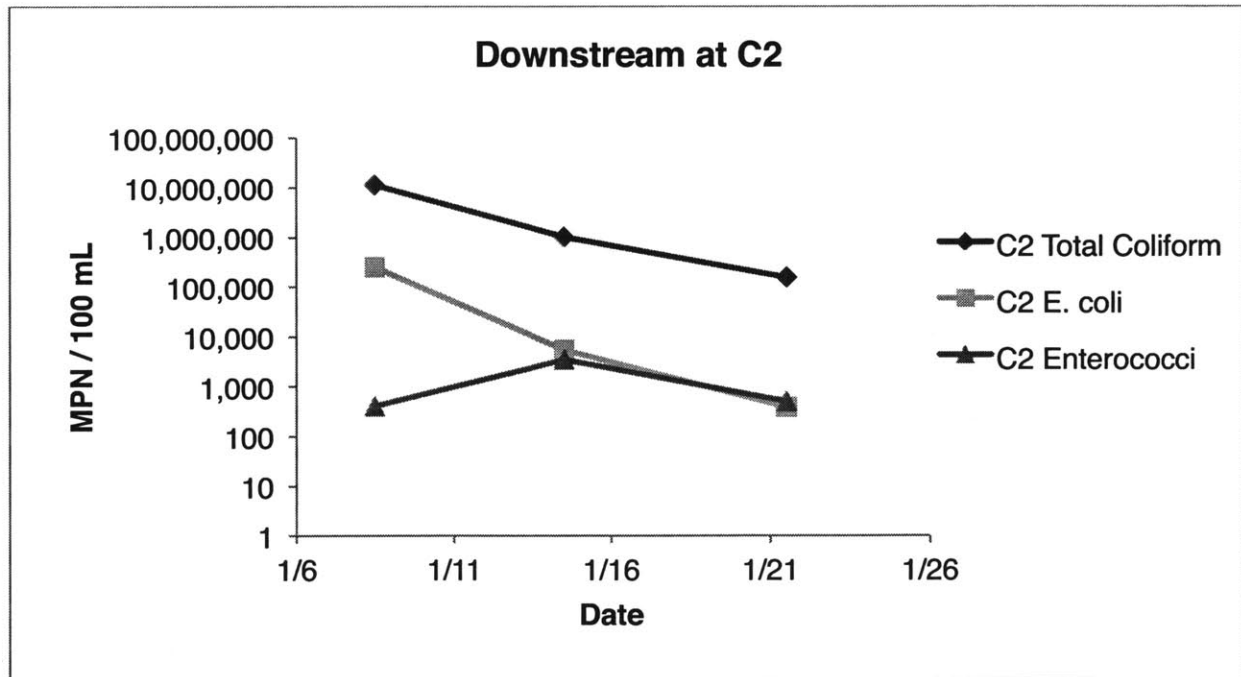


Figure 15. Fecal indicator bacteria concentrations over time in the stormwater immediately downstream of the downstream coupon (C2) at Lorong 8 Toa Payoh.

4.4 Relationships Between Biofilms and Storm Drainage Waters

The FIB concentrations on the coupon surfaces were compared with the FIB concentrations in the storm waters. These measurements were taken at the same locations and times. Figures 16 through 18 compare the concentrations of FIB in stormwater with concentrations on the tops and bottoms of concrete coupons.

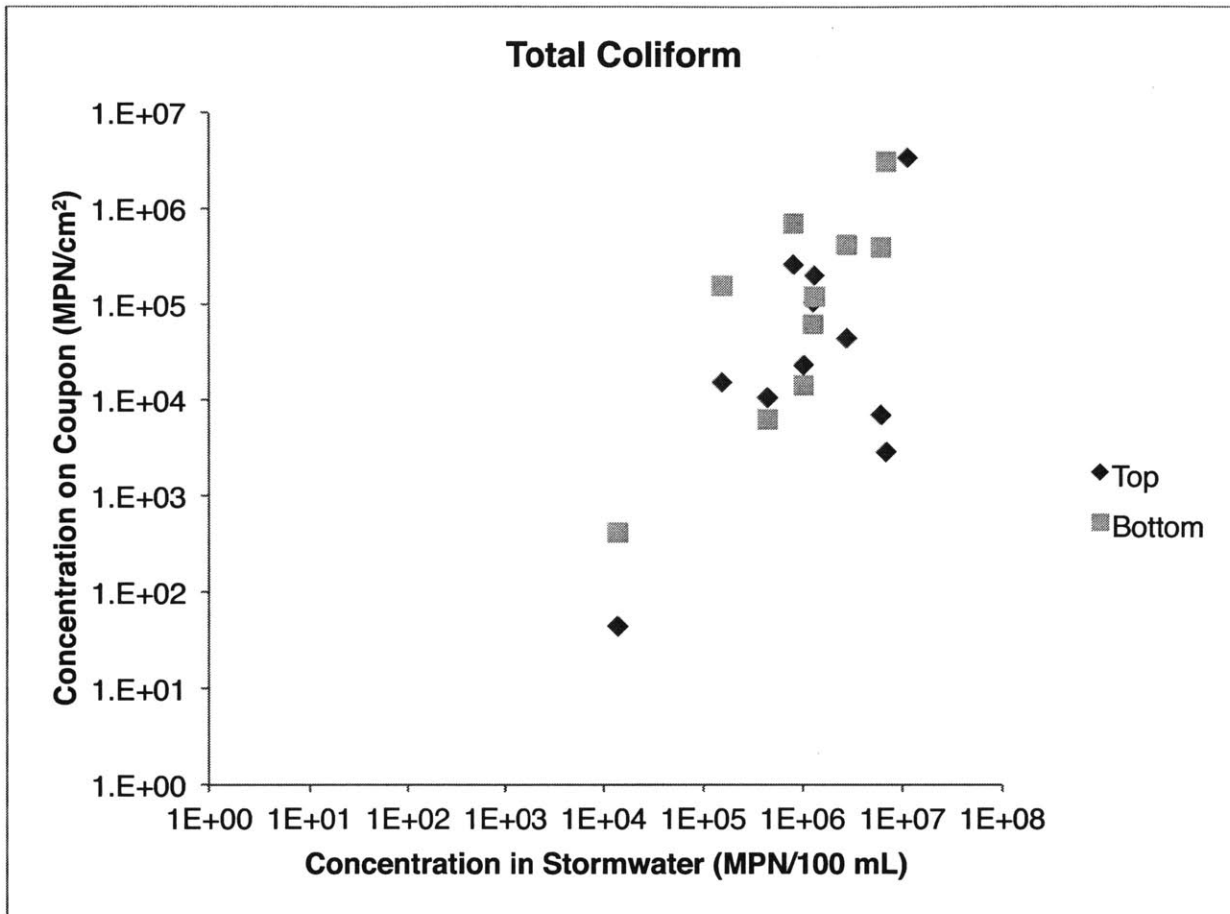


Figure 16. Total coliform concentrations in stormwater and on coupon surfaces.

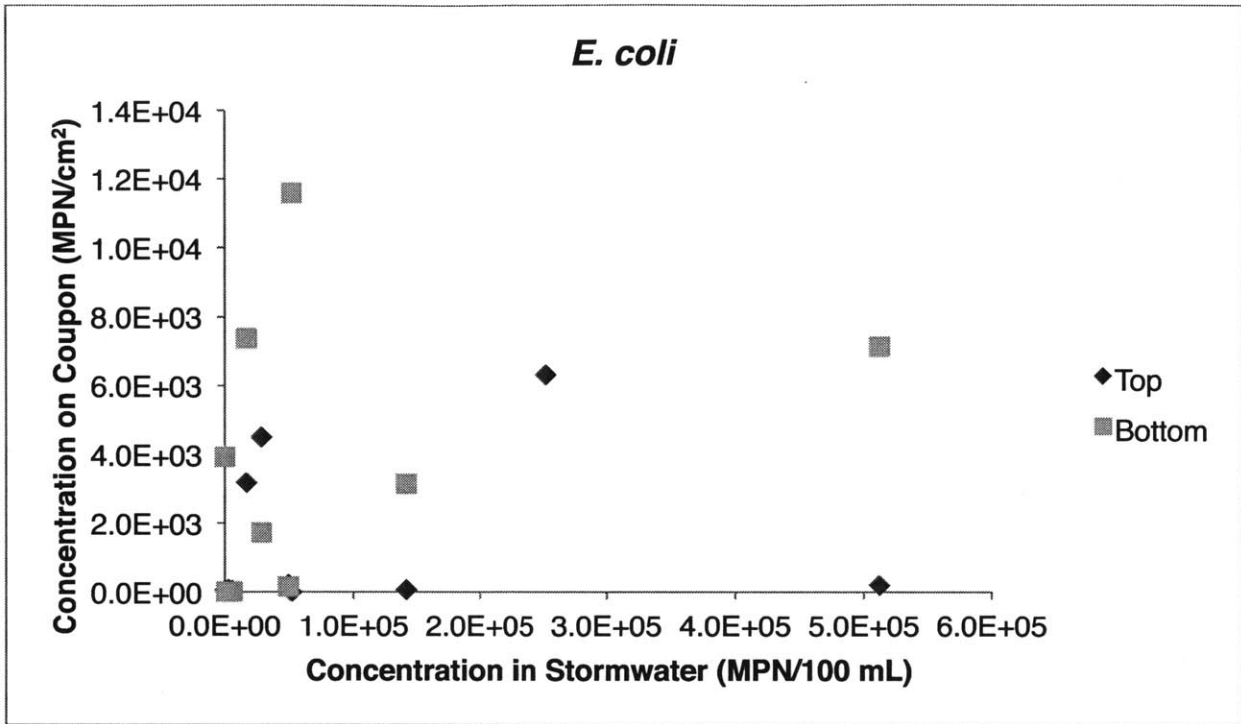


Figure 17. *Escherichia coli* concentrations in stormwater and on coupon surfaces.

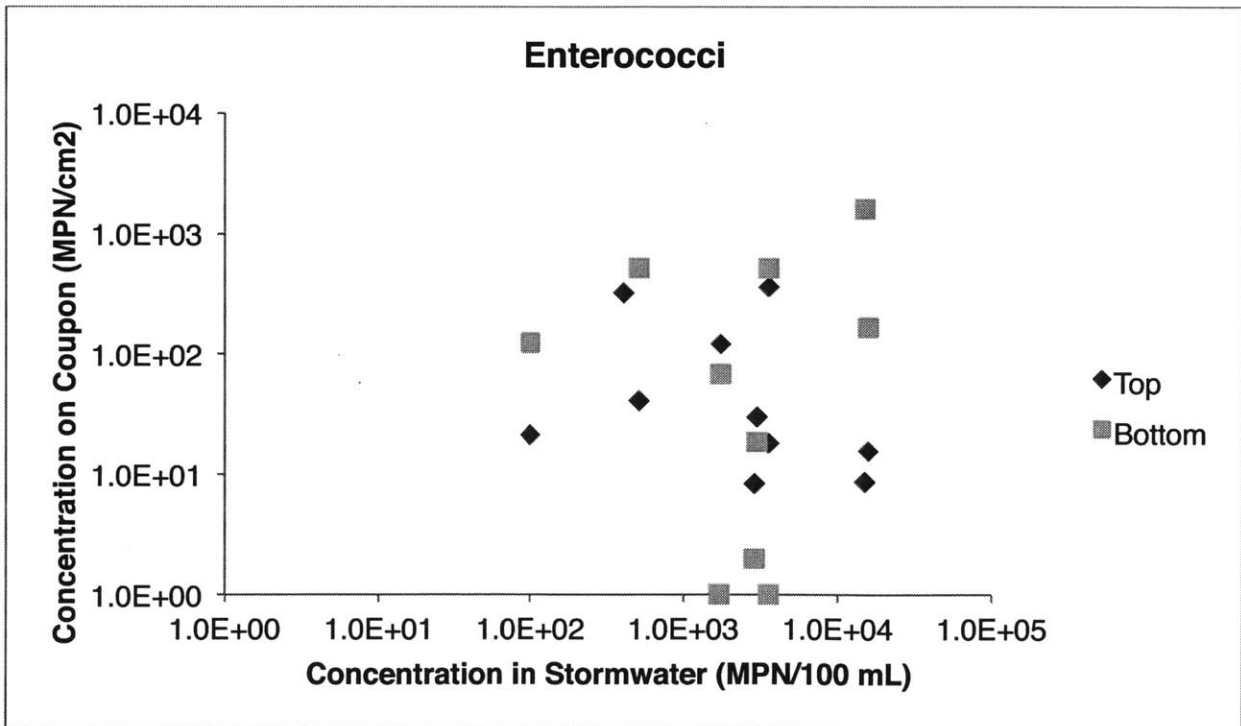


Figure 18. Enterococci concentrations in stormwater and on coupon surfaces.

4.5 Limitations

Several limitations arise from the laboratory procedures and calculation methods used. In the fecal indicator bacteria laboratory analysis, human errors were likely to occur. When the Quanti-Tray/2000® sample trays were sealed, some wells contained more liquid than others due to the presence of air bubbles. On two occasions, one well of the Quanti-Tray/2000® sample tray was empty of fluid due to unusually large air bubbles. In the cases where a well was empty, the well was counted as negative for bacteria presence unless all other wells were counted as positive. Additionally, errors could occur in counting the number of colored or fluorescing wells, as there was a spectrum of color and fluorescence intensity and only visual comparisons with the IDEXX standards were performed.

In the calculation of the MPN per 100 mL for four of the samples (total coliform analyses of C1BOT3 and C1WAT3, and *E. coli* analyses of C5WAT2 and C1WAT3), a value that was not the most representative out of each sample's three dilutions (1:1, 1:100, or 1:100,000 for stormwater, and 1:10, 1:1,000, or 1:10,000,000 for biofilms) was selected by following the enumeration methods specified by IDEXX for the use of the MPN conversion tables. However, these values indicated by the IDEXX procedure were considered not the most representative because the selected values, from the set of results for the three dilutions completed for each sample, had the greatest ranges for the confidence intervals. A value with the smallest confidence interval is considered most representative. The MPN-per-100-mL values for the four samples used in the final analysis were instead selected as larger MPN-per-100-mL values of the dilutions with smaller confidence intervals. The four samples were analyzed from the field samples collected on January 21, 2013 at Lorong 6 Toa Payoh and Lorong 8 Toa Payoh.

5 Analysis and Interpretation

5.1 Biofilm Structure

From the visual examination of biofilm samples, it is clear that the biofilm is varied in structure and composition. The organic matter observed with light microscopy in the biofilm likely provides nutrients and shelter to microorganisms living within the biofilm. By observing a more detailed structure using gram staining, gram-positive and gram-negative bacteria are observed to attach to the larger structures of the biofilm. Some of the structures present within the biofilm sample appear to be very similar to some attached algae depicted in Figure 19, which is Plate II from *Algae and Water Pollution* (USEPA 1977). A more detailed examination of the biofilm with fluorescence microscopy confirms the presence of bacteria clusters within the biofilm. These clusters of bacteria indicate that certain parts of the biofilm are more favorable for growth than others, and the bacteria may be multiplying at those locations.

5.2 Fecal Bacteria Concentrations in Biofilms and Storm Drainage Waters

A comparison of concentrations of fecal indicator bacteria (FIB) over time illustrates the environmental variability for both the biofilm and the stormwater. By examining the concentrations of FIB over 2 to 3 days on the coupons at Lorong 8 Toa Payoh, three interpretations can be made.

The first interpretation is that there is a clear variation over time, as each measurement of the concentrations of FIB increases or decreases compared to the previous measurement (Figures 12 through 15). The high variation is likely due to large fluctuations in stormwater flows with precipitation events, which cause erosion and sloughing of biofilm from the storm drain surface. As most of the ground at Lorong 8 Toa Payoh is covered with impervious materials, such as pavement and concrete, any rain will quickly be transported to the storm drains. At the upstream coupon (C1), the stormwater flows are smaller in magnitude than the downstream coupon (C2) because the drain in which C1 was placed joins the larger drain where C2 was located. The stormwater flow above C1 on a typical day in March 2013 was measured to be 67 cubic centimeters per second (Alkaff, 2013). Many intense rainstorms were observed between sampling times, so that high frequency may contribute to the generally decreasing concentrations over time of FIB on the downstream coupon C2 (Figure 13) while the concentrations of FIB observed on the upstream coupon C1 (Figure 12) did not decrease between the first sample day and the last sample day. The concentrations of FIB in the stormwater at C1 and C2 (Figures 14 and 15) did not fluctuate much over time, but did follow a generally decreasing trend over time. The decrease in FIB concentration in the stormwater over time could likely be due to dilution effects as fresh rainwater runoff mixes with the storm drainage water. However, all measured biofilm FIB concentrations at Lorong 8 Toa Payoh during January 2013 were greater than those observed on unused “clean” coupons.

ATTACHED ALGAE



Figure 19. Examples of attached algae (USEPA 1977).

A further analysis of these trends focuses on the individual FIB types that were analyzed. On both the coupons and in the storm drainage waters, the concentrations of total coliform were the greatest and the concentrations of enterococci were the least. The concentrations of *Escherichia coli* were in the middle of the range of FIB concentrations. This makes sense because *E. coli* is a subset of fecal coliforms. By observing the gram-stain photograph at 1000x magnification in Figure 6 (a), most of the biofilm material is stained red, indicating gram-negative cell dominance in the biofilm structure. As total coliform and *E. coli* are gram-negative bacteria, the measured concentrations correspond well with what was visually observed.

Finally, for the upstream coupon (C1) at Lorong 8 Toa Payoh, the measured concentrations of FIB on the top surface were always less than those measured on the bottom surface. The upstream location at C1 had relatively lower stormwater flows than the downstream location at C2, and occasionally the top surface of the coupon at C1 was not submerged in stormwater. Also, the bottom surface of a coupon provided shelter to biofilm because low flows of stormwater passed between the coupon and the storm drain surface and sunlight did not reach the bottom surface. These factors are in favor of bacteria survival and less detachment of biofilm on a coupon bottom. However, for the downstream coupon (C2), the measured FIB concentrations on the bottom surface were less than the FIB concentrations on the top surface. This result suggests that up to a threshold, the surface of a coupon that is always submerged in the stormwater encounters more bacteria, leading to a better-developed biofilm. Several environmental factors such as stormwater flow rate, temperature, and sunlight could contribute to the threshold above which the shelter the bottom surface provides biofilm is not advantageous in bacterial survival over the top surface environment.

5.3 Biofilm Detachment to Storm Drainage Water

Biological, chemical, and physical processes control the development of a biofilm (Picioreanu et al. 2001). For a biofilm in a stormwater drain, bacteria are transported to the drain surface, attach to the drain surface or existing biofilm, detach from the biofilm, and are generated by cell growth. As bulk stormwater containing fecal bacteria flows over a stationary biofilm, some fecal bacteria may attach to the exposed biofilm surface while the remaining fecal bacteria continue on downstream in the bulk flow. Correspondingly, fecal bacteria may detach from the biofilm either from the biofilm surface or from some depth in the biofilm as a cluster and join the bulk flow. The difference between attachment and detachment contributes to the development of a biofilm on the storm drain surface in addition to the in situ growth of bacteria cells in the biofilm. If the biofilm system operates as a steady-state system, the biofilm volume will be constant over time despite the gains and losses of bacteria.

Bacteria attachment to a biofilm is assumed to occur by virtue of the fact that a biofilm has developed in the drain. A biofilm grows as bacteria attach to the drain surface and eventually the biofilm can reach a steady-state thickness as more microorganisms are attached to the existing biofilm and grow than are lost from the biofilm. The mechanisms of biofilm detachment are more complex than biofilm attachment. As stormwater moves across the biofilm surface, internal stress is created on the biofilm and detachment occurs (Picioreanu et al. 2001). Biofilm detachment is illustrated in Figure 20. The detachment of bacteria from biofilms may be due to shearing forces increasing in the water, starvation of the bacteria, or a colonization strategy (Hall-Stoodley and Stoodley 2005). The major mechanisms for biofilm detachment are abrasion,

erosion, grazing, and sloughing (Rittmann 1989). Abrasion and grazing are neglected in this analysis.

Biofilm can detach from a surface by erosion (Rittmann and McCarty 2001). Biofilm erosion is a continuous process that occurs due to shear stress on the biofilm. High turbulence causes perpendicular forces on the biofilm surface causing pieces of biofilm to detach from the surface of the biofilm. Single cells or a small number of cells are removed from a biofilm surface during erosion (Stewart 1993). The specific biofilm-detachment loss coefficient for erosion can be calculated quantitatively for fixed media.

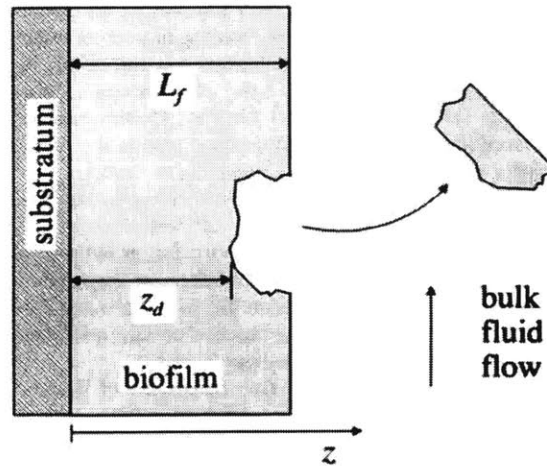


Figure 20. Detachment of a biofilm particle to the bulk fluid flow (Stewart 1993).

Sloughing is an important parameter in the operation of biological wastewater treatment systems (Elenter 2007). Biofilm sloughing occurs whether fluid shear is constant or increasing and can occur within hours after biofilm development. Biofilm detachment can be modeled in several ways. One-dimensional surface detachment, one-dimensional volume detachment, multi-dimensional fracture mechanics, multi-dimensional decay, and multi-dimensional erosion have been used in the literature to model detachment of biofilm. In this research, a simple one-dimensional surface detachment model was used to examine biofilm detachment from concrete coupon surfaces to the overlying stormwater.

Trickling filters are used in biological wastewater treatment for biochemical oxygen demand (BOD) oxidation (Rittmann and McCarty 2001). Wastewater is distributed over a filter consisting of plastic packing or rocks (Metcalf and Eddy 2003). Biofilms develop on the media of the filter and can consist of microorganisms such as facultative bacteria, algae, protozoa, and others. As the environmental conditions of the filter change, there occur sloughing events in which pieces of biofilm detach (Rittmann and McCarty 2001). A sloughing event is when the biofilm structure breaks down and detaches from the packing. Trickling filter effluent has been seen to contain more BOD and total suspended solids after sloughing occurs (Hawkes 1963). Sloughing is not often studied quantitatively because the mechanism of sloughing is difficult to quantify.

In a conventional trickling filter wastewater treatment plant, a secondary clarifier follows the trickling filter and removes sludge from the trickling filter effluent. The volumetric loading criterion for secondary treatment of trickling filter applications is 0.3-1.0 kg BOD/m³/d (Metcalf and Eddy 2003). An example of a trickling filter and secondary clarifier process is depicted in Figure 21.

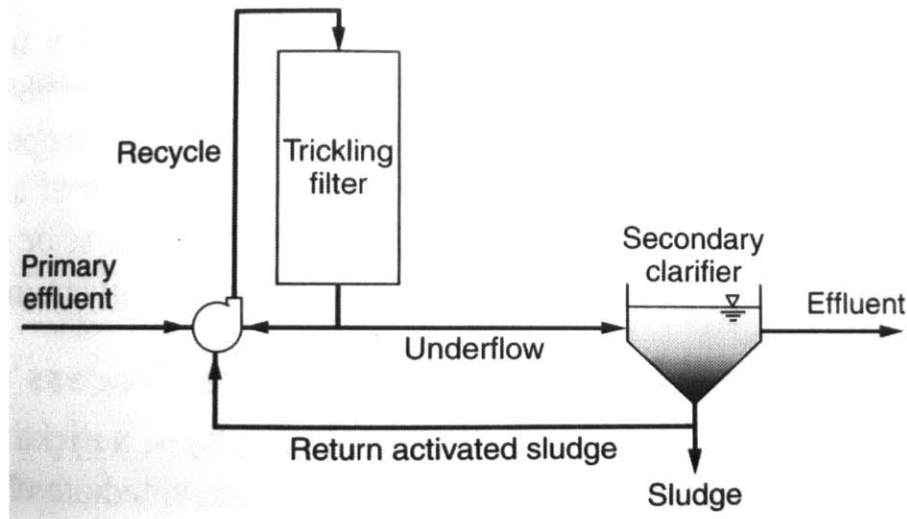


Figure 21. An activated biofilter schematic (Metcalf and Eddy 2003).

5.4 Model of Biofilm in Stormwater Drain

In this thesis, the relationship between fecal bacteria concentrations in biofilms and associated stormwater is further analyzed through the derivation of equations relating the concentration of FIB present in a biofilm as a function of several parameters. The five terms relevant for the FIB mass balances in the biofilm-to-stormwater detachment model are as follows and are illustrated in Figure 22.

- Inflow: a function of stormwater flow rate and the inflow concentration of FIB.
- Outflow: a function of the stormwater flow rate and the outflow concentration of FIB.
- Attachment: a function of the biofilm surface area in contact with stormwater, the settling velocity of bacteria cells, and the inflow concentration of FIB.
- Detachment: a function of the biofilm surface area in contact with stormwater, the FIB concentration per unit thickness of biofilm, and the biofilm residence time.
- Growth: a function of the FIB concentration per unit thickness of biofilm, the first-order growth kinetics, and the biofilm surface area in contact with stormwater.

All terms have units of bacteria counts per time in most probable number (MPN) per day. The parameters used in the terms and their respective designations and dimensions are:

$$\text{Time} = t [T]$$

$$\text{Water flow rate} = Q \left[\frac{L^3}{T} \right]$$

$$\text{Inflow concentration} = C_{in} \left[\frac{MPN}{L^3} \right]$$

$$\text{Outflow concentration} = C_{out} \left[\frac{MPN}{L^3} \right]$$

$$\text{Biofilm surface area} = A_{bio} [L^2]$$

$$\text{Settling velocity} = u_s \left[\frac{L}{T} \right]$$

$$\text{Biofilm concentration} = C_{bio} \left[\frac{MPN}{L^2} \right]$$

$$\text{Biofilm residence time} = t_{res} [T]$$

$$\text{First order growth rate} = k_{grow} [T^{-1}]$$

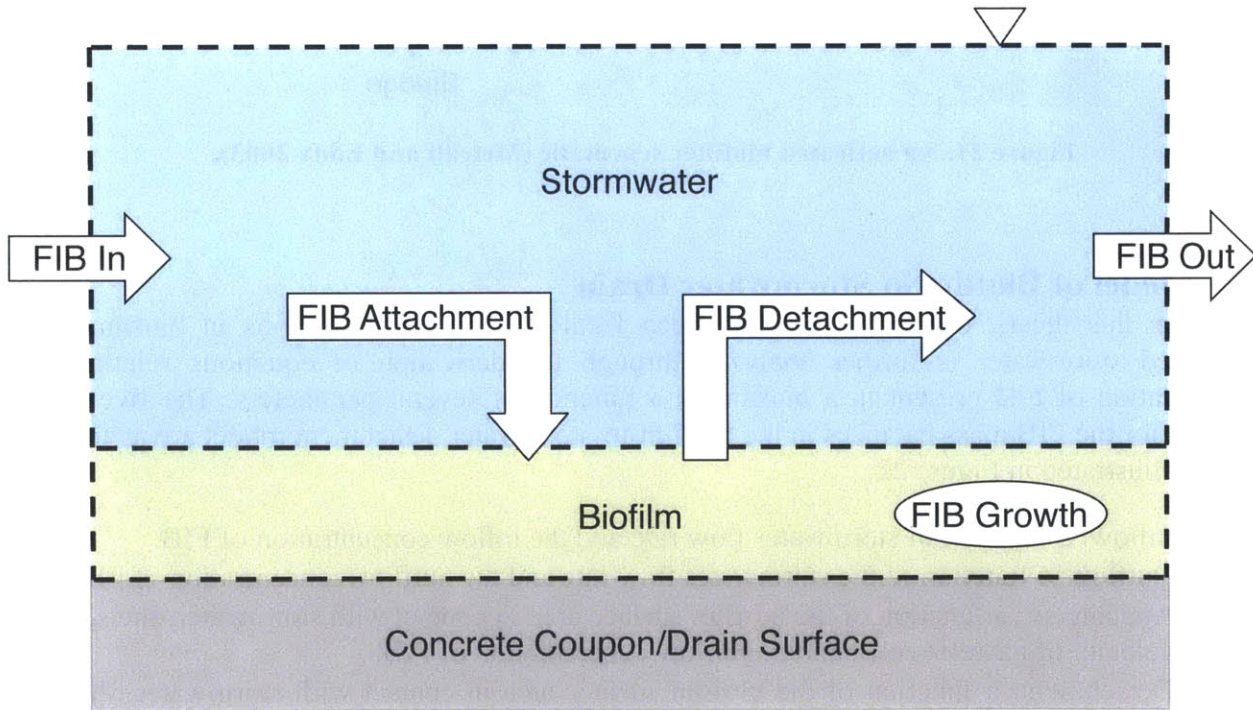


Figure 22. FIB mass balance control volumes for stormwater and biofilm.

5.4.1 Stormwater FIB Mass Balance Equation

The following equation was developed for the mass balance of FIB in the stormwater:

$$C_{bio} = \left[\frac{Q}{A_{bio}} (C_{out} - C_{in}) + u_s C_{in} \right] t_{res} \quad (1)$$

Below is the derivation of equation (1) for FIB in the stormwater. The overall mass balance for the water column considers the following mass fluxes:

$$(Inflow) - (Attachment) + (Detachment) = (Outflow) \quad (2)$$

$$(QC_{in}) - (A_{bio}u_sC_{in}) + \left(\frac{A_{bio}C_{bio}}{t_{res}}\right) = (QC_{out}) \quad (3)$$

Rearranging equation (3) yields:

$$\frac{A_{bio}C_{bio}}{t_{res}} = QC_{out} - QC_{in} + A_{bio}u_sC_{in} \quad (4)$$

Equation (1) is the result of solving equation (4) for C_{bio} . The stormwater FIB mass balance is not examined further because C_{out} data was not collected in this research.

5.4.2 Biofilm FIB Mass Balance Equation

Likewise, equation (5) was developed for the mass balance of FIB in the biofilm:

$$C_{bio,t} = C_{bio,0} e^{\left(k_{grow} - \frac{1}{t_{res}}\right)t} + \frac{1}{\left(k_{grow} - \frac{1}{t_{res}}\right)} \left[e^{\left(k_{grow} - \frac{1}{t_{res}}\right)t} - 1 \right] u_s C_{in} \quad (5)$$

Note that the biofilm concentration, C_{bio} , is in terms of mass per unit area while the water-column concentrations, C_{in} and C_{out} , are in terms of mass per unit volume. Equation (5) for FIB in the biofilm is derived as follows.

(Rate of bacteria biomass change with time) =

$$(Attachment\ rate) - (Detachment\ rate) + (Growth\ rate) \quad (6)$$

$$\left(A_{bio} \frac{dC_{bio}}{dt}\right) = (A_{bio}u_sC_{in}) - \left(\frac{A_{bio}C_{bio}}{t_{res}}\right) + (k_{grow}C_{bio}A_{bio}) \quad (7)$$

$$\frac{dC_{bio}}{dt} = u_sC_{in} - \frac{C_{bio}}{t_{res}} + k_{grow}C_{bio} \quad (8)$$

$$\frac{dC_{bio}}{dt} = u_sC_{in} + \left(-\frac{1}{t_{res}} + k_{grow}\right)C_{bio} \quad (9)$$

$$\frac{dC_{bio}}{dt} = \left(k_{grow} - \frac{1}{t_{res}}\right)C_{bio} + u_sC_{in} \quad (10)$$

Equation (10) is a first-order linear ordinary differential equation and C_{bio} is a function of time. The relationship $\frac{d}{dt} \left[\left(k_{grow} - \frac{1}{t_{res}}\right)C_{bio} + u_sC_{in} \right] = \left(k_{grow} - \frac{1}{t_{res}}\right) \left(\frac{dC_{bio}}{dt}\right)$ is only valid if C_{in} is assumed to be constant over time. Equation (10) is rearranged as followed to derive equation (5):

$$\frac{\left(\frac{dC_{bio}}{dt}\right)}{\left[\left(k_{grow} - \frac{1}{t_{res}}\right)C_{bio} + u_sC_{in}\right]} = 1 \quad (11)$$

$$\int \frac{\left(\frac{dC_{bio}}{dt}\right)}{\left[\left(k_{grow} - \frac{1}{t_{res}}\right)C_{bio} + u_sC_{in}\right]} dt = \int_0^t 1 dt \quad (12)$$

By integrating equation (12), a constant of integration (C) is introduced, as shown in equation (13).

$$\frac{\ln\left[\left(k_{grow} - \frac{1}{t_{res}}\right)C_{bio,t} + u_s C_{in}\right]}{\left(k_{grow} - \frac{1}{t_{res}}\right)} + C = t \quad (13)$$

Equation (13) is simplified to equation (15) by substituting in the following variable:

$$k' = k_{grow} - \frac{1}{t_{res}} \quad (14)$$

$$\frac{1}{k'} \ln(k' C_{bio,t} + u_s C_{in}) + C = t \quad (15)$$

The initial condition $C_{bio}(t = 0 \text{ days}) = C_{bio,0}$ is introduced in equation (16) to determine the value of the constant of integration (C).

$$\frac{1}{k'} \ln(k' C_{bio,0} + u_s C_{in}) + C = 0 \quad (16)$$

Simplifying equation (16) results in equation (17), the expression for C.

$$C = -\frac{1}{k'} \ln(k' C_{bio,0} + u_s C_{in}) \quad (17)$$

Equation (17) is substituted into equation (15) to give equation (18).

$$\frac{1}{k'} \ln(k' C_{bio,t} + u_s C_{in}) + \left[-\frac{1}{k'} \ln(k' C_{bio,0} + u_s C_{in})\right] = t \quad (18)$$

Equation (18) is simplified and solved for $C_{bio,t}$ in equation (19).

$$C_{bio,t} = C_{bio,0} e^{k't} + \frac{1}{k'} u_s C_{in} (e^{k't} - 1) \quad (19)$$

The expression in equation (14) is substituted into equation (19) to derive equation (5).

5.4.3 Parameter Designation

Several parameters require further definition: the settling velocity, the biofilm residence time, and the first-order growth rate.

5.4.3.1 Settling Velocity

The settling velocity was estimated using Stokes' law. In Stokes' law, the settling velocity of a particle (or bacteria cell) is reached as the drag force and the buoyant force on a particle balance the force caused by gravity. Stokes' law is given as follows in equation (20):

$$u_s = \frac{\left(\frac{2}{9}\right)g\left(\frac{\rho_{cell}}{\rho_{water}} - 1\right)r^2}{\nu_{water}} \quad (20)$$

The mean water sample temperature was 26.3 ± 2.4 °C, so a value of 26.3 °C was used as the environmental temperature in the calculation of the settling velocity. The density of water, ρ_{water} , is estimated to be 996.7055 kg/m³ at 26.3 °C (Haynes 2013). At 300 K (or 26.9 °C), the kinematic viscosity of water, ν_{water} , is estimated to be 8.5669×10^{-7} m²/s.

The bacteria included in the total coliforms group are generally rod-shaped. *E. coli* cells are rod-shaped, while enterococci are spherical. Bacteria have an average length of 1-2 μm or an average diameter of 0.5-1 μm (Maier, Pepper, and Gerba 2009). Therefore, in assuming that bacteria cells are spherical to simplify calculations and considering 1 μm to be an average diameter, the radius of a bacteria cell is approximately 0.5 μm or 5×10^{-7} m. The volume of a bacteria cell is calculated using the radius value to be $0.52 \mu\text{m}^3$ or $5.2 \times 10^{-19} \text{m}^3$. The mass of a single *E. coli* cell is 9.5×10^{-13} g or 9.5×10^{-16} kg (Neidhardt 1996). As a result, the density of a model bacteria cell is approximately $1.8 \times 10^3 \text{kg/m}^3$. The settling velocity is thus:

$$u_s = 5.13 \times 10^{-7} \frac{m}{s} = 4.4 \times 10^{-2} \frac{m}{d}$$

5.4.3.2 Biofilm Residence Time

An average solids residence (or retention) time is applicable to a steady-state biofilm (Rittmann and McCarty 2001). The solids residence time is the ratio of the active biomass in the biofilm to the active biomass production rate, averaged over an entire biofilm. Simplified, the solids residence time is the inverse of the biofilm specific detachment rate. The biofilm detachment rate depends on the water shear stress on the biofilm surface. The biofilm residence time was estimated to equal a common value for an aeration basin solids residence time (Daigger and Boltz 2011). The value is a typical design criterion for a roughing filter and activated sludge process. Therefore:

$$t_{res} \cong 8.0 \text{ days}$$

5.4.3.3 First-Order Growth Kinetics Rate Factor

The first-order growth rate of fecal indicator bacteria in a biofilm was estimated from biofilm model studies. Horn et al. (2003) used a mean maximum growth rate of 5 per day for heterotrophic biofilm and this value is assumed to be sufficient for this study's purposes. Thus:

$$k_{grow} \cong 5 \text{ day}^{-1}$$

5.4.4 Solution

The equation for FIB mass balance within the biofilm becomes equation (21). The values of the parameters were substituted into the equation with the following units: $C_{bio,t}$ and $C_{bio,0}$ in MPN/cm^2 , t in days, and C_{in} in MPN/100 mL .

$$C_{bio,t} = C_{bio,0} e^{4.875 t} + (9.026 \times 10^{-3})(e^{4.875 t} - 1)C_{in} \quad (21)$$

In equation (21), the growth of FIB in biofilm dominates the detachment of FIB from the biofilm. Without occasional erosion and sloughing events, the growth in the biofilm would be unlimited. By examining the relationship between the stormwater total coliform concentration in MPN/100 mL and the biofilm total coliform concentration in MPN/cm^2 , one sees that the values for the biofilm concentrations increase and exceed the values for the stormwater concentrations within a few days. If $C_{bio,t}$ is to be less than C_{in} in equation (21), the timescale t must a fraction of a day.

6 Summary, Conclusions, and Recommendations

6.1 Summary

In this research, three fecal indicator bacteria (FIB), total coliform, *Escherichia coli*, and enterococci, were measured once per week in biofilms and storm drainage waters at six locations in Singapore. Well-developed biofilm conditions were observed at Choa Chu Kang Crescent, Verde View, Lorong 6 Toa Payoh, and two points in Lorong 8 Toa Payoh. An initial biofilm growth condition was observed for a secondary study at Nanyang Technological University. All samples were collected between January 8, 2013 and January 22, 2013. The main objectives of the research were to conclude if biofilms are reservoirs for FIB, to measure FIB over time within biofilms and stormwater, and to examine the relationships between FIB in biofilms and FIB in stormwater. All three of the original objectives were accomplished in this research and aided in the development of a model for FIB in storm drain biofilms.

6.2 Conclusions

The biofilms in storm drains were observed to be reservoirs for FIB. In all of the samples taken from the well-developed biofilms, total coliform, *E. coli*, and enterococci concentrations were countable and prominent. The analysis confirms what Ekklesia observed previously in Singapore storm drains (2012). As debris and sediments were commonly observed in the storm drains, the additional structure, nutrients, and protection these provided to biofilms promotes survival of microorganisms. The analysis supports the conclusions of studies of sediment biofilm behavior in various aquatic environments, particularly studies of beach sand and beach wrack. In those studies, beach sand and beach wrack were found to be reservoirs for fecal bacteria because these media were observed to prolong fecal bacteria survival (Craig et al. 2002a; Alm et al. 2003; Lee et al. 2006; Hartz et al. 2008; and Imamura et al. 2011). The presence of FIB within biofilms indicates that sewage leakage may not be the only pathway of stormwater contamination.

The measured FIB concentrations fluctuated over time in both the biofilm and the storm drainage waters during the three weeks of observation. These changes could be due to processes within the biofilms or phenomena in the storm drain environment. The concentrations of FIB in biofilms could change over time due to the natural growth and death processes of bacteria. FIB concentrations could increase as bacteria deposit from the overlying stormwater and attach to the biofilm surface. Otherwise, the stormwater could inflict shearing and erosion on the biofilm during high stormwater flows, leading to decreases in FIB concentrations.

The detachment of bacteria from biofilms is a likely pathway for FIB contamination of stormwater. Greater fluctuations in FIB concentrations in biofilms than in storm drainage waters indicate that the stormwater is more stable and has additional sources of FIB contributing to the contamination. However, with a preliminary model of FIB detachment from biofilms to storm drainage waters, the biofilms may be significant, at least locally, in contributing to bacteria contamination in the stormwater drainage system. Nevertheless, the results confirm poor stormwater quality and that fecal contamination can originate from within the storm drains.

6.3 Recommendations for Future Work

For future studies of the contamination of storm drainage waters by fecal bacteria that originate from within the storm drains, I would suggest both detailed research in biofilms and expanding the research to other locations.

First, the environment of the storm drain can be better characterized. Relationships among observed FIB concentrations with parameters such as temperature, pH, and salinity may provide better insight into why certain locations within the drains have different types and quantities of biofilm growth. The biofilms present in different storm drains can be visually examined for comparisons as well. As only one biofilm sample from Lorong 8 Toa Payoh was examined, the observations were assumed to be applicable to all samples collected, even though they were collected from different locations. Further research would involve the confirmation of this assumption that all the biofilms in Singapore are essentially the same for the purposes of the project. The research might involve determining different methods for examination, such as alternative microscopy techniques or using biofilm and algae field guides. Other fecal indicators such as fecal *Bacteroides* can be pursued in an equivalent research project. By examining other fecal indicators, the results observed in the research can be reinforced or expanded.

Another area requiring further studies is to produce more detailed time series of FIB concentrations at a single point and correspond those with stormwater flow measurements. Biofilms can be sampled more frequently over time, such as over a 12-hour period so that the opportunity to record multiple rain events and the resulting effects observed in the storm drain may arise. Alternatively, multiple locations along a storm drain can be sampled during a relatively short period to better examine upstream influences on the downstream environment. By visiting a well-studied location, such as at Lorong 8 Toa Payoh, more biofilm and stormwater sampling points could be added for this research. Therefore, more detailed contributions of each tributary into the main drain or other features of the drain, such as leaks, can be determined.

Finally, the research can be expanded to encompass other sampling locations around Singapore. More detailed studies can be focused within single neighborhoods such as Toa Payoh or Choa Chu Kang, or more sampling locations can be investigated in other districts of Singapore. The study can also encompass samples at locations within the Charles River basin in Massachusetts, U.S.A.

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Appendix I Maps



Figure 23. Map of Lorong 8 Toa Payoh sampling locations, the upstream coupon C1 marked with a blue pin, and the downstream coupon C2 marked with a yellow pin (Google Maps 2013).

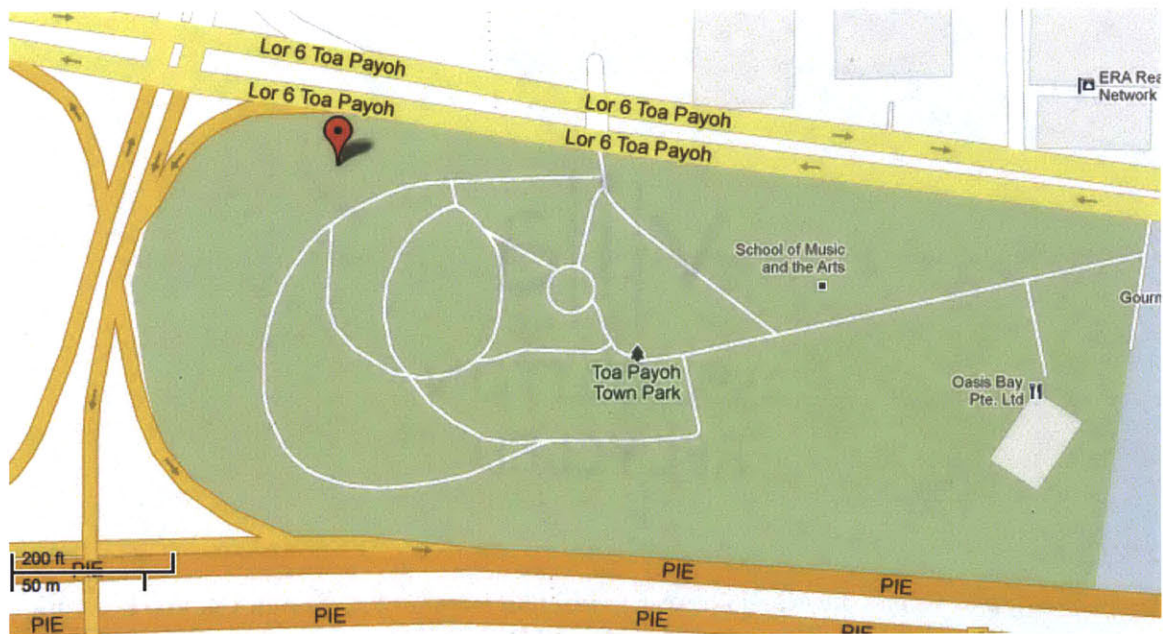


Figure 24. Map of Lorong 6 Toa Payoh sampling location for coupon C5, marked with a red pin (Google Maps 2013).



Figure 25. Map of Verde View sampling location for coupon C3, marked with a green pin (Google Maps 2013).

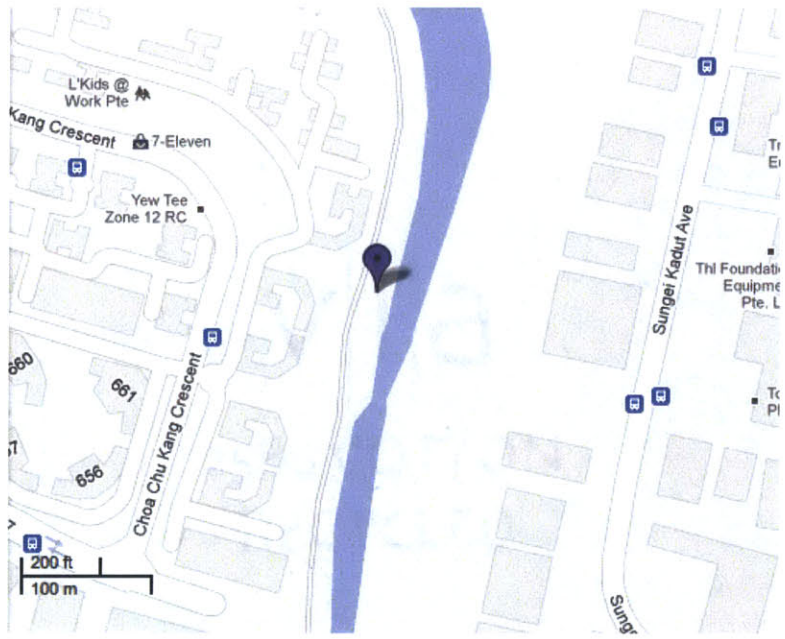


Figure 26. Map of Choa Chu Kang Crescent sampling location for coupon C4, marked with a purple pin (Google Maps 2013).