Laboratory studies of physical transformation processes in sewers by wastewater-grown biofilms

By:

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Abstract

Changes in pipe flow capacity with time have been reported to be caused by biofilm formation, sediment accumulation and pipe deterioration. Biofilm has been demonstrated to cause increasing hydraulic roughness in natural water flows thus changing the hydraulic properties of the system. However, little work has been done in sewer pipes with heavily polluted wastewater. Sediment accumulation, deposition and erosion processes in sewers have also been reported to be influenced by microbial activity.

The thesis reports on the development of a novel method for investigating the influence of wastewater-grown biofilm on pipe flow characteristics and bed sediment stability. This work presents systematic laboratory studies of the biofilm growth under different conditions, pipe flow characterisation at different hydraulic configurations, deposit characteristics for different consolidation periods, with changes of organic matter concentration being monitored for all tests. All laboratory tests were conducted using wastewater.

The results obtained indicate that biofilm growth changes flow behaviour in pipes by decreasing flow depth, thus decreasing pipe hydraulic roughness, and increasing average flow velocity. This finding depends on the level and character of biofilm growth conditions in the pipe, as different characteristics of biofilm were obtained at different conditions. For sediment deposits, biofilm growth was observed to increase bed stability with longer consolidation phase, thus reducing bed erosion at higher shear stress. These results vary with the duration and character of the consolidation phase of the sediment bed.

The findings obtained provided a better understanding of the role of biofilm in sewer pipes and may contribute to the development of more accurate modelling of pipe flow and sediment accumulation and transport processes in sewers.

Dedications

This is for Mom and Dad.

and Myself.

-I cried a lot and laughed a lot, but it was so beautiful-

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Publications

Conference papers;

1. Abd Rahim, A., Tait, S.J., Biggs, C.A. and Jensen, H.S. (2014) The effect of wastewater-grown biofilm on pipe surface roughness. *Proceedings of 13th International Conference on Urban Drainage (ICUD2013),* Sarawak, Malaysia.
2. Abd Rahim, A., Seco, I., Tait, S.J. and Jensen, H.S. (2016) Influence of biofilm on sewer sediment deposit*. Proceedings of 8th International Conference on Sewer Processes and Networks (SPN8),* Rotterdam, The Netherlands.

Nomenclatures

A	Mass of filter plus dried residue	[g]
A_b	Area of sediment bed	[m^2]
A_w	Flow wetted area	[m^2]
B	Mass of filter	[g]
B_o	Outside surface width of flow	[m]
B_i	Flow surface width	[m]
C	Mass of residue plus dish before ignition	[g]
C_{SS}	Suspended sediment concentration before dilution	[g/L]
$C_{SS,i}$	Suspended sediment concentration of diluted samples	[g/L]
$C_{SS,i+1} - C_{SS,i}$	Suspended sediment concentration difference between sample i+1 and i	[g/L]
D	Mass of residue plus dish after ignition	[g]
D_i	Pipe inner diameter	[m]
D_f	Sedimentological particle diameter	[m]
D_h	Hydraulic diameter	[m]
d_{min}	Minimum particle diameter	[m]
d_{max}	Maximum particle diameter	[m]
d_{av}	Average particle diameter	[m]
d_w	Flow depth	[m]
e_i	Eroded bed thickness at step i	[mm]
e_{cum}	Cumulative eroded bed thickness	[mm]
f	Darcy-Weisbach friction factor	[-]
F_D	Dilution factor	[-]
F_{cr}	Critical Froude number	[-]
g	Acceleration due to gravity	[m/s^2]
G	Relative centrifugal force	[-]
h_f	Energy loss due to friction	[m]
h_i	Energy loss at pipe entrance	[m]

h_L	Energy loss due to pipe fittings	[m]
h_o	Energy loss at pipe exit	[m]
K_d	Discharge loss coefficient	[-]
K_L	Minor loss coefficient	[-]
k_s	Pipe hydraulic roughness	[m]
$\overline{k_s}$	Average pipe hydraulic roughness	[m]
K_m	Half saturation constant	[mg/L]
L_p	Length of pipe	[m]
p	Bed porosity	[-]
P_{wo}	Pipe outside wetted diameter	[m]
P_{wi}	Pipe inside wetted perimeter	[m]
Q_f	Discharge flowrate	[L/s]
q_i	Average erosion rate during step i	[g/m^2/s]
R_c	Radius of rotors	[cm]
Re	Reynolds number	[-]
R_h	Flow hydraulic radius	[m]
r_o	Pipe outside radius	[m]
r_i	Pipe inner radius	[m]
S	Centrifugal speed	[RPM]
S_o	Bed slope	[m/m]
S_f	Water slope	[m/m]
t	Pipe thickness	[m]
u	Flow velocity	[m/s]
u_i	Flow velocity at pipe entrance	[m/s]
u_o	Flow velocity at pipe exit	[m/s]
$\bar{u}$	Average flow velocity	[m/s]
V_{cum}	Cumulative volume of water extracted	[L]
V_i	Sample volume collected at step i	[L]
V_s	Sample volume	[L]
V_w	Water volume in the column	[L]
Δt	Duration of time step	[s]
ρ_r	Relative density	[-]

ρ_f	Fluid density	[kg/m^3]
ρ_b	Bed density	[kg/m^3]
ν	Kinematic viscosity	[m^2/s]
τ	Applied shear stress	[N/m^2]
τ_o	Mean boundary shear stress	[N/m^2]
τ_{cr}	Critical shear stress	[N/m^2]
μ_{max}	Maximum specific growth rate	[hr^{-1}]
θ_o	Outside angle of flow	[rad]
θ_i	Inside angle of flow	[rad]
μ	Water dynamic viscosity	[kg/ms]

Abbreviations

COD	Chemical Oxygen Demand
TSS	Total suspended solids
VSS	Volatile suspended solids
PDF	Probability density function
SRB	Sulphate reducing bacteria
VFA	Volatile fatty acids
TOC	Total organic carbon
RI	Refractive index
AI	Absorbance index
DNS	Dinitrosalicylic acid
A/V	Area over volume ratio
PE	Population equivalent

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Chapter 1 Introduction

1.1 Background

Biofilm is regarded as a natural consequence of bacterial existence in natural environments (Romanova and Gintsburg, 2011). Biofilm has also been found to have a major role in in-sewer processes, such as oxygen uptake in sewer pipes, odour formation and pollutants released from combined sewer overflows events (Chen *et al*. 2003; Sharma *et al.* 2014).

Biofilm activity in a pipe has been found to have a direct influence on pipe surface roughness and sediment physical stability. However, only a few investigations on the influence of biofilm formation on pipe hydraulic roughness and bed deposit have been carried out (Guzmán *et al*. 2007; Lewandowski *et al*. 1992).

Hydraulic roughness is one of the most important parameters in sewers, as it determines how the pipe surfaces influence hydraulic flow capacity in the pipe and its determination is crucial in sewer flood risk modelling. Sediment accumulation, transport and erosion are also important in modelling and designing networks to minimise environmental impacts from sewer overflows and in preventing sewer blockages (Ashley *et al*. 2004).

Biofilm coverage on any wastewater-submerged surfaces in sewer pipes may influence processes taking place in the sewer and also sewer hydraulics. A study by Guzmán *et al*. (2007) on biofilm grown with tap water enriched with methanol and glucose with COD concentration of 800 mg/L demonstrated that biofilm growth increased pipe surface roughness. This experiment did not able to represent the complexity of biofilm in sewer pipes, due to the multi-substrate and multi-species composition of real wastewater.

In addition to that, biofilm has been demonstrated to influence bed deposits. Vollertsen and Hvitved-Jacobsen (2000) have reported that sediment deposit properties such as bed strength could be influenced by microbial transformation in sediments. Several studies have reported weakening of bed deposits due to changes in physical and biochemical properties of the sediment (Le Hir *et al*. 2007) while others have claimed to observe a stronger bed due to biological activities (Righetti and Lucarelli, 2007).

These differences can be speculated to be caused by the differences in organic matter concentration available, type of bacteria presents, and hydraulic characteristics in both systems. This topic, however, has not been studied in detail and thus will be included in this work.

Understanding changes in pipe flow and bed stability that were caused by the biofilm is a challenging yet intriguing question. Various environmental and hydraulic conditions were tested in this study to obtain a better understanding of biofilm growth under different conditions. Novel methods for determining these changes were also developed and implemented in this work.

1.2 Hypothesis

The author believes that wastewater-grown biofilm influences the pipe flow profile by changing pipe hydraulic roughness. These changes may depend on biofilm characteristics grown under various conditions. If these changes can be estimated, it might be possible to determine the changes in parameter values to be implemented in existing sewer networks models to take biologically derived effects into account. Other than that, biofilm was also believed to influence bed sediment stability, depending on the conditions of the bed during consolidation period.

1.3 Work focus

This work focuses on biofilm growth effects on both pipe flows and bed sediment stability. This relationship can be presented in Figure 1.1.

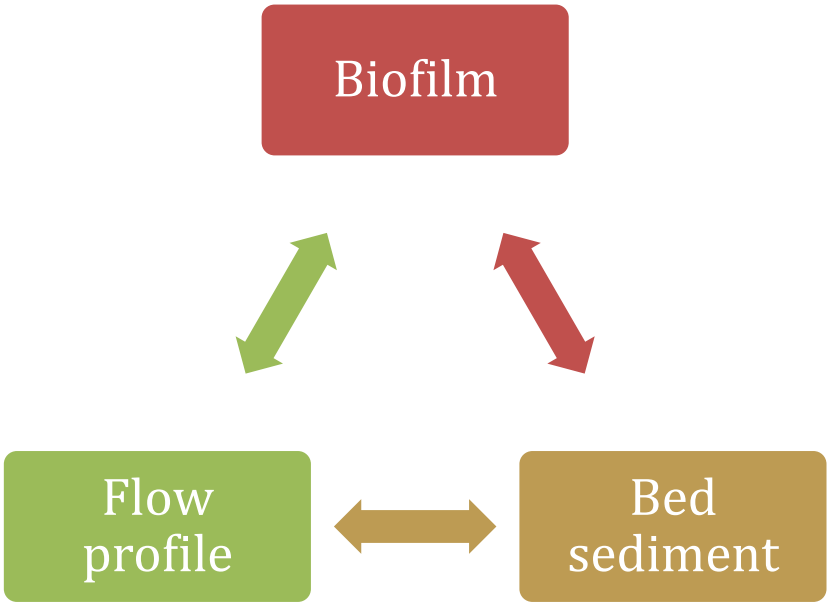

Figure 1.1. The relationship between biofilm, flow profile and bed sediment.

These three-way interactions illustrated by Figure 1.1 shows the relationship between biofilm growth, flow profile and bed sediment. Bed sediment and sewer's wall serve as surfaces for biofilm growth in a typical sewer environment, and grown biofilm has been found to influence bed sediment stability (Schellart *et al*. 2005; Tait *et al*. 2003a). The bed sediment limits flow capacity of a sewer by increasing hydraulic roughness of sewer and reduce the flow area (Mark, 1992). These changes will affect sewer flow parameters which have further influence on sediment bed transport and suspension processes (Banasiak and Tait, 2008). The relationship between flow profile and biofilm growth is biofilm characteristic depends on hydraulic conditions of the flow (Rochex *et al*. 2008; Wäsche *et al*. 2002) while grown biofilm has been reported to cause changes in the flow profile (Guzmán *et al*. 2007). This relationship was further investigated in three stages of study;

1. Influences of flow conditions on biofilm growth, where biofilm was grown at different hydraulic and environmental conditions in order to obtain different biofilm characteristics.
2. Biofilm growth effects on flow conditions were studied by linking changes of pipe hydraulic roughness and flow velocity with different biofilm characteristics obtained.
3. Influences of biofilm growth on bed sediment stability were investigated by understanding the changes in bed particle eroded when subjected to higher shear stress after consolidated at different time period.

A novel approach to measure the influences of biofilm on flow conditions and sediment stability were developed and implemented. These involved hydraulic measurements of the flow, analysis of eroded bed particle and quantification of organic matter which will provide a further understanding of organic matter degradation within sewer environments.

1.4 Aims and objectives of research

The aim of this research is to investigate influences of wastewater-grown biofilm on pipe flow profile and bed stability under various conditions. The data will be collected using laboratory scale reactors. Objectives of this research are to;

I. Develop a facility for biofilm growth in partially filled pipes.
II. Understand flow profile characteristics of partially filled pipes under various hydraulic and environmental conditions (pipe length, bed slope, dissolved oxygen concentration and wastewater initial organic matter concentration was varied).
III. Investigate biofilm growth and characteristics under different hydraulic and environmental conditions.
IV. Develop relationships of biofilm growth to pipe hydraulic roughness and flow velocity values.

V. Develop a novel approach to investigate biofilm growth on sediment bed by implementing various consolidation periods for the biofilm growth.

VI. Investigate the changes in bed stability caused by biofilm growth under different conditions and develop relationships from results obtained.

VII. Understanding organic matter consumption with biofilm growth and develop an understanding of the factors that link these two parameters.

1.5 Thesis outline

The thesis has six chapters; Chapter 1 (Introduction) provides an overall background of the work, aims and objectives of the study. Chapter 2 (Literature review) presents relevant information on biofilm, wastewater, sediment, organic matter, and sewer networks. Chapter 3 (Materials characterisation) describes characteristics of materials used in this study and provide background information for all tests conducted. Chapter 4 (Pipe tests experiment) provides an in-depth description of the relationship between biofilm growth and flow. Overall experimental setup, conditions and results obtained are also presented. Chapter 5 (Bed stability experiment) presents experimental works, conditions and obtained experimental results for the influence of biofilm growth on bed stability. Chapter 6 (Conclusions and future works) provides a summary of findings and achievements of the work has achieved, and recommendation for future studies.

Chapter 2 Literature review

This study focuses on biofilm and sediment in partially filled pipes, which are commonly found in sewer pipes. This chapter will provide an overview of some key fundamentals and characteristics of biofilm and sediment in sewer pipes. Sewer pipes have been reported to be affected by biofilm formation (Guzmán *et al.* 2007; Grengg *et al*. 2015), sediment accumulation and pipe deterioration (Romanova *et al.* 2011). Sediment built up in the sewer causes changes in pipe flow capacity and may cause severe problems such as flooding and delay during wastewater transportation. The release of pollutants from sediment built up during storm event can cause serious health and environmental problems (Butler and Davies, 2004). Biofilm growth has been reported to increase pipe hydraulic roughness (Guzmán *et al.* 2007), and only a few investigations have been carried out in this area (Guzmán *et al*. 2007; Lewandowski and Beyenal, 2005) . Changes in pipe hydraulic roughness can cause changes in flow conditions in the pipe, thus, may alter any flow predictions obtained through modelling. These changes, however, has not been included in any developed models design for flow in a pipe.

2.1 Overview of sewer network

A sewer network is a system that is designed to transport sewage from sources of production to locations for treatment before the subsequent release of the treated effluent to the environment. Sewers have existed for many years, as the earliest sewer-like system has already been developed on the Orkney Islands around 3200 BC. Other well-known examples were Babylonia (4000 to 2500 BC) and Mohenjo Daro (3000 to 2000 BC) (Schladweiler, 2017).

Fast forward to modern history, in the 19th century, raw sewage used to be dumped directly into the River Thames. This practice was conducted up until the middle of the 19th century when an outbreak of cholera killed 10,000 people and prompted the government to create new legislation to combat these issues which led to the development of sewer networks that we have presently.

The total length of sewers in the UK is approximately 624,000 km (Defra, 2012) where the majority of existing sewers are combined sewers, which compromise approximately 70% of the total sewerage length (Butler and Davies, 2004). These existing facilities have been reported to have various problems, such as leaking, blockage, groundwater infiltration and misconnection (Geovation, 2017).

Two main sewerage systems exist; combined sewers and separate sewers. Combined sewers transport wastewater and stormwater in the same pipe whilst separate sewers convey wastewater and stormwater separately (Butler and Davies, 2004). Wastewater is water originated from various sources including residential and industrial areas while stormwater is the product of precipitation, such as rain and snow. Both wastewater and stormwater have been reported to cause health and safety related issues to humans and the environment (Butler and Davis, 2004; Tchobanoglous *et al*. 2002).

The sewer can be considered as a complex system, as it changes with distance and time. For example, changes can occur due to seasonal factors such as more stormwater obtained during wet weather periods or increasing flows of wastewater in the sewer during peak hours of the day. Sewers also change with distance, for example, changing of pipe slope and pipe diameter with distance.

The sewer can be described as a system with various components where each component has its own role, but at the same time, the components work together as a system. Four main components have been identified; in-sewer atmosphere, wastewater, biofilm and sediment.

Wastewater, biofilm and sediment are the focus of this study which will be discussed further. However, solid transport of sediment and biofilm detachment processes will not be dealt with, thus, were not considered in the process description. The main focus is directed towards biofilm growth and its influences on pipe hydraulic roughness, sediment deposit stability, and organic matter degradation in the system.

2.2 Introduction to biofilm

One of the main components of a sewer is biofilm. Biofilm is defined as a layer of bacteria that stick to a surface and made of 90 to 99% of water, living cells, dead cells, and extracellular polymeric substances (EPS) (Melo and Frias, 2004). Biofilm plays an important factor in the natural environment and in technical applications such as in trickling filters for wastewater treatment. However, some types of biofilm are undesirable, for examples, biofilm can contaminate medical devices and can cause serious health problems if it grows on living tissues (Fitzpatrick *et al*. 2005; Kokare *et al.* 2009). Other examples of undesirable biofilm are biofilm on ship hull (Andrewartha *et al*. 2010; Teng *et al*. 2008). Study of biofilms have been evolving at a fast pace and recent advancement in regards of influence of biofilm on drugs transformation in sewers (McKall *et al*. 2016), biofilm dynamics under varying shear stress (Ai *et al.* 2016), and changes in bacterial communities in combined sewers (Jensen *et al*. 2016) have been reported in this area of research.

A study conducted on a single species of bacteria in the human body by Jefferson (2004) provided the reasons for the transition of the bacteria from planktonic to sessile mode. Based on the study, biofilm formation are for protection and defence mechanism against harmful conditions, to utilise the benefits of a community, allowing more possession and dominance in the nutrient-rich area, and act as the default mode of bacterial growth.

Biofilm growth can be described in five main steps; 1) reversible attachment of bacteria, 2) irreversible attachment of bacteria, 3) development of biofilm

architecture, 4) biofilm maturation and 5) biofilm detachment to the environment (Stoodley *et al*. 2002). These processes can be presented in Figure 2.1.

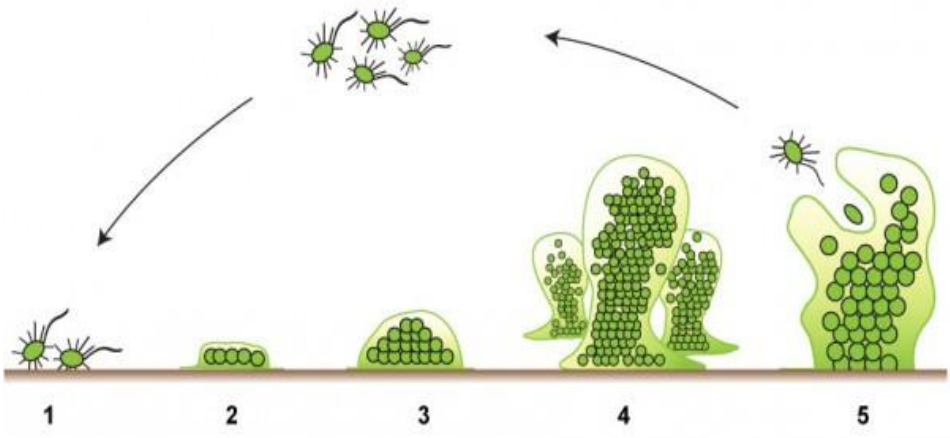

Figure 2.1. Processes of biofilm formation (adapted from Aqua-tech, 2017).

Figure 2.1 (number 1) shows the first step in biofilm growth processes. When a surface is in contact with water, surface charges are neutralized by the organic molecules that adhered to the surface. This condition allows bacteria to stick to the surfaces via electrostatic attractions and physical forces (Renner and Weibel, 2011; Toole *et al*. 2000). This adhesion is weak and reversible as the adhesion can be affected by many factors, such as physical and chemical characteristics of bacteria (hydrophobicity and surface charge), surface properties (roughness, texture and chemical composition) and environmental factors (temperature, pH and bacteria concentration) (Simões *et al*. 2010).

In step 2, bacteria start producing EPS that secures the cells firmly onto the surfaces. The EPS is mainly made of polysaccharides, proteins, uronic acid, DNA and cell fragments (Späth *et al.* 1998). EPS composition differs according to several factors including microorganisms present in the system, temperature and nutrient availability (Flemming and Wingender, 2010). The EPS functions as a barrier against desiccation and anti-microbial agent, toxicity and shock load to the bacteria (Andersson *et al*. 2008), while at the

same time also contributes in cell communication by facilitating in-situ quorum sensing signals among microbial cell within a biofilm (Decho, 2015).

The biofilm maturation processes start in Step 3 and 4. This process can be characterized by colonies formation in the biofilm thus created a three-dimensional structure with pores and channels. Some interesting biofilm structures that have been observed in previous studies are cauliflower-like structures which were obtained under high denitrification flux (Derlon *et al*. 2013), honeycomb-like structures which were produced by biofilm grown in a rotating disk reactor using domestic wastewater (Okabe *et al*. 1998), mushroom shape biofilm was reported on biofilm grown of *Legionella pneumophila* under different temperatures (Piao *et al*. 2006), and finger-like biofilm structures were observed on aerobic heterotrophic biofilm (Derlon *et al*. 2013).

Biofilm physical characteristics such as thickness are a crucial parameter as it influences on the dissolved oxygen diffusion mass transfer processes between biofilm and wastewater. Biofilm thickness has some influence on the type of bacteria in the biofilm, as sulphate reducing bacteria (SRB) usually can be found on biofilm top layer and bottom layer usually consists of methanogen bacteria (Sun *et al.* 2014). High concentration of SRB at the top layer of surfaces was due to depletion of substrates at the deeper layer of the surface which prompt the SRB to colonize the top layer in order to obtain more substrate (Jørgensen, 1982). Biofilm thickness in gravity sewers has been reported to be about 1 to 3 mm thick while biofilm in pressure sewer is thinner, approximately between 0.1 to 0.5 mm thick (Nielsen *et al.* 1992).

The last stage in biofilm formation processes is biofilm detachment as depicted by Figure 2.1 (number 5). During this stage, pieces of biofilm are detached from the surfaces due to high hydrodynamic forces that exceed biofilm cohesion strength (Coufort *et al*. 2007). This process can occur in many different ways depending on the characteristics of detached biofilm.

Erosion is defined when a small piece of biofilm is lost to the bulk phase while sloughing is referring to the removal of large pieces of biofilm (Wang and Zhang, 2010). Abrasion is biofilm removal due to collisions between particle and the biofilms (Gjaltema *et al*. 1997) while grazing is defined as loss of biofilm due to predators (Romaní *et al*. 2012). Detached biofilm has also been reported to attach to another available surface and starts a new biofilm layer (Gomes *et al.* 2014).

Biofilm detachment has been reported to occur due to changes in physicochemical properties of the biofilm. Some examples that have reported in the literature are production of extracellular enzymes that degrade the biofilm substrate (Pecharki *et al*. 2008) and also biofilm matrix (Kaplan *et al*. 2003). Factors such as changes in nutrient concentration and biofilm starvation have been reported to cause biofilm detachment for a single species biofilm (Gjermansen *et al*. 2009; Hunt *et al.* 2004). Other factors such as pH, temperature and oxygen availability have also been reported to have influence in biofilm detachment processes (Karatan and Watnick, 2009; Huang *et al*. 2012).

It has been agreed that biofilm total removal is almost impossible to occur in the sewer (Balmer and Tagizadeh-Nasser, 1995). This statement gives more weight to the responsibility to embrace this creation and understand its role and importance in the sewer for a more accurate approach to understand in-sewer processes.

2.2.1 Factors influencing biofilm growth, formation and detachment processes

As discussed above, several factors have been identified in influencing biofilm growth formation and detachment processes. Environmental factors that have been identified include temperature, nutrient availability, oxygen level, toxicity, pH, and type and number of bacteria presents in the system while hydrodynamic condition refers to shear stress, flow velocity, substrate type, substratum type and roughness and flow conditions.

Holá *et al*. (2006) reported that more biofilm formation was obtained at a higher temperature (37^{o}C) and nutrient-rich environmental for pure media culture using *Staphylococcus epidermidis*. The study also reported lower biofilm production at a lower temperature (25^{o}C) with high nutrient level. This finding was consistent with a study by Hostacká *et al*. (2010), where less bacterial growth was obtained at low temperature (30^{o}C and 37^{o}C) for three different pure culture biofilm (*Pseudomonas aeruginosa, Klebsiella pneumoniae, and Vibrio cholera*). Another study conducted on seawater-grown biofilm provides a consistent result, as thicker biofilm was observed at conditions where the temperature was increased by 5^{o}C (Rao, 2009).

The humidity level was reported to have less influence on the biofilm growth as compared to temperature. Else *et al*. (2003) reported highest biofilm growth for the temperature of 30^{o}C at a relative humidity of 100% for a study conducted using crushed rock samples. The study was conducted by growing biofilms under varying humidity concentration using different concentration of salt solutions at 30^{o}C, 60^{o}C and 70^{o}C. The effects of temperature on biofilm production were significant, as the temperature can delay biofilm growth as certain temperature limit has been studied to cause protein denaturation of the bacteria thus may stop or slow down the bacterial growth process (Ahmed and Vafai, 2012).

The presence of multiple species was also observed to change biofilm characteristics. Dual species biofilm was observed to be more resistant to antimicrobial agents, as compared to single species biofilm as reported by Simões *et al*. (2009) from the study using *Bacillus cereus* and *Pseudomonas fluorescens*. The study also reported that more biofilm production was observed in dual species biofilm, as compared to single species biofilm, which was speculated to be caused by bacterial survival to antimicrobials agent. Ohashi *et al*. (1999) reported that biofilm density varies with microbial composition and shows that more biofilm production was obtained for denitrifying biofilm under high substrate load conditions.

pH and solid surfaces type and characteristics have also been reported to influence biofilm production. Hostacká *et al*. (2010) demonstrated that increasing pH from 5.5 to 8.5 leads to 139 to 244% increase in biofilm production for *Pseudomonas aeruginosa* biofilm. Pederson (1990) reported more biofilm growth was observed on rougher substratum surface (matt stainless steel surface). Biofilm was grown using municipal drinking water for 167 days on stainless steel and PVC surfaces. The study suggested that the finding was due to the reduction of biofilm detachment as biofilm was shielded from the flow and increasing substratum surface area for the biofilm growth.

Wäsche *et al*. (2002) reported that biofilm structure, density, and thickness were influenced by hydrodynamic and substrate load during biofilm growth phase. Smooth biofilm cultivated from activated sludge samples were obtained under high shear stress and low substrate conditions (Reynolds number = 6000, flow velocity = 0.231 m/s, glucose concentration = 2.5 g/m^2d). This result was also obtained by another study conducted on pure media culture, where biofilm density was reported to increase with increasing shear stress and decreasing substrate load from 7.70 to 0.94 g COD/m^3d (Kwok *et al*. 1998). Melo and Vieira (1999) reported similar findings, as physical stability for pure culture biofilm made of *Pseudomonas fluorescens* was observed to be increasing with flow velocity (ranging between 0.34 to 0.97 m/s, shear stress between 3.4 and 9.7 N/m^2). Other than that, the study also found that thicker and less stable biofilm was obtained under turbulent flow.

Rochex *et al*. (2008) reported that diversity of biofilm grown from industrial water was decreasing under increasing shear stress (from 0.055 to 0.27 Pa). Higher shear stress was also observed to slow down biofilm maturation process thus mostly produced only young biofilm at high shear stress level. Mixed culture biofilm grown under high shear stress value ranging between 1.1 to 3.1 N/m^2 has shown to have higher density (Choi and Morgenroth, 2003) and biofilm produced was observed to be thinner, denser and have a smoother outer layer (Liu and Tay, 2001).

Another interesting finding was increasing of biofilm thickness with substrate loading rate and biofilm growth was not affected by increasing shear stress as reported in pure culture biofilm made of *Pseudomonas aeruginosa* as reported by Peyton (1996). The studies reported an increase of biofilm thickness as much as 30 μm with substrate loading rate made of glucose ranging from 0.0102 to 0.0922 g/m^2h.

Beyenal and Lewandowski (2002) reported that biofilm re-arrange their structure based on flow velocity in the system to ensure that they are able to withstand the shear stress of the fluid flowing past them and to control the rate of nutrient transportation process into the biofilm. Low velocity biofilm showed low density and highly effective diffusivity but was not able to withstand higher shear stress level while biofilm obtained at higher shear stress level shows higher density and ability to withstand higher stress values but have a lower effective diffusivity. The study was done on two different bacteria, *Pseudomonas fluoresens* and *Klebsiella pneumonia* at flow velocities ranging from 0.8 to 28 cm/s. In addition to that, a study by Lau (1995) suggested that increasing flow velocity helps in improving biofilm growth condition by enhancing the supply of nutrient and oxygen from the liquid phase to biofilm.

It is interesting to note that although biofilm growth under high shear stress level is possible, a sudden increase in shear stress has the ability to initiate sloughing process. However, maintaining constant shear stress does not actually prevent biofilm detachment from occurring (Elenter *et al.* 2007).

In general, most studies agreed that more biofilm production was observed at higher temperature conditions. Humidity level does not have a significant influence on biofilm, as compared to pH and microbial composition in the biofilm. Substrate concentration is also observed to have less influence on biofilm compared to temperature. Increasing shear stress has been demonstrated to produce thinner and smoother biofilm. However, these findings seem to rely on the type of bacteria presents in the system.

There were unlimited possibilities on the biofilm obtained under different conditions which further illustrates the complexity of biofilm growth processes. More studies are required to obtain a better understanding of the influence of each of these factors to the biofilm growth.

2.2.2 Biofilm in sewers

Biofilm in pipes has been studied intensively for drinking water problems, biofouling, its role in the degradation of organic matter and its contribution to in-sewer processes. However, the importance and effects of biofilm growth onto pipe flow and sewer hydraulics have only been researched recently, due to difficulty and limitation in designing experiments that are able to mimic the conditions of a real sewer.

Beyenal and Lewandowski (2005) reported that biofilm growth smoothed wall surface under low velocity values of 0, 30, 60, and 90 mL/min using biofilm grown from activated sludge sample taken from municipal wastewater treatment plant (Lewandowski *et al*. 1992). Images taken using Nuclear Magnetic Resonance Imaging (NMRI) method show systems with biofilm have more stable flow pattern. Another study conducted by Guzmán *et al.* (2007) found that biofilm grown in potable water enriched with glucose and methanol with COD values of 800 mg/L increases pipe surface roughness in a 13 m pipe length with a diameter of 150 mm and 200 mm configuration lab scale setup. The biofilm was grown for 45 days at three different slopes; 0.1%, 0.3% and 0.5%. The pipe roughness was obtained through estimation of Manning's n coefficient.

However, no additional studies can be found on this subject that can be used to clarify these results. Other than that, wastewater-grown biofilm is likely to produce a different set of results, due to multiple substrate and species of organisms that exist in the wastewater. This may be caused by increasing competition and survival between bacteria for space, oxygen and nutrients. Several studies have reported that more EPS were detected in mixed culture biofilms as compared to pure culture biofilm (Andersson *et al*. 2011) which

further supported the possible different biofilm growth one might obtain due to the usage of wastewater for biofilm growth.

2.3 Sediment in sewers

Sewer sediment has been a popular topic for a discussion and research due to several related current environmental issues such as flush events where changes in environmental conditions have direct effects on the sediment transport processes in sewers (Sakrabani *et al*. 2009).

Other than that, sewer sediment has also been studied due to problems that it may pose to the environment and society. It has been established that sewer sediment deposits can causes blockage, reducing hydraulics capacity in the sewer and act as storage to pollutants (Creaco and Bertrand-Krajewski, 2009). Solids have been identified as the main source of pollutions in wet weather conditions, which made up 83 to 92% of the total pollution COD values (Chebbo *et al.* 1995). These problems may lead to more serious issues such as surface flooding and production of corrosive gases in the atmosphere.

Sediment is commonly made of solids that can enter sewers from various sources including but not limited to atmosphere (dust particles and aerosols), ground surfaces (accumulated solids washed off during storm events), below ground surfaces (infiltration and exfiltration), sewage and from processes inside the sewage (degradation and decaying process of solids) (Ashley and Crabtree, 1992).

Butler and Davies (2004) defined sewer sediment as any settleable particulate materials that found in stormwater or wastewater and able to form bed deposits in sewers or other associated hydraulic structures under appropriate conditions.

There are four categories of solids in the sewer based on particle sizes as shown in Figure 2.2 (Butler and Davies, 2004).

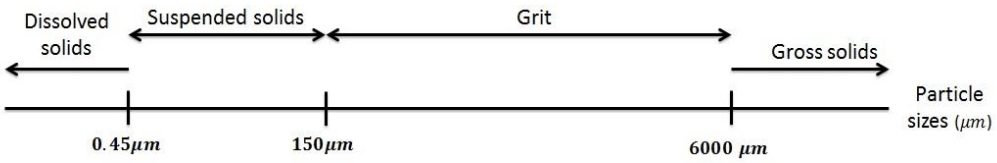

Figure 2.2. Basic classification of solids in wastewater and stormwater (modified from Butler and Davies, 2004).

Two mains solid transport modes have been established from the literature. Bed load occurs near the bed and consists of mainly coarse particle while suspension loads refer to smaller and finer particle that are transported via suspension. Sediment is transported via both method during storm events, however, during dry weather periods, the sediment will settle and accumulate a high organic layer on the bed causing the development of cohesive-like bonds in the sediment (Banasiak and Tait, 2008; Fernandez Luque and Van Beek, 1976). The strength of this bond depends on sediment input, bed consolidation phase, and organic matter presence in the system.

Several studies have reported changes in the sediment deposit caused by microbial activity in the system. Tait *et al*. (2003a) reported that aerobic biofilm growth reduced the strength of sewer deposits and exhibits two-stage erosion process. This study was conducted with two sediment types; substitute sediment made of crushed olivestone and sand, and real sewer from Dundee and Loenen under aerobic conditions at 4 different periods; 18, 42, 56, and 80 hours. The first layer refers to an active aerobic top layer which may change bed material and strength while the second layer is a bottom or inner layer that consists of anaerobic/anoxic bulk phase. The erosion of the second layer was reported to depend on the initial removal of the first layer.

Schellart *et al*. (2005) reported that sediment deposit strength was reduced due to microbial activity in the sediment and from increasing the consolidation period during the formation phase of the bed. The study also suggested that 18 hours consolidation period was sufficient for bacteria processes to influence deposit strength as demonstrated from the

experiments done on real sediment from the UK and The Netherlands under both aerobic and anaerobic conditions. The tests were conducted at 2 different temperatures (4^oC and 14^oC) for 18, 90 and 138 hours consolidation periods.

Another study by Tait *et al*. (2003b) shows contradictory results, as they reported an increase in deposits resistance with duration of consolidation. The increases were speculated to cause by biofilm growth and bed physical consolidation. The experiments were conducted on a single size particle of crushed olivestone at consolidation period of 16, 66, 144 and 162 hours. Two temperatures were used for the tests; 4^oC and 14^oC and both aerobic and anaerobic conditions were tested.

Fang *et al*. (2014) reported a similar finding on a study using real sediment, as biofilm growth on nutrient-rich mixture was found to increase sediment stability. The study was conducted using 4 different sediment particle sizes, ranging from <0.05 mm to 0.1 mm. The sediment was collected from a stabilization pond, and 8 tests were conducted at each sediment sizes, where the biofilm was grown for 1 week to 8 weeks duration. The study reported that biofilm growth has a strong influence on sediment characteristics by changing the morphology and structure of the biofilm.

The changes in sewer deposits due to microbial activity were found to be inconclusive as the changes obtained was not consistent. This may occur due to various factors that may affect the biofilm growth such as the type of sediment used, environmental conditions in the system and period of the bed consolidation phase.

These findings were also in agreement for marine grown biofilm. A lot of studies that have been done on effects of marine, river and fluvial grown biofilm on sediment agreed that biofilm growth increased bed stability thus increasing sediment strength. This phenomenon, or also known as biostabilization was first studied by Grant and Gust (1987) and concluded

marine sediment-bound biofilm requires five times higher shear stress level than a system without biofilm to disturb the bed.

These findings are supported by Vignaga *et al*. (2012) and Vignaga *et al*. (2013) that found that marine-grown biofilm was more resistant to shear stress from fluid motion and detachment processes. The study also reported better biofilm growth on porous media, which is commonly found in the river and sea beds. Biofilm has been reported to increase bed stability by modifying sediment structure and bed surface structure. This caused changes in sediment behaviour, as the sediment developed a more elastic membrane on the bed surfaces. Other than that, Fang *et al*. (2012) reported that biofilm growth has a significant influence on rheological properties of cohesive sediment after 3 weeks period, as shown by increase yield stress, viscosity and shear stress values over time for tests conducted with samples obtained from lotus pond and enriched with different nutrients.

On the other hand, several studies have also reported a reduction in bed strength with biofilm growth for marine and fluvial environment (Le Hir *et al.* 2007; Mermillod-Blondin and Rosenberg, 2006). Both studies reported a decrease in bed resistance due to biological processes, which is known as bioturbation. The process was described as the destruction of the internal sediment structure due to microorganism activities that cause an additional disturbance on physical and biogeochemical processes in the sediment.

Based on the author knowledge, there is no known comparison has been studied on the changes observed for sediment in sewer and marine and fluvial environment due to biofilm growth. It is hypothesized that the sediment characteristics may present a level of differences between the two as these two conditions provide very different growth conditions for the biofilm.

Microorganism presents in sewer biofilm are mainly made of complex multiple species of bacteria that are still not clearly understood and identified. Wagner and Alexander (2002) reported that *Beta-, Alpha-, Bacteroidetes*, *Actinobacteria* and *Gammaproteobacteria* were found from activated sludge

and biofilm samples obtained from sewage treatment systems. Kaevska *et al*. 2016) reported bacteria communities consisted of *Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes* and *Fusobacteria* was found from river sample at a city in the Czech Republic. Similar bacteria composition was reported from samples of Santa Ana River at California, USA (Ibekwe *et al*. 2016) and Ganjiang River, China (Wang *et al*. 2016).

Nutrient concentration also largely differs between sewer and marine system. Nutrient concentration of sewer is high, as 350 mg/L to 750 mg/L COD values has been reported for wastewater (Butler and Davies, 2004) while 200 to 800 mg/L COD values were reported for sewer networks in United States, Europe and Australia (Hvitved-Jacobsen *et al*. 2013).

On the other hand, nutrient concentration for a marine system is lower than sewer system, with values of 1.6 to 20.6 mg/L COD was reported from rivers in South Korea (Hur and Cho, 2012) and 0.7 to 1.13 mg/L COD was obtained from seawater (Liu *et al*. 2005). Another study conducted using Mediterranean seawater at Egypt obtained COD values of 3.0 to 4.0 mg/L (Faragallah *et al*. 2009). This was speculated to be caused by lack of organic matter presents in the marine systems as compared to sewer systems.

These factors were taken into consideration in the works that were conducted that aims to understand the effects of biofilm growth on the bed sediment characteristics when subjected to increasing shear stress level.

2.4 In-sewer processes

This section will discuss processes that occur in the sewer, which related to the different sewer components, sewer conditions and organic matter.

Physical, chemical, electrochemical and biological processes have been reported to occur in different sewer phases; sediment, biofilm, sewer atmosphere, sewer walls and bulk phase (Boltz and Daigger, 2010; Hvitved-Jacobsen *et al*. 2002; Kaijun *et al*. 1995). The main factor for these

processes to occur is organic matter. Organic matter is the electron donor for these processes while the electron acceptor can be dissolved oxygen for aerobic condition, nitrate for anoxic condition and sulphate and carbon dioxide for anaerobic conditions (Rauch *et al*. 1999).

Organic matter can be defined in many different ways; a chemistry definition of organic matter is any compound that contains carbon. To a biologist, organic matter is a living material or a material that was once alive. For an environmental engineer, organic matter definition is material that burns at 550 ^{o}C. In wastewater terms, organic matter can be defined as the nutrient loads of the wastewater. Organic matter plays important roles as it determines the quality of the wastewater in the sewer thus allowing adjustments to be made in the wastewater treatment plant to achieve optimal treatment of the wastewater before the release of the treated effluent to the environment.

The major constituents of organic matter present in the sewer are proteins that constitute between 40 to 60% of the overall organic matter present, carbohydrates (25 to 50%) and fats (10%) (Haldane and Logan, 1994). Minor groups of organic matter include volatile fatty acids and amino acids (Raunkjær *et al*. 1994). These values, however, vary depending on other factors including sewer type, wastewater residence time, climate, wastewater sources and location of the sewer (Nielsen *et al.* 1992).

Physically, organic matter can be grouped into four categories depending on its sizes. Settleable is when organic matter is less than 800 μm, supra colloidal is between 1 to 100 μm, colloidal is between 0.8 to 1 μm and soluble is for organic matter less than 0.08 μm (Huang *et al*. 2010). An easy method used to separate soluble and particulate organic matter is by filtering the sample with 0.45 μm pore size filter papers which were usually used in wastewater applications (Patel *et al*. 2005; Rao, 2009). However, these pore sizes may vary with the sample, and the definition of ‘dissolved’ itself depends on the intended purposes of the analysis. Processes that occur in a sewer can be summarized in Figure 2.3.

Figure 2.3 shows that sulphur oxidation mainly takes place in the sewer atmosphere. In this process, sulphuric acid is produced by oxidation of H_2S at the concrete surfaces, which is also known as sewer corrosion (Hvitved-Jacobsen *et al*. 2013). A 90% decrease of sulphide concentration in gravity sewer was found to be caused by sulphur oxidation, and only a small fraction was released to the environment and caused odour problems (Nielsen *et al.* 2006)

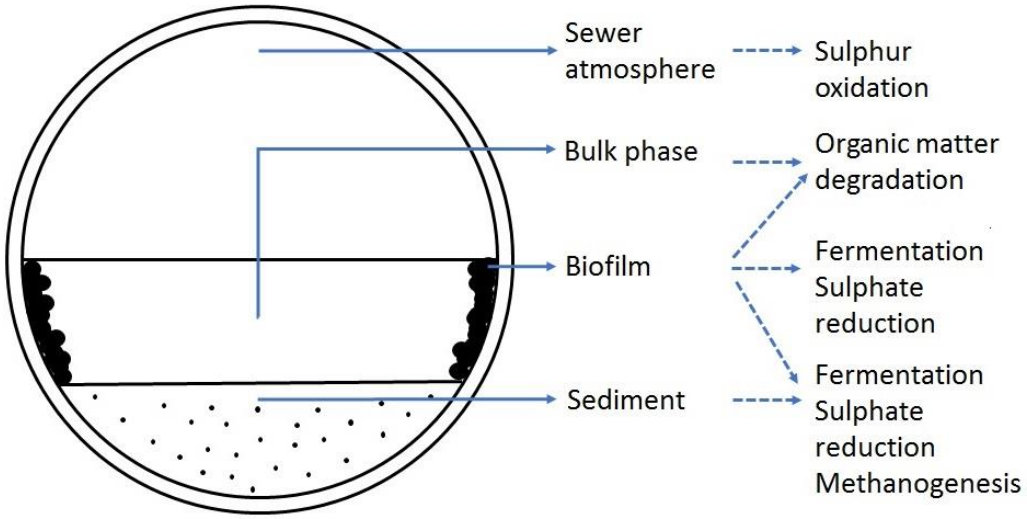

Figure 2.3. Summary of in-sewer process for each of the sewer component (adapted from Ashley *et al.* 2004).

Organic matter degradation in wastewater takes place mostly in the bulk phase and biofilm as reported by Jahn and Nielsen (1998) and Raunkjær *et al*. (1995). Microorganisms are responsible for these processes to occur, where dissolved oxygen is consumed during the process. These processes depend on the electron donor, electron acceptor and sewer conditions, for example, anaerobic or aerobic sewer conditions.

High activity of heterotrophic microorganisms during aerobic condition leads to the growth of biomass, hydrolysis and organic matter degradation in the sewer (Hvitved-Jacobsen *et al*. 2002). Hydrolysis is a temperature dependent process assisted by enzymes where large molecules are broken down to small ones with the presence of water (Butler and Davis, 2004). During this

process, the easily biodegradable substrate is removed while the slowly biodegradable substrate is produced.

Several studies have found that dissolved organic matter is used more quickly as compared to the total organic matter presents in the wastewater (Raunkjær *et al*. 1995). This is because large particles need to undergo a hydrolysis process first before being used by microorganisms in the system.

Anoxic conditions rarely exist in the sewer, unless if nitrate is added in the sewer to prevent anaerobic conditions (Hvitved-Jacobsen *et al*. 2002). Under anaerobic conditions, several processes occur in the sewer. Anaerobic hydrolysis, fermentation, and methanogenesis have been reported to occur in wastewater, biofilm and sediment phase. Fermentation is a process where readily biodegradable substrate is converted into volatile fatty acids (VFA). Methanogenesis is a process that transformed fermentation product into methane by bacteria known as methanogens. Sulphate reduction is a process that can occur both in aerobic and anaerobic conditions. In this process, H_2S is produced from a chemical reaction where organic carbon or H_2 is oxidised while sulphate is reduced by SRB (Hvitved-Jacobsen *et al.* 2013).

Organic matter transformation in aerobic conditions can occur in three different ways; growth of biomass, hydrolysis and consumption of dissolved oxygen in the system (Hvitved-Jacobsen *et al*. 1998). In order to evaluate these changes, two different approaches were used in this study. The first approach was to measure any changes in the total amount of organic matter by determining Chemical Oxygen Demand (COD) values of the sample (Ginestet *et al*. 2002; Vollertsen and Hvitved-Jacobsen, 2002). The second approach was by measurement of any changes in the concentration of specific organic pools, as for this study, determination of changes in protein and carbohydrates concentration of the sample (Raunkjær *et al*. 1995; Zhang *et al*. 2008).

As previously stated, solid transport of sediment and biofilm detachment processes will not be considered in the process described in this work. The approaches mentioned above are used to study biofilm growth and its influences on pipe hydraulic roughness, sediment deposit stability, and organic matter degradation in the system.

2.5 Overview of sewer modelling works

Hydraulic roughness is defined as the measurement of resistance the flow experienced due to pipe roughness which refers to physical irregularities of the surface. Hydraulic roughness is regarded as one of the most important parameters required for sewer modelling (Stanić *et al*. 2017) as it determines the flow velocity profile in the pipe or channel which has a direct influence on free surface position, biofilm growth, and bed sediment transportation. Generally, pipe roughness values of new material have been established and can be summarized in Table 2.1.

Table 2.1. Typical hydraulic roughness values for different material commonly found and in new condition (Chadwick, 2004).

Pipe material	Pipe roughness (mm)
Slime concrete sewer	6.0
Galvanized iron	0.15
Wrought iron	0.05
Asbestos cement	0.03
Plastic	0.03
Bitumen-lined ductile iron	0.03
Spun concrete line ductile iron	0.03
Brass, copper, glass, Perspex	0.003

There are a lack of information available on pipe and hydraulic roughness values for used pipes, as pipes have been reported to deteriorate due to ageing, and corrosion (Bennis *et al.* 2003), biofilm growth (Guzmán *et al.*

2007) and sediment accumulation (Romanova *et al*. 2011a). Several studies conducted on changes of pipe roughness values due to biofilm growth have shown conflicting findings, as Guzmán *et al*. (2007) have reported an increase in Manning's n coefficient for biofilm growth on potable water enriched with methanol and glucose at COD concentration of 800 mg/L. The biofilm was grown for 45 days. Manning's n values were reported to increase from 0.011 (clean pipe) to 0.015 to 0.020 for biofilm-coated of 200 mm diameter PVC pipe at 0.5% slope.

An earlier study conducted by Lewandowski *et al.* (1992) reported a smoother pipe with biofilm formation grown with activated sludge sample collected from municipal wastewater treatment plant. The study was conducted under low-velocity conditions, where laminar flow was achieved.

No details of hydraulic roughness values adjusted by biofilms in existing sewer modelling studies can be found from the literature. The changes of pipe hydraulic roughness values due to microbial growth are still to be included in the existing models description. Some examples of models that have been developed and reported from the literature can be summarized in Table 2.2. The vast majority of current sewer models have been shown to integrate process description of several sewer components in one model. This is deemed logical and necessary, as none of the sewer components is able to exist independently.

In-sewer modelling studies are difficult due to insufficiency of available data from the sewer system to support the build and calibration of such models. For sewer sediment models, the model requires great numbers of parameters such as sewer geometries and particle characteristics in order to obtain a model that is able to represent the required processes with a high level of confidence, accuracy and reliability. The most challenging part in modelling sediment transport is due to the complexity of the processes involved such as erosion and deposition. It is also challenging to compare results for different models as each model usually are calibrated using their

own set of data, and the calibration was conducted only using a few available data.

Adding biological derived effects parameter in the existing models is hypothesized to increase the accuracy of models in predicting flow parameters in the sewers. This will further help with a better estimation of in-sewer processes such as organic matter degradation and sediment re-suspension and consolidation processes.

Table 2.2. Summary of currently in used modelling works for sewer processes.

Models	Overview	Advantages	Disadvantages
MouseTrap	• Deals with sewer transport processes[1] and include biochemical processes in the description[10]. • Advection dispersion module: use to model wash load processes[1]. • Sediment transport module: use to model suspended solids and bed load processes[1]. • Water quality module: use to model transformation processes in the sewer[1]. • Derived from a study based on uniform non-cohesive sewer sediment[1].	• More flexible than InfoWorks [1]. • Can simulate various hydraulic performances over time with high accuracy if given the sufficient field data[7]. • Modelling can be conducted with various sediment size fractions[10] for both uniform and non-uniform sediment particles[1].	

InfoWorks/Hydroworks	• Deals with few of sewer transport processes[1]. • Derived from a study based on uniform non-cohesive sewer sediment[1]. • Has been used to calculate urban catchment runoff, flow in the sewer and quality and quantity of the effluent wastewater[8]. • Provides full hydraulic solutions and able to predict sediment buildup in sewer theoretically[10].	• 2 sediment fractions are defined; Organic fraction and mineral fraction. Both can be modelled dependently or independently[1]. • Sufficient field data are required in order to simulate changes in hydraulic parameters with time[7].	• Bed load transport[1] and biochemical processes are not included in the model descriptions[10]. • Not recommended for sewer accumulation prediction in sewer due to limited sedimentation depth in model descriptions[1]. • Computational time has been reported to take up to a year of continuous run[4].
Storm Water Management Model (SWMM)	• Deals with planning, analysis, and design related issues with stormwater runoff, sewers, and another type of drainage system[9]. • Differentiate between bed, suspended and wash load for 10 different sediment sizes[2].	• Can be used for various processes including rainfall, accumulation and melting of snow, and interflow between drainage system and groundwater[9]. • Vast ability and flexibility in hydraulic modelling which	• Unrealistic assumptions: single and one size distribution of deposits and suspended loads[2]. • In need of a great number of parameters including flow profile and sewer or water body characteristics [2].

		include various flow regime in the water body, and fittings such as orifice and pumps[9]. • Can be used in the estimation of pollutant release due to stormwater runoff[9].	

Activated Sludge Model (ASM)	• Deals with transformation processes in the sewer[3] and biochemical processes in biofilm[5].	• Include various biochemical processes in the sewer such as aerobic growth of heterotrophs, the decay of heterotrophs and hydrolysis of particulate organic matter[3].	• Include various restrictions, limitations and assumptions[11];
			  • Constant temperature, pH and nitrification coefficient values.
			  • No consideration in changes in organic matter concentration over time.
			  • Homogenous and constant heterotrophic biomass.

Wastewater aerobic/anaerobic transformation in sewer (WATS)	• Deals with transformation processes in wastewater and biofilm phase[5]. • Has been validated and	• Includes sulphide production in the process descriptions[5]. • The model descriptions include major biological processes in	• Hydrogen sulphide production and in-sewer denitrification during nitrate dosing process are not included in the model

	calibrated against field measurements data[6].	sewer such as sulphur cycle, aerobic/anoxic heterotrophic transformation of organic matter and aerobic/anoxic heterotrophic transformation of organic matter[5]. • Allow predictions of sulphide concentration and consequent problems[6].	description[5].

[1] Bouteligier *et a*l. 2002
[2] Bertrand-Krajewski *et al*. 1993
[3] Bjerre *et al*. 1998
[4] Ashley *et al*. 2000
[5] Jiang *et al*. 2009
[6] Nielsen *et al*. 2008
[7] Tait *et al*. 2003a
[8] Schellart *et al.* 2010
[9] EPA *et al.* 2017
[10] Field *et al*. 2004
[11] Henze *et al*. 2000

2.6 Conclusions

This chapter provides background information on the knowledge and approaches that have been taken from previous studies to obtain a better understanding of biofilm growth and its effect on pipe hydraulic roughness, sediment stability and organic matter transformation in a sewer. Important findings that are relevant to this study are also included in this chapter.

A lot of research has been conducted on biofilms. Biofilm growth and development processes have been investigated countless times thus provide valuable information on biofilm characteristics under different conditions, factors that are affecting the processes, biofilm modelling and biofilm observation methods. However, most of these studies were done under controlled conditions where one or two substrates and up to three species of bacteria were considered or any combinations of them. This simplification is deemed necessary to obtain the intended objectives of these studies, however, with more knowledge obtained from new research, it is possible to further expand this limitation for a better representation of processes in a sewer.

Further studies conducted on biofilm provide evidence that biofilm has the ability to influence hydraulic conditions of flow. These findings, however, are not relevant in representing wastewater-grown biofilm, which has been demonstrated to have high complexity of structure, composition and characteristics due to multi-species and multi-substrate nature of the wastewater.

Other than that, the study of the influence of biofilm on bed stability has shown conflicting outcomes, which suggested a more thorough investigation are needed for a better understanding of these findings. A controlled method for determining these changes is constructed and implemented in this work, which involves the measurement of organic matter presents in the system, as an indication of biofilm growth and organic matter consumption. From literature, countless studies have used this approach in measurement

(Ginestet *et al*. 2002) and modelling of organic matter transformation processes in a sewer (Rudelle *et al*. 2012), however, no attempts by other authors have been found to link the relationship between organic matter degradation with changes observed in pipe flow and bed sediment stability caused by wastewater-grown biofilm.

Chapter 3 Materials characterisation

This chapter aims to provide insight on the materials that were used in this study. Wastewater and substitute sewer sediment made of clean sand and crushed olivestone were subjected to several tests to determine their properties. This information is an important foundation for this study, as it will help in understanding the wastewater composition obtained and the processes of organic matter degradation that occur in any experimental work during testing.

3.1 Wastewater characterisation

3.1.1 Wastewater sampling procedure

Wastewater was collected from a local wastewater treatment plant that is situated an hour return trip by car from the University of Sheffield. The plant is treating both domestic and industrial wastewater with a design capacity of 185,000 PE (population equivalent). Wastewater was collected at the inlet of the plant after the raw sewage has been physically screened to remove large solids but has not been treated chemically and biologically. Wastewater was collected at the same time of the day using a bucket (see Figure 3.1) and stored in air-tight jerricans until it arrived at the laboratory. The temperature of the sewer wastewater was taken during the sampling, and COD and pH were determined right after the wastewater arrived at the laboratory.

Figure 3.1. The site of wastewater sampling at Woodhouse Mills treatment plant.

3.1.2 Materials used as bed sediment substitute

In order to provide a level of control in the tests conducted, substitute sewer sediment made by crushed olivestone and sand (Fraction D after Standard BS 1881, part 131) were used instead of real sewer sediment. This is to ensure that the bed sediment used in the tests was uniform, with known properties for a better understanding of the results obtained. The properties of both materials can be summarized in Table 3.1 (Camuffo, 2001; Tait *et al*. 2003b).

Table 3.1. Material characteristics of surrogate sediment.

	Sand	Crushed olivestone
Characteristics particle diameter (d_{50})	150 to 300 μm	47 to 54 μm
Density (kg/m^3)	2650	1445

Both materials were provided by local companies in the UK; David Ball Specialist sands and BWLCH TOCYN Farmhouse. Clean sand was prepared by rinsing the sand with 5% H_2O_2 solutions followed by distilled water and

dried at 105^{o}C. The sand was cleaned to remove any impurities or contamination obtained during storage.

3.2 Analytical procedures

3.2.1 Pump calibration

In order to establish a link between pump speed and discharge flowrate at different hydraulic conditions, a calibration of the peristaltic pump was conducted before each test.

A peristaltic pump (Cole Palmer, Masterflex 07258-10, USA) was used for this work. The pump was installed with two heads (Cole Palmer, Masterflex WZ-77200-50, USA) in order to provide higher pumping ability of the liquid. Norprene tubing with an internal diameter of 7.9 mm (Cole Palmer, USA) and silicone tubing with an internal diameter of 8.0 mm (Cole Palmer, USA) were connected to the pump and used to transport the liquid.

The main setup for this procedure was a peristaltic pump which was connected to two tanks with a different water level in them using norprene tubing and silicon tubing. The calibration was done by measuring the time taken for 1 L of tap water to travel from one tank to the other at increasing pump speed. The measurement was done 10 times in order to calculate uncertainties of the flow rate calculated by dividing the volume of water travelled (1 L) with the time it takes to move from one tank to another (in seconds).

3.2.2 Oxygen sensor calibration

For a more reliable measurement of dissolved oxygen concentration, oxygen sensor was calibrated before the start of each experiment. The principle involved in this method is based on the effect of dynamic luminescence quenching by molecular oxygen. The relationship between dissolved oxygen concentration and luminescence intensity and lifetime is described by the

Stern-Volmer equation. Optical properties of the analyte in the sensors changes when interacting with the level of dissolved oxygen in the sample. These changes may cause changes in the colour (absorbance or spectral distribution) or in the luminescence properties which include intensity, lifetime and polarisation. This information was transmitted by the light, produced by the LED in the sensor (Presens, 2017).

Dissolved oxygen sensor (Presens, TX3, Germany) used in this study was a needle-type fibre optic oxygen sensor. The fibre optic cable had a diameter of 140 μm and was housed in a microlance syringe with dimensions of 0.8 mm x 40 mm. The sensor was connected to the transmitter which connects to a computer to give a real-time data during usage by pre-installed software named 'TX3'.

Calibration was conducted using two different solutions; 100% oxygen solution was made by aerating tap water for 2 hours while 0% oxygen solution was made by dissolving 1 g of sodium sulphite (NA_2SO_3) into 1 L of distilled water.

Oxygen saturation level was measured for both solutions for 10 minutes, with 1 s intervals between each measurement. The measurement was conducted at room temperature, 20.0 ± 1.0 ^{o}C. The average for phase and temperature values obtained were then calculated and added manually to the software to overset any previous values. The phase refers to changes in the light optical path (Gholamzadeh and Nabovati, 2008). The software will have a soft reset afterwards which indicates the calibration was a success.

3.2.3 Chemical oxygen demand (COD) protocols

Chemical oxygen demand (COD) is the main method used in quantifying the concentration of organic matter present in the sample in this study. This method was able to provide information on oxidizable material presents in the sample, thus offer some degree of understanding of organic matter consumption and transformation in this study.

Chemical oxygen demand refers to the measurement of organic and inorganic material available in a sample that can be oxidized chemically. COD values in this study were determined by Hach Lange method (Hach Lange, LCK 514, Germany). The method was a simplified version of COD quantification based on closed reflux colourimetric method by Standard Method (APHA *et al*. 1999).

In this method, 2 mL of sample was added to a pre-mixed solution in a vial and heated at 150 ^{o}C for 2 hours. After the solution cooled down, the absorbance of the solution was read using a spectrophotometer (Hach Lange, DR3900, Germany) at 605 nm. The sensitivity of this vials is 0.0005 Abs/(mg/L) with lower detection limit values at 4.6 mg/L (Hach, 2017).

The theory behind this method is to measure the changes of Cr^{3+} and $\mathrm{Cr_2O_7^{2-}}$ of the sample after oxidisation. The former is applicable for COD values within 100 to 900 mg/L, where the sample was measured at 600 nm regions while the latter is for COD values of less than 90 mg/L and measurement was done at 420 nm.Sample with low COD concentration yield yellow to orange colour spectrum after oxidation while green colour is observed for sample with high COD concentration (APHA *et al*. 1999).

Hach Lange method was preferred as a safer option as it possesses a lower risk of injuries due to minimal volume of dangerous chemical. This method is suitable for a general measurement of organic matter in the sample and is limited to samples that have a low volume of insoluble suspended matter as this will influence the spectrophotometer reading as less light could pass through.

3.2.4 Protein determination protocols

As discussed previously, protein has been suggested as one of the main groups of organic matter presents in the wastewater. Protein was also recognised as the largest fraction of grown biofilm using raw wastewater with

a low concentration of easily biodegradable matter and COD values of 110 mg/L (Raunkjær *et al*. 1997).

Protein is defined as a polymer that is made of amino acids linked by peptide bonds. It is one of the main components found in wastewater, as 40 to 60% of organic matter in the wastewater is made of protein (Haldane and Logan, 1994). All protein in this study was determined using a modified Lowry method, originally developed by Gerhardt *et al.* 1994.

In general, this method measures changes of $Cu+$ when reacted with a Folin reagent which resulted in a blue coloured solution caused by oxidation of amino acids by copper. This method is best used for sample with protein concentration from 1 to 1000 mg of protein/L (Walker, 2012). In this method, the sample changes colour to greenish blue depending on the concentration of amino acids composition of protein in the sample.

A standard calibration curve was constructed from bovine serum albumin (BSA) solutions ranging from 0 mg/L to 400 mg/L of protein. 0.5 mL of sample was used in each assay, and each sample was done in triplicate. A blank test was conducted using distilled water before any test samples measurement. Lower detection limit value obtained from the blank tests conducted on 60 samples was 5.9 ± 0.4 mg/L.

Three solutions were prepared beforehand; Solution A was made by dissolving 2.86 g of sodium hydroxide (NaOH) and 14.31 g of sodium carbonate (Na_2CO_3) with distilled water to make 500 mL of solution. Solutions B and C were made by dissolving 1.42 g of copper sulphate pentahydrate ($CuSO_4.5H_2O$) and 2.86 g of sodium tartrate with distilled water to make 100 mL of solution.

The Lowry solution was made by mixing Solution A, Solution B, and Solution C with a ratio of 100:1:1. This solution was made fresh on the day of any testing. Folin reagent solution was prepared by diluting 5 mL of 2N Folin and Ciocalteau's Phennol Reagent with 6 mL of distilled water during the test.

0.5 mL of sample was pipetted into a small tube, and 0.7 mL of Lowry solution was added. The solution was mixed using a vortex and incubated at room temperature for 20 minutes in the dark. Then 0.1 mL of Folin reagent solution was added, and the mixture was mixed and incubated for another 30 minutes in the dark. After the incubation, the sample was mixed, and absorbance was obtained using a spectrophotometer at 750 nm. This method can be summarized in Figure 3.2.

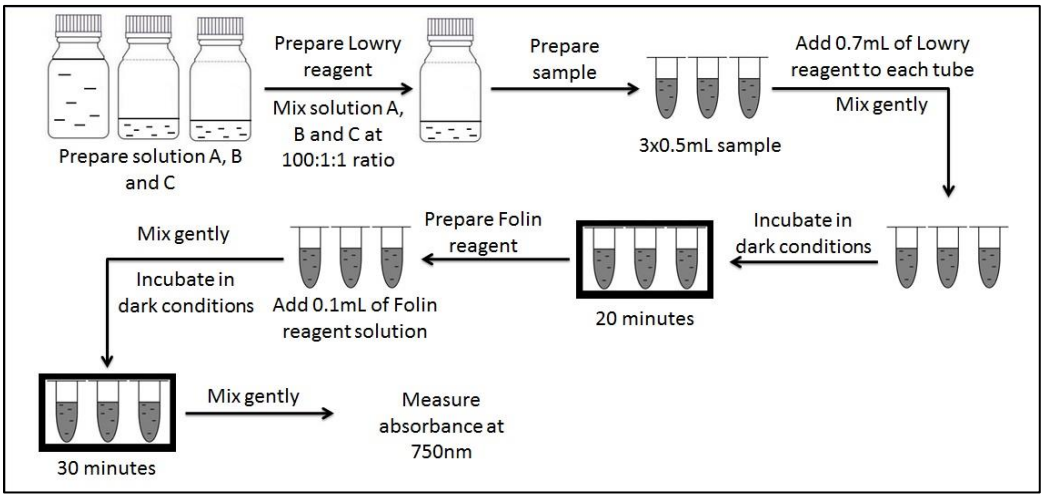

Figure 3.2. Summary of protein quantification protocol (Lowry method).

This method has been widely used in wastewater application due to its sensitivity to low protein concentration in the sample and its straightforward procedure. However, protein quantification using this method depends heavily on the sample pH (Walker, 2012). This limitation can be disregarded for a small volume of samples as the changes will be insignificant.

3.2.5 Reducing sugar quantification protocols

Carbohydrate is the second largest component of organic matter in wastewater with approximately 20 to 40% of wastewater consists of carbohydrates (Haldane and Logan, 1994). Carbohydrates can be group into monosaccharide, disaccharide, oligosaccharide, and polysaccharide. A monosaccharide is the simplest form of carbohydrates, as it exists as a single molecule of saccharides. A disaccharide is defined when two molecules linked together by a covalent bond. An oligosaccharide is

described as a small group of monosaccharide tied together while polysaccharide refers to a long polymer of monosaccharide link together.

In this study, reducing sugar was determined using a modified colourimetric method known as dinitrosalicylic acid (DNS) method, originally developed by Miller (1959). This method only measures reducing sugars, which are made of monosaccharide and some of the disaccharides, such as lactose.

The theory of this method is that DNS reagent will react with the aldehyde group in the sample under alkaline conditions and produce 3-amino-5-nitrosalicylic acid which resulted in orange colour. The intensity of the colour after the reaction is an indicator of the concentration of reducing sugar in the sample. This method was able to determine reducing sugar with concentration from 100 to 500 μg/mL (de Toledo *et al*. 2012).

A standard calibration curve was obtained using glucose solution with a concentration of 0 mg/L to 400 mg/L. 0.5 mL of sample was used for the assay, and each sample was done in triplicate. Two solutions were made before the assay started. Solution A was made by dissolving 300 g of sodium potassium tartrate ($KNaC_4H_4O_6.4H_2O$) with distilled water to make 500 mL, and Solution B was prepared by diluting 10 g of 3,5-dinitrosalicylic acid with 2 N NaOH solution to make a 200 mL of solution.

A DNS reagent was prepared fresh on the day by mixing solution A, solution B and the volume was raised to 1 L using distilled water. 0.5 mL of sample was added with 0.5 mL of DNS reagent and was heated for 5 minutes at 100^oC using a heating block.

After the heating process, the sample was allowed to cool down to room temperature. This was done in a quick manner, to avoid any precipitation in the tube. Once it reached room temperature, 1 mL of distilled water was added to the tube to stop the reaction. The absorbance was obtained using a spectrophotometer at 540 nm. This procedure can be illustrated in Figure 3.3.

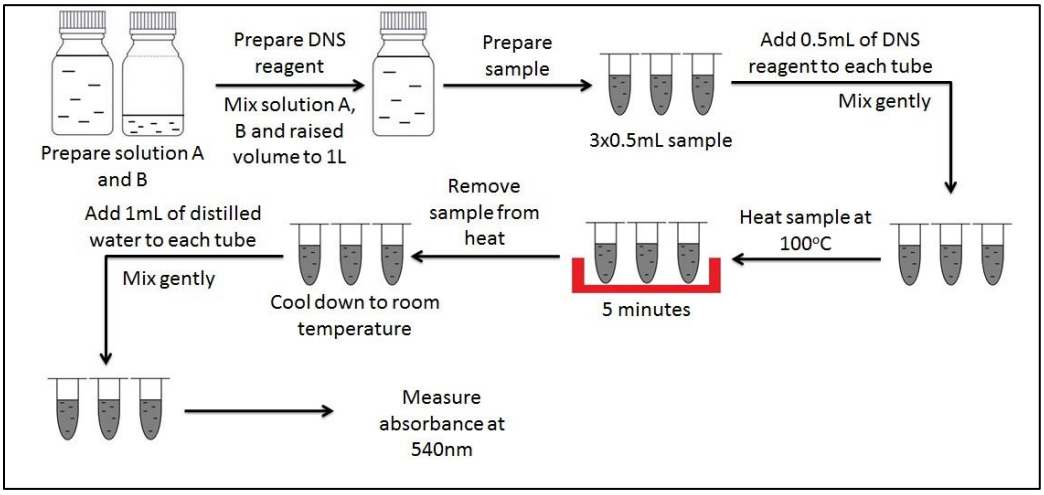

Figure 3.3. Summary of reducing sugar quantification protocol (DNS method).

A blank test with 60 samples was conducted using distilled water before measurement of any samples. The tests conducted obtained the values of the lower detection limit of 11.4 ± 0.3 mg/L. The reducing sugar concentration was used as an estimation of carbohydrate concentration in the sample. Only a few studies have used this method for wastewater application as Anthrone method is preferable due to a large range of carbohydrates that it can measure and no interference from other organic matter presents in the sample (Raunkjær *et al.* 1994).

This method was chosen as it is easily handled, have low analysis cost, and was sufficient for the intended analysis. Although reducing sugar was a fraction of the overall carbohydrates in wastewater, determination of reducing sugar was assumed to have a direct relation with total carbohydrates concentration in the wastewater, and the analysis should be able to demonstrate any changes in carbohydrates concentration in the system.

3.2.6 TSS and VSS measurement

Total suspended solids (TSS) is defined as a portion of solids that is retained by a filter while volatile suspended solids (VSS) is defined as weight loss of residue from ignition (APHA *et al.* 1999). Microfibre filters were used in this procedure, with pore size of 1.5 μm and 47 mm diameter (Whatman, 934-AH,

Germany). This procedure was conducted following Standard Method (APHA *et al*. 1999).

Before any measurement was conducted, the filter was rinsed using distilled water and ignited at 550^{o}C overnight. Each filter was numbered and weighted before used in the analysis. The dish and crucibles were cleaned prior to the test using tap water and dried at 550^{o}C overnight.

A well-mixed sample solution was filtered and residue collected was placed into a weighing dish which was then dried in an oven at 105^{o}C overnight. The dried sample was immediately stored in a desiccator to avoid moisture on the sample while the temperature was reduced to room temperature before the mass measurement was taken.

Once the measurement was taken, the sample was then ignited at 550^{o}C using a furnace for 2 hours. Due to the samples have different volumes, 2 hours was deemed adequate for the sample to achieve a constant mass condition. Once ignited, the sample was cooled in a desiccator until measured.

TSS and VSS were calculated following Equation 3.1 and Equation 3.2 as shown below;

$$TSS = \frac{(A-B)x1000}{Vs} \qquad \text{(Equation 3.1)}$$

Where; V_s is sample volume (L), A is mass of filter plus dried residue (g) and B is mass of filter (g).

$$VSS = \frac{(C-D)x1000}{V_s} \qquad \text{(Equation 3.2)}$$

Where; C is mass of residue plus dish before ignition (g) and D is mass of residue plus dish after ignition (g).

The sample was homogeneously mixed before the procedure to ensure that the result was representative of the environment being sampled. This method was sensitive for TSS values ranging from 2.5 to 200.0 mg of dried residue.

Both TSS and VSS measurements are prone to negative errors due to loss of volatile organic matter during drying. (APHA *et al*. 1999).

3.3 Results of organic matter present in wastewater sample

Organic matter present in the wastewater after each sampling session was obtained by determining COD, protein and reducing sugar concentrations. These results were used to observe organic matter variation with weather conditions and at the same time served as background information before the wastewater were used in any experimental works.

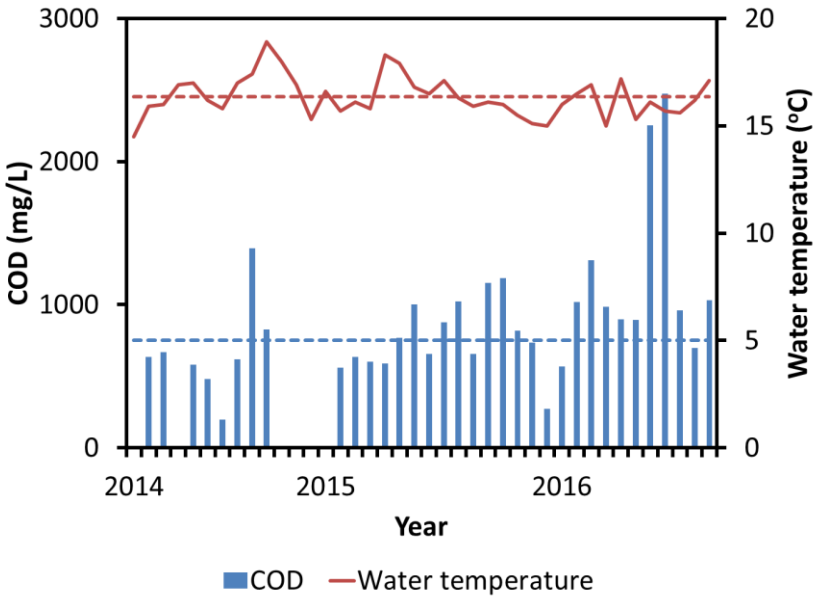

Figure 3.4. Average COD concentration and water temperature of wastewater during sampling.

Figure 3.4 illustrates the average COD values and wastewater temperature obtained during wastewater sampling. Sampling was done all year round, with no regard to dry weather or wet weather periods. Sampling was also conducted at the similar time of the day, to ensure some control over obtained wastewater. Average wastewater temperature obtained was 16.3 ^{o}C with the lowest value was 14.5^{o}C, obtained in January 2014. The highest temperature recorded was 18.9^{o}C in July 2014.

Excluding high COD concentrations obtained during winter 2016, the highest recorded COD value was 1392 mg/L which was obtained in July 2014. Lowest COD concentration was recorded in May 2014 with a value of 195 mg/L. No explanation can be provided for the sudden increase of COD values during winter 2016. The average of the COD concentration gave a value of 665 mg/L which suggests that the wastewater was in the normal range of COD concentration obtained for sewer networks in United Kingdom, Europe and Australia (Hvitved-Jacobsen *et al*. 2013).

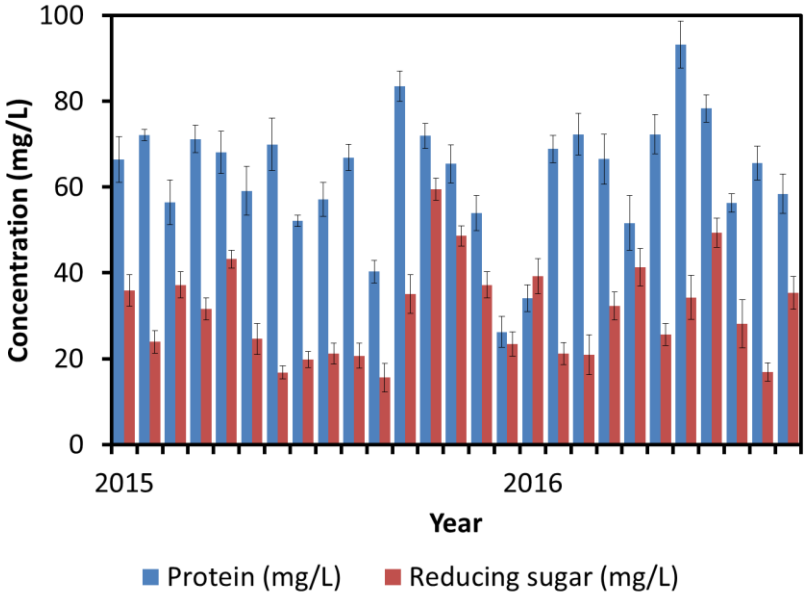

Figure 3.5. Protein and reducing sugar concentration obtained during wastewater sampling (error bars representing standard deviation from triplicate of sample measurements).

Several studies from the literature have reported various COD values of wastewater. Raunkjaer *et al.* (1994) found COD values of 28 mg/L from wastewater collected at the inlet of Aalborg East wastewater treatment plant. No known catchment area and PE were provided in the study. Sophonsiri and Morgenroth (2004) reported a higher value of 309 mg/L, where the wastewater was collected from the primary effluent of municipal wastewater. Gopala Krishna *et al*. (2008) reported a COD value of 1000 mg/L for municipal wastewater. From the literature, the wastewater collected was

found to have similar COD values, and thus relevant comparison was deemed possible.

pH obtained for the wastewater samples were ranging between 7.0 and 7.5 and were deemed in the normal range of pH as reported from the literature. Sharma *et al.* (2014) reported pH values of 7.2 to 8.5 for wet well for rising sewer mains. Nielsen *et al*. (1998) reported pH values of 7.0 to 8.5 for domestic sewage collected from pressure mains. Pai *et al*. (2010) reported pH values of 6.2 to 7.4 for gravity sewers in Taiwan and 7.7 to 9.8 was reported for sewer networks in Nancy, France (Houhou *et al.* 2009).

Wastewater temperature obtained from the results agreed with a study by Dürrenmatt and Wanner (2014) which reported that wastewater temperature originated from household varies between 10 to 20^{o}C all year around. High wastewater temperature has been reported to occur in some part of Europe, as wastewater temperature of 27^{o}C was observed in the Netherlands (Hoes *et al*. 2009) and 22^{o}C was reported in Italy (Cipolla and Maglionico, 2014). Cipolla and Maglionico (2014) also reported that wastewater temperature varies with seasons, as 18 to 22^{o}C was observed in summer periods while 10 to 14^{o}C was obtained in winter periods. Raunkjaer *et al.* (1995) reported 14.4^{o}C and 15^{o}C during September 1991 for the city of Dronninglund, Denmark.

Figure 3.5 shows result obtained for protein and reducing sugar concentration for wastewater collected during this study. From the graph, protein shows higher values of concentration as compared to reducing sugar. This finding was expected, as reducing sugar was only a fraction of total carbohydrates present in the wastewater. The graph shows that protein concentration varies from 30 to 90 mg/L while reducing sugar was lower, at approximately 20 to 60 mg/L. A lot of studies have conducted measurement of organic matter in wastewater. However, this information is hard to reach as the values vary greatly between each wastewater sample.

From the literature, wastewater composition usually presented as COD fraction in percentages. Protein COD fraction obtained in this study was 5 to 12% while reducing sugar COD fraction was from 2 to 9%. Some values reported from the literature can be summarized in Table 3.2.

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Table 3.2. Organic matter composition in wastewater obtained from various studies.

References	Total COD (mg/L)	COD fraction (%)			
		Protein	Carbohydrate	Lipid	Unknown
Haukelekian and Balmat (1959)	203	31	16	45	8
Narkis *et al*. (1980)	813	30	n.d[1]	10	60
Henze *et al*. (1982)	530	8	12	10	70
Tanaka *et al*. (1991)	259	12	6	19	63
Raunkjaer *et al.* (1994)	n.d[1]	28	18	31	22
Dignac *et al*. (2000)	967	18	16	7	59
Sophonsiri and Morgenroth (2004)	309	12	6	82	0

[1] Not determined

The literature suggests that there is no wastewater that is the same, as it can be influenced by unlimited factors. For this work, wastewater was deemed suitable for used in the tests, as COD, protein and reducing sugar concentration of the wastewater were within the reported values from the literature.

3.4 Determination of protein and reducing sugar for substitute sewer sediment materials

In order to establish the influence of protein and reducing sugar from the substitute sewer sediments, background tests were conducted on sand, crushed olivestone, tap water and distilled water. These samples were

subjected to protein and reducing sugar analysis where the relationship between different concentration of materials to the measured protein and reducing sugar concentrations was obtained.

Serial dilutions of three different materials (crushed olivestone, clean sand and combination of 20% crushed olivestone and 80% clean sand by dry mass) were prepared by diluting the sample with tap water or distilled water to the volume of 0.05 L. The sample mass and final concentrations can be summarized in Table 3.3.

Table 3.3. Serial dilution of surrogate sediment samples.

Mass of sample (mg)	Final concentration (mg/L)
0.0	0.0
2.0	40.0
4.0	80.0
6.0	120.0
8.0	160.0
10.0	200.0

All materials were subjected to protein and reducing sugar analysis as previously discussed in Section 3.2.4 and 3.2.5. Each sample was analysed in triplicate, in order to quantify uncertainties in the results obtained. A plot of sample concentration (mg/L) against protein and reducing sugar concentration (mg/L) was constructed, and the slope of the graph was obtained. The slope represents the mass of protein or reducing sugar obtained from the sample (mg/ mg of sample).

For tap water, no protein concentration was observed, and only 0.0005 ± 0.0003 mg of reducing sugar concentration was obtained in 0.0005 L of sample. This result suggested that tap water does not contain any measurable protein but does have a very small amount of reducing sugar in it. This value, however, was not considered to be significant for this study, as

it was too small to cause any effects to organic matter analysis of samples. For distilled water, very small negative values of protein concentration were observed, and no reducing sugar concentration was obtained in the sample. The slope values obtained from calibration curve for materials diluted with tap water and distilled water has been summarized in Table 3.4.

From Table 3.4, it can be observed that the protein concentrations were higher for all material in comparison to reducing sugar concentrations. Highest contributor of protein concentration was crushed olivestone, as 0.1231 mg of protein/mg of sample was obtained when olivestone was diluted with tap water. This value was higher than protein concentration for crushed olivestone with distilled water.

Table 3.4. Results of protein and reducing sugar concentrations for a different type of materials used in this study.

	Tap water		Distilled water	
	Protein (mg/mg)	Reducing sugar (mg/mg)	Protein (mg/mg)	Reducing sugar (mg/mg)
Olivestone	0.1231	0.0017	0.0657	0.0082
Clean sand	0.0049	0.0029	0.0021	0.0020
20% olivestone and 80 % clean sand	0.0125	0.0010	0.0124	0.0040

This result suggested that tap water may contain some form of organic matter. Other than that, olivestone was able to produce high protein and reducing sugar concentration because it is made of organic material, thus, suitable to be used as easily degradable organic matter in the system for microbial growth.

Clean sand produced the lowest protein and reducing sugar concentration, which was due to its inorganic properties as sand was made mostly of quartz and silicate (Camuffo, 2001). The mixture of 20% olivestone and 80% sand

produced a higher protein concentration as compared to clean sand. This is due to the presence of olivestone in the mixture, which contributes to both protein and reducing sugar concentration.

3.5 Conclusions

This chapter provides information on the material used and a number of analysis methods that were implemented in this study. Several conclusions can be made from the results obtained;

- Wastewater collected was tested and found to be representative of combined sewers.
- Wastewater COD values were in the normal ranges of as obtained from the literature. The average COD values obtained was 655 mg/L.
- Higher values of protein concentration were obtained as compared to reducing sugar concentration for wastewater sample. Protein concentration ranging from 30 to 90 mg/L while reducing sugar varies from 20 to 60 mg/L. To put into perspective, protein COD fraction was 5 to 12% while reducing sugar COD fraction was 2 to 9%.
- Tap water contains no protein concentration while reducing sugar concentration obtained was 0.0005 ± 0.0003 mg for 0.0005 mL of sample.
- Distilled water contained no organic matter concentration.
- The highest protein and reducing sugar concentration obtained was from a solution of olivestone diluted with tap water. Protein concentration obtained was 0.1231 mg/mg and reducing sugar concentration was 0.0117 mg/mg.
- The mixture of 20% of crushed olivestone and 80% of sand by mass diluted with tap water show protein concentration of 0.0125 mg/g while reducing sugar obtained for the sample was 0.0010 mg/mg.

From the result, it can be concluded that the wastewater was deemed suitable to be used in this study. Other than that, background protein and reducing sugar values of each material used for surrogate sediment bed were deemed significant, thus, needs to be taken into consideration when dealing with data interpretation of organic matter analysis of the sample.

Chapter 4 Effects of biofilm growth on pipe hydraulic roughness

Pipe hydraulic roughness is important in determining mean in-pipe flow velocity and water depth in sewer networks. Values of hydraulic roughness can be obtained from standard values that were published for different pipe materials or from existing calibrated sewer network hydrodynamic models. These values however excluded biofilm formation in the values estimated due to the complexity of incorporating chemical and biological processes in hydraulic network models, for example, Infoworks/Hydroworks modelling do not include any biological processes in the model description (Field *et al*. 2004). To understand the effects of biofilm growth on pipe flow, a number of tests have been carried out using a small-scale pipe reactor where biofilm was grown at a set period of times under various conditions. All tests conducted under steady, uniform flow conditions. Changes in pipe flow were determined by measurement of pipe hydraulic roughness and flow velocities during the tests. This chapter will provide a better understanding of different biofilm growth characteristics obtained at each condition and its influence on the pipe flow.

4.1 Experimental setup for pipe experiments

All tests were carried out under laboratory conditions. The tests were conducted using a pipe rig as shown in Figure 4.1. The system consists of 1 m artificially roughened clear Perspex pipe with an internal diameter of 50 mm and thickness of 5 mm and two tanks with a height of 205 mm and an internal diameter of 180 mm. A butterfly valve was fitted at the downstream end of the pipe.

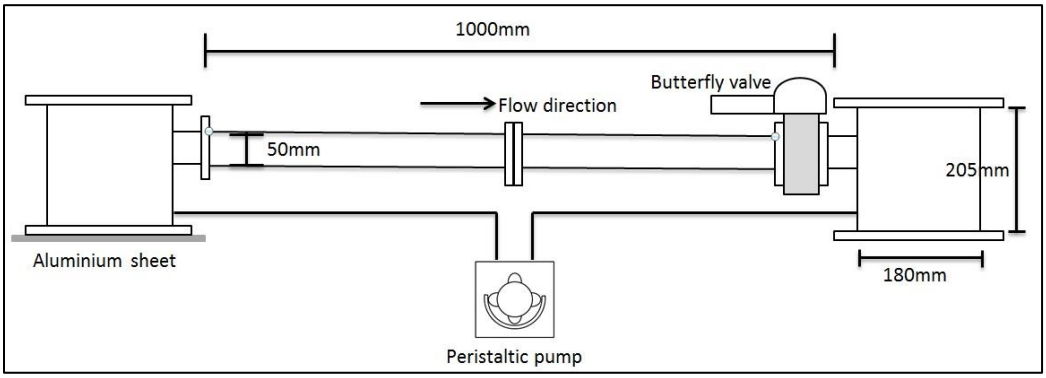


Figure 4.1. Schematic diagram of pipe setup for 1.0 m pipe configuration (Flow direction to the right).

6 L of tap water was circulated in the system using a pre-calibrated peristaltic pump (Cole Palmer, Masterflex 07258-10, USA,) that was connected by two different tubings; norprene tubing with 7.9 mm internal diameter (Cole Palmer, USA) and silicone tubing with 8.0 mm internal diameter (Cole Palmer, USA). Water level along the pipe was controlled using a butterfly valve (Durapipe, 425960, UK) which was installed at the downstream end of the pipe. Pipe inclination was introduced in the system using an aluminium sheet with a height of 3mm placed underneath of the bottom of the upstream tank.

Pre-calibrated oxygen sensors (Presens, TX3, Germany) and a temperature sensor were installed at the upstream tank. Both sensors were set to log data every 1 minute during the test.

The setup was secured to the bench using tape to avoid any movement during the test. The whole set up was placed on a bench in a 20.0 ± 1.0 ^{o}C temperature-controlled laboratory.

4.2 Pipe tests experimental procedures

4.2.1 Pipe characterisation experiments

Before biofilm was grown in the pipe, the pipe was characterised by determination of flow profile at the intended hydraulic conditions, which was

controlled to be as close as possible to sewer conditions. This step was to demonstrate that uniform flow could be obtained and to obtain measurements of hydraulic roughness for pipes with different levels of biofilm growth.

The pipe was equally divided into twelve equal sections, each 80 mm in length. Pipe outside wetted perimeter,P_{wo}, was measured using a measuring tape (± 1 mm) and then used to calculate flow depth, d_w. Before the aluminium sheet was added into the system, bench slope was measured by plotting flow depths of stationary water against pipe length at fully opened valve conditions to determine the slope of the bench. This is important in order to ensure that the bench was horizontally flat. The aluminium sheet was then added under the upstream tank to control the bed slope, S_o, of the system. Bed slope was determined by obtaining slope value from plot of flow depth of stationary water against pipe length at a fully open valve.

Water slope, S_f was calculated by adding slope from graph of flow depths of moving water against pipe length at various pump speed and valve opening positions to known values of S_o. S_f should be within 15% higher or lower values to bed slope in order to ensure that the flow can be considered uniform. This was an assumption made to satisfy the requirement below;

$$\sin\theta_i = \tan\theta_i = S_o and \cos\theta_i = 1$$

The equation shows the relationship between sin, cos and tan functions with θ_i in order to achieve uniform flow (Chadwick, 2004), where, θ_i is the inside angle of flow. The ideal water slope was observed to be within 10% of bed slope, however, after taking into consideration of pipe length and uncertainties during P_{wo}measurements, 15% is deemed acceptable to obtain similar bed and water slope values.

Discharge flowrate, Q_f was obtained by measuring the volume of water collected at the downstream tank during a 5 s period. The measurements were repeated ten times to reduce uncertainties on average flow rate. The

height of water in both tanks was measured using a ruler (± 1 mm) during each measurement.

4.2.2 Biofilm growth experiments

Once uniform flow was obtained, the pipe was run for 168 hours to allow for biofilm growth, although, some tests were ended early due to biofilm detachment. P_{wo}, pH, Q_f, and water height in tanks were measured regularly during the test. Oxygen level saturation and system temperature were monitored constantly from logged data. 10 mL of sample was collected daily from the downstream tank during the test and was used for organic matter analysis. Each sample collection was replaced with the same amount of fresh wastewater.

Total COD was determined for estimation of substrate concentration and consumption during bacterial growth. Temperature and pH were measured to ensure that it is within a desirable range for biofilm growth, which is between 5.5 to 8.5 (Hostacká *et al*. 2010). pH was measured using pH paper (Fisher Scientific, 1033501) and was conducted on a daily basis to determine whether a buffer is needed in the system.

Aeration was provided at the downstream tank using an aeration stone connected to an aquarium pump, and the dissolved oxygen level was maintained between 60 to 80% oxygen saturation at all times for experiments with aeration. All experiments were conducted as soon as possible after wastewater was obtained from a nearby wastewater treatment plant. The oxygen sensor and temperature sensor were started once uniform flow was obtained, which was marked as time zero, T = 0 hours for the tests. Once the tests ended, the biofilm was scrapped off the pipe using sponges and was analysed for total solid, following Section 3.2.6, for total suspended solids analysis procedure.

The reactor was left untouched with the exception of sampling and P_{wo} measurements in order to maintain the uniform flow and to avoid disrupting

the biofilm growth. The pipe was cleaned using soft brush and sponges, and multiple rinsing using boiling water were also implemented.

4.3 Pipe hydraulics preliminary experiments

These tests were conducted to determine the valve opening setting in order to achieve uniform flow depth, which occurred when the water slope is equal or very similar to bed slope. This flow condition is essential to ensure an accurate calculation of pipe hydraulic roughness, k_s.

All tests were conducted using dyed tap water. In addition to established flow hydraulics, the tests were also aimed to estimate biofilm growth period for tests with wastewater. Three sets of test were conducted;

1. 1.0 m pipe length with 3 mm bed elevations
2. 1.5 m pipe length with 3 mm bed elevations
3. 1.0 m pipe length with 6 mm bed elevations

Each set was done in triplicate to determine uncertainties in values obtained. For each test, the pump was run from 150 RPM to 600 RPM at 50 RPM increment for each different valve positions. This test was done to obtain information on the flow profile at different pump speed and valve positions and also to determine at which conditions will uniform flow likely to occur.

50 and 100 RPM was not included as the flow was too slow, causing full flowing flow at small valve openings. The flow was allowed to stabilise after each change by leaving it running for 30 minutes after each change. Water slope for each condition was calculated, and values that were within 15% of bed slope values were accepted as uniform flow and recorded for used in further tests.

4.4 Experimental conditions for pipe tests

Once the hydraulic performance of the system using tap water was established, further tests were conducted using wastewater. Four variables were considered in this study, namely pipe length, bed slope, dissolved oxygen concentration in the system and wastewater COD initial concentration.

Two different pipe lengths were used; 1.0 m and 1.5 m. The longer pipe was speculated to produce more biofilm in the pipe, as larger area over volume ratio (A/V) was obtained. 1.0 m pipe produced an A/V ratio with the value of 26.83 m^{-1} while 39.92 m^{-1} was calculated for 1.5 m pipe length. McKall *et al*. (2016) reported an A/V value of 33 m^{-1} for medium-sized gravity sewers, which is in agreement with the proposed A/V values in this study. O'Brien *et al.* (2017) reported a value of 70.9 m^{-1}, which was found to be higher than average for large diameter pipes.

Different bed slope values were used in this test in order to obtain a different level of shear stress. Higher bed slope generally produced higher shear stress which has been found to produce biofilm with different characteristics as compared to low shear stress conditions (Xu *et al*. 2017). The shear stress values obtained for all the tests were typically found in the sewer, as the shear stress of 2 to 4 N/m^2 have been reported by Nielsen *et al*. (1992) for gravity sewers.

The dissolved oxygen concentration in the tests was manipulated by running the tests with and without aeration. Tests without aeration were speculated to cause a level of stress to the biofilm which may further influence the biofilm growth. The lowest dissolved oxygen concentration of 2.80 mg/L was obtained for tests without aeration, which was deemed sufficient to sustain aerobic conditions for biological activity in the system, as oxygen concentration of 1 to 4 mg/L has been reported for gravity sewers (Nielsen *et al*. 1992).

All tests were conducted using fresh wastewater collected on the day without any additional organic matter except for 2 tests (Test 12 and Test 16). These were done in order to grow the biofilm at a similar condition as the sewer, where variation in the organic matter concentration of wastewater has been discussed in Chapter 3. Table 4.1 summarized all tests that have been conducted in this study.

Table 4.1. Summary of tests conducted.

Test Number	Pipe length (m)	Aeration	Bed slope (m/m)	Dischar-ge Flowrate (L/s)	Flow depth at T=0 hours (m)	Flow depth at T=168 hours (m)	Mean boundary shear stress (N/m^2)
1	1.0	No	0.0032	0.0515	0.0186	0.0193	0.2842
2	1.0	No	0.0042	0.0510	0.0170	0.0167	0.2926
3	1.0	No	0.0040	0.0524	0.0166	0.0169	0. ^{o}C 72
4	1.0	No	0.0038	0.0533	0.0172	0.0174	0.2719
5	1.0	No	0.0034	0.0535	0.0169	0.0172	0.2434
6	1.0	No	0.0033	0.0579	0.0202	0.0210	0.3531
7	1.0	No	0.0034	0.0652	0.0184	0.0188	0.3409
8	1.0	No	0.0036	0.0673	0.0185	0.0187	0.3490
9	1.0	Yes	0.0034	0.0614	0.0192	0.0187	0.3384
10	1.0	Yes	0.0037	0.0675	0.0167	0.0179	0.4670
11	1.0	Yes	0.0041	0.0633	0.0182	0.0186	0.4020
12	**1.0**	**Yes**	**0.0041**	**0.0652**	**0.0181**	**0.0179**	**0.3979**
13	1.5	No	0.0040	0.0694	0.0201	0.0195	0.4200
14	1.5	No	0.0041	0.0707	0.0184	0.0202	0.4240
15	1.5	Yes	0.0034	0.0687	0.0195	0.0204	0.3570
16	**1.5**	**Yes**	**0.0039**	**0.0690**	**0.0189**	**0.0204**	**0.4236**
17	1.0	No	0.0067	0.0925	0.0172	0.0176	0.5437
18	1.0	No	0.0072	0.1003	0.0163	0.0157	0.6416

These tests were grouped into five categories;

1. Test 1 to Test 5 – 1.0 m pipe length, no aeration, low shear stress.

2. Test 6 to Test 8 – 1.0 m pipe length, no aeration, high shear stress.
3. Test 9 to Test 12 – 1.0 m pipe length, aeration, high shear stress (Test 12 was conducted at constant 800 mg/L COD concentration, was shown as bold in the table).
4. Test 13 to Test 16 – 1.5 m pipe length, aeration and non-aeration, high shear stress (Test 16 was conducted at constant 800 mg/L COD concentration, was shown as bold in the table).
5. Test 17 to Test 18 – 1.0 m pipe length, no aeration, 6 mm bed elevations.

Biofilm was grown for 168 hours (7 days) for all tests. This was due to previous knowledge obtained from feasibility studies conducted that shows biofilm grown using wastewater was visible after 18 to 24 hours period and 168 hours was assumed to be sufficient to obtain mature biofilm in the pipe.

4.5 Analysis

4.5.1 Calculation of flow hydraulic characteristics

Only two parameters can be obtained physically in this test; pipe outside wetted perimeter, P_{wo} and discharge flowrate, Q_f. These parameters were used for determination of other flow hydraulic parameters using a series of equation as shown below, with reference to Figure 4.2;

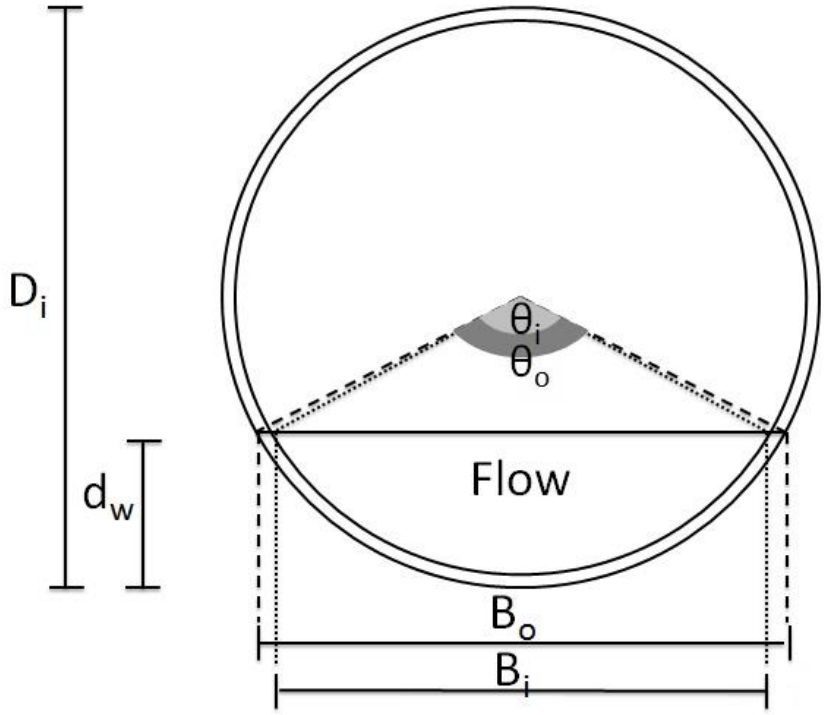


Figure 4.2. Parameters of the pipe, as viewed from the front.

Outside angle of the flow, θ_o was determined by Equation 4.1.

$$\theta_o = \frac{P_{wo}}{r_o} \quad \text{(Equation 4.1)}$$

Where; r_o is pipe outside radius (m).

Outside surface width of flow, B_o was calculated by Equation 4.2.

$$B_o = 2r_o sin\frac{\theta_o}{2} \quad \text{(Equation 4.2)}$$

Flow surface width, B_i was then obtained using Equation 4.3.

$$B_i = B_o - 2t \quad \text{(Equation 4.3)}$$

Where; t is pipe thickness (m).

Next, inside angle of the flow, θ_i was obtained by Equation 4.4.

$$\theta_i = 2sin^{-1}\left(\frac{0.5B_i}{r_i}\right) \quad \text{(Equation 4.4)}$$

Where; r_i is pipe inner radius (m).

Flow depth, d_w was then obtained by Equation 4.5.

$$d_w = r_i - \left(\frac{0.5B_i}{tan\left(\frac{\theta_i}{2}\right)}\right) \quad \text{(Equation 4.5)}$$

Flow wetted area, A_w was calculated by Equation 4.6.

$$A_w = \left(\frac{\theta_i - \sin\theta_i}{8}\right) D_i^{\,2} \quad \text{(Equation 4.6)}$$

Where $D_i$is pipe inner diameter (m).

Next, flow hydraulic radius, R_h was then determined by Equation 4.7;

$$R_h = 1 - \left(\frac{\sin\theta_i}{\theta_i}\right)\left(\frac{D_i}{4}\right) \quad \text{(Equation 4.7)}$$

Flow velocity,u was obtained using Equation 4.8 and Reynolds Number, Re was calculated using Equation 4.9. The Reynolds number will determine the condition of the flow, as Re less than 500 is considered as laminar flow while Re more than 1000 is considered as turbulent flow for open channel flow conditions (Chadwick, 2004).

$$u = \frac{Q_f}{A_w} \quad \text{(Equation 4.8)}$$

$$Re = \frac{\rho_f u R_h}{\mu} \quad \text{(Equation 4.9)}$$

Where Q_f is discharged flowrate (m^3/s), ρ_f is fluid density (kg/m^3) and μ is water dynamic viscosity (kg/ms).

Pipe hydraulic roughness, k_s was calculated using Equation 4.10, modified from the Colebrook-White equation for free surface flow in pipes (following Colebrook, 1939).

$$k_s = 14.8R_h\left(10^{-\frac{1}{2\sqrt{f}}} - \frac{2.51}{Re\sqrt{f}}\right) \quad \text{(Equation 4.10)}$$

Where; f is the Darcy-Weisbach friction factor, obtained from Equation 4.11;

$$f = \frac{8gR_hS_f}{u^2} \quad \text{(Equation 4.11)}$$

Where; g is acceleration due to gravity (m/s^2) and S_f is water slope (m/m).

Gravity forces and frictional resistance forces are assumed to be equal in uniform flows and can be presented using Equation 4.12 as shown below (Chadwick, 2004);

$$\tau_o PL = \rho g A_w L \sin\theta \quad \text{(Equation 4.12)}$$

Where τ_o is mean boundary shear stress (N/m^2).

Small bed slope, S_o is assumed in order to satisfy Equation 4.13;

$$\sin\theta \approx \tan\theta = S_o \quad \text{(Equation 4.13)}$$

Equation 4.13 was then substituted into Equation 4.12 to yield Equation 4.14;

$$\tau_o = \rho g A_w S_o / P_{wi} \quad \text{(Equation 4.14)}$$

Where P_{wi} is pipe inside wetted perimeter (m).

As R_h is equal to A_w/P_{wi}, Equation 4.14 can be re-arranged as;

$$\tau_o = \rho g R_h S_o \quad \text{(Equation 4.15)}$$

In uniform flows, S_o is equal to S_f, thus final equation used to determine mean boundary shear stress was shown below;

$$\tau_o = \rho g R_h S_f \quad \text{(Equation 4.16)}$$

4.5.2 Calculation of energy losses in the system

Energy losses are defined as loss of energy due to resistance when fluid is flowing through a pipe. The losses are categorized into two groups; major losses which caused by resistance while minor losses caused by the changes in geometry or addition of components to the pipe setup. Minor losses include sudden expansion and contraction of pipe, pipe fittings, bend and any obstruction in the pipe (Bansal, 2008). For a long pipeline, minor losses can be neglected. However, the values can be more significant for shorter pipes where the value may also higher than the value of major losses (Chadwick, 2004). Energy loss due to friction (major losses), h_f was calculated following Equation 4.17 (Bansal, 2008);

$$h_f = f\frac{L_p}{D_h}\frac{u^2}{2g} \quad \text{(Equation 4.17)}$$

Where L_p is length of pipe (m) and D_h is hydraulic diameter (m).

Energy losses due to fittings and joints in the pipe rig were calculated using various equations as below (Bansal, 2008);

For energy loss at the sharp edge pipe entrance, h_i ;

$$h_i = 0.5\ \frac{u_i^2}{2g} \quad \text{(Equation 4.18)}$$

Where u_i is the flow velocity at the pipe entrance (m/s).

Some examples of K_L values for most common pipe fittings can be obtained from Table 4.2.

Table 4.2. Minor loss coefficient values for pipe fittings.

Pipe fittings	K_L
Gate valve (fully open)[1]	0.19
90^o elbow[1]	0.9
45^o elbow[1]	0.4
Butterfly valve (30^o opening)[2]	3.9
Butterfly valve (40^o opening)[2]	10.8
Butterfly valve (50^o opening)[2]	32.6
Butterfly valve (60^o opening)[2]	118.0
Butterfly valve (70^o opening)[2]	256.0
Butterfly valve (80^o opening)[2]	751.0

[1] Bansal (2008)

[2] Chapallaz *et a*l. (1992)

Energy loss at the pipe exit, h_o was calculated by Equation 4.19;

$$h_o = K_d\frac{u_o^2}{2g} \quad \text{(Equation 4.19)}$$

Where u_o is the flow velocity at the pipe exit (m/s), K_d is discharge loss coefficient (-).

Energy loss due to pipe fittings, h_L was obtained following Equation 4.20;

$$h_L = K_L\frac{u^2}{2g} \quad \text{(Equation 4.20)}$$

Where K_L is the minor loss coefficient (-).

Minor energy losses for pipe entrance, pipe exit and pipe fittings were calculated for all tests conducted. Major energy loss due to friction was also obtained and percentages changes between the values at T = 0 hours and 168 hours were calculated in order to obtain the percentage changes of energy loss in the pipe due to biofilm growth.

4.5.3 Quantification of average biofilm dry mass

Biofilm dry mass was determined to estimate average biofilm growth rate during each test. This dry mass represents the whole pipe, where the biofilm was assumed to grow uniformly along the pipe. Biofilm dry mass over the wetted area was calculated to give an estimation of biofilm growth in the pipe, thus, was used to correlate changes in pipe hydraulic roughness with biofilm growth.

In this test, biofilm sample was collected from the inner pipe surface using sponge right after the tests ended. Sponges were dried beforehand at 105^{o}C overnight and weighted before the collection. Biofilm and sponges combination were placed in a tray, with dimensions 200 mm x 160 mm as shown in Figure 4.3.

Figure 4.3. Image of biofilm collected using sponges.

The sample was dried overnight in an oven at 105^{o}C. Once the drying finished, the sample was stored in a desiccator and cooled down to room temperature before measurement.

4.5.4 Determination of COD

Wastewater samples collected during the test were subjected to COD analyses following Section 3.2.3.

4.6 Results of pipe hydraulic preliminary experiments

Figure 4.4 (a) and (b) illustrate results obtained for background experiments conducted at 3 mm bed elevation for both 1.0 m and 1.5 m long bed configurations while Figure 4.4 (c) show results obtained from tests conducted on 1.0 m long pipe configuration at 6 mm bed elevation.

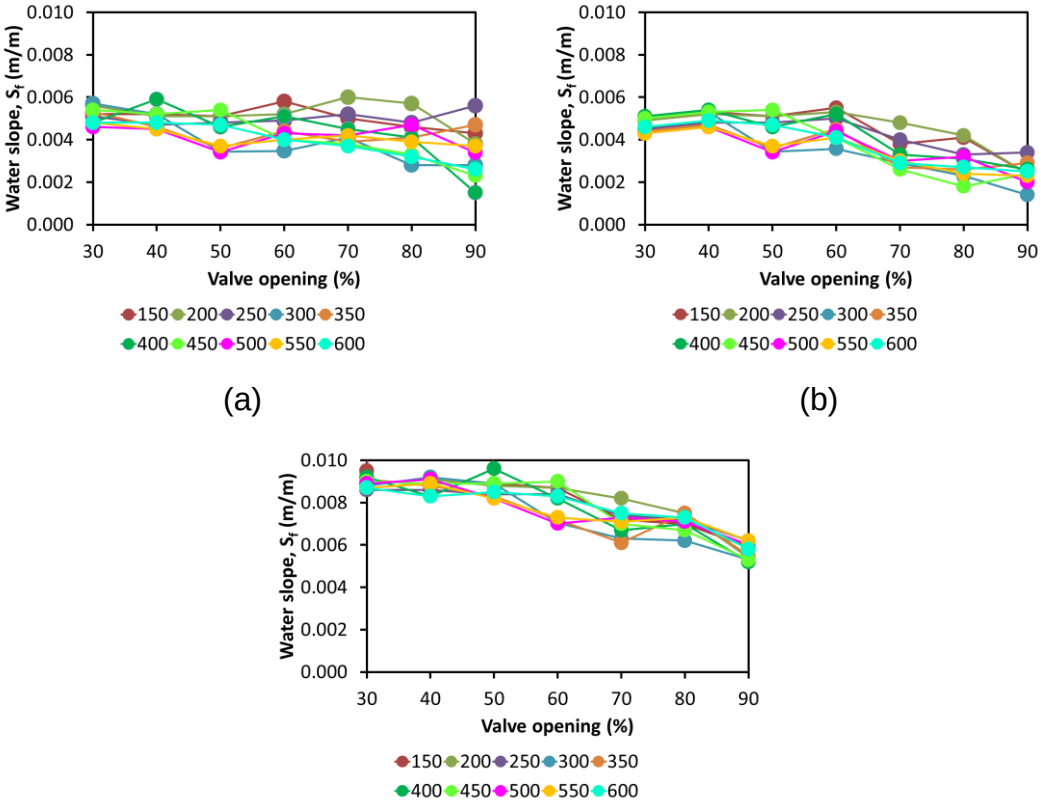


Figure 4.4. Results of background experiments at 3 mm bed elevation for (a) 1.0 m (S_o= 0.0035 ± 0.0004 m/m), (b) 1.5 m (S_o= 0.0032 ± 0.0003 m/m) and

(c) 6 mm bed configuration at 1.0 m pipe length (S_o= 0.0071 ± 0.0004 m/m). The legend represents pump speed, in RPM.

In general, Figure 4.4 shows decreasing S_f values with increasing valve opening position. This was due to decreasing flow depth at the downstream ends as the valve opening position was increased. At small valve opening position, flow depth at the upstream end was higher than downstream end, thus resulted in larger S_f values.

From the 1.0 m pipe long configuration at 3 mm bed elevation (Figure 4.4a), $S_o$value calculated was 0.0035 ± 0.0004 m/m. To obtained uniform flow, S_f values need to be from 0.0030 to 0.0040 m/m and these values were obtained at conditions as below;

- 50% valve position – 300 RPM, 350 RPM, 500 RPM, 550 RPM
- 60% valve position – 300 RPM, 450 RPM, 550 RPM and 600 RPM
- 70% valve position – 350 RPM, 450 RPM, 600 RPM
- 80% valve position – 450 RPM, 500 RPM
- 90% valve position – 500 RPM, 550 RPM

Discharge flowrate, Q_f obtained at each different RPM in this condition were shown by Table 4.3;

Table 4.3. Summary of discharged flowrate with standard deviation values obtained corresponding to different pump speed applied.

Pump speed (RPM)	300	350	400	450	500	550	600
Discharge Flowrate (L/s)	0.0326 ± 0.0007	0.0378 ± 0.0022	0.0449 ± 0.0014	0.0512 ± 0.0008	0.0556 ± 0.0025	0.0564 ± 0.0021	0.0603 ± 0.0041

For the 1.5 m pipe long configuration at 3 mm bed elevation (Figure 4.4b), S_o obtained was 0.0032 ± 0.0003 m/m. Accepted S_f values for uniform flow to

occur were between 0.0027 to 0.0037 m/m. This range of S_f values was achieved at below conditions;

- 50 % valve position – 300 RPM, 350 RPM, 500 RPM, 550 RPM
- 60 % valve position – 300 RPM, 350 RPM, 400 RPM
- 70 % valve position – 350 RPM, 400 RPM, 450 RPM, 500 RPM, 550 RPM
- 80 % valve position – 400 RPM, 500 RPM, 550 RPM.

Q_f obtained in this condition is shown in Table 4.4.

An independent t test was conducted to compare S_f values obtained at different valve opening position for pipe configuration of 1.0 m and 1.5 m at 3 mm bed elevation. For 40, 50 and 60% valve opening position, the results were not statistically significant ($p>0.05$).

Table 4.4. Discharge flowrate values obtained at this condition for different pump speed.

Pump speed (RPM)	300	350	400	450	500	550	600
Discharge Flowrate (L/s)	0.0366 ± 0.0008	0.0401 ± 0.0001	0.0475 ± 0.0009	0.0521 ± 0.0072	0.0533 ± 0.0022	0.0581 ± 0.0053	0.0600 ± 0.0083

Other than that, the majority of uniform flows were obtained at 50 and 60% valve opening position for both pipe lengths. The uniform flow was more achievable in 1.5 m pipe configuration, as a longer pipe length helps reducing flow depth differences at upstream and downstream ends.

At 6 mm bed elevation, S_o obtained was 0.0071 ± 0.0004 m/m. S_o values need to be within 0.0060 to 0.0081 m/m to obtain uniform flow in this condition. Uniform flow was achieved at these conditions;

- 60% valve position – 300 RPM, 350 RPM, 500 RPM, 550 RPM
- 70% valve position – 300 RPM, 350 RPM, 400 RPM
- 80% valve position –350 RPM, 400 RPM, 450 RPM

Discharge flowrate obtained at this setup was higher as compared to previous tests with lower S_o values. These values can be summarized by Table 4.5.

Table 4.5. Discharge flowrate values obtained at 1.0 m pipe length with 6 mm bed elevation.

Pump speed (RPM)	300	350	400	450	500	550	600
Discharge Flowrate (L/s)	0.0507 ± 0.0006	0.0568 ± 0.0008	0.0599 ± 0.0033	0.0675 ± 0.0012	0.0726 ± 0.0015	0.0766 ± 0.0003	0.0781 ± 0.0009

Another set of independent t test was conducted on 1.0 m pipe configuration at 3 mm and 6 mm bed elevations. The result shows that flow at all valve position was significantly different between these two conditions.

Once the uniform flow has been identified at each bed elevations, another background test was conducted using dyed tap water following different conditions, as previously discussed in Chapter 4.4. This test aims to determine $\overline{k_s}$ values at each respective condition to be used as a comparison with $\overline{k_s}$ values obtained from tests with wastewater. These five conditions were;

1. 1.0 m pipe length, no aeration, low shear stress
2. 1.0 m pipe length, no aeration, high shear stress
3. 1.0 m pipe length, aeration, high shear stress
4. 1.5 m pipe length, aeration and non-aeration, high shear stress
5. 1.0 m pipe length, no aeration, 6 mm bed elevations

S_o, Q_f and $\overline{k_s}$ values obtained at each condition using tap water can be summarized by Table 4.6.

All tests show no biofilm growth in the pipe after a period of one week. However, biofilm growth was observed in the pipe after 3 weeks periods. This may occur due to a limited nutrient in the system as no additional

organic matter was added. These findings imply that biofilm growth was possible under low nutrient concentration condition and that faster biofilm growth rate was expected for tests with wastewater due to higher nutrient content concentration.

S_o values for tests conducted at 1.0 m pipe long configuration obtained similar values, with exception of Condition 5. This value was higher due to high bed slope causing large differences in flow depth calculated at the upstream and downstream end of pipe. This also caused less uniform flow obtained at this condition. $\overline{k_s}$ values obtained were also similar at T = 0 and T = 168 hours, which suggests that $\overline{k_s}$ values remained unchanged due to no biofilm growth in the system. t test conducted for $\overline{k_s}$ values at T = 0 hours and 168 hours for all conditions presented in Table 4.6 shows the changes were not significant ($p>0.05$).

Table 4.6. Bed slope, discharge flowrate and average pipe hydraulic roughness values obtained for each condition for tests conducted using tap water at T = 0 hours and T = 168 hours.

Conditions	S_o (m/m)	Q_f (L/s)	$\overline{k_s}$ values at T = 0 hours (m)	$\overline{k_s}$ values at T = 168 hours (m)
1	0.0036 ± 0.0002	0.0501 ± 0.0003	0.0036 ± 0.0002	0.0034 ± 0.0003
2	0.0035 ± 0.0004	0.0568 ± 0.0004	0.0031 ± 0.0003	0.0032 ± 0.0002
3	0.0037 ± 0.0003	0.0602 ± 0.0008	0.0033 ± 0.0006	0.0034 ± 0.0004
4	0.0038 ± 0.0005	0.0630 ± 0.0006	0.0031 ± 0.0004	0.0033 ± 0.0001
5	0.0069 ± 0.0003	0.0805 ± 0.0008	0.0044 ± 0.0005	0.0043 ± 0.0002

From the results, several relationships can be observed. Discharged flowrate values were observed to increase with increasing bed slope at similar water

depths. A longer pipe configuration has shown to produce smaller bed slope values and thus creates more opportunities for the uniform flows to occur in the pipe. No biofilm growth was observed in tests conducted with tap water which suggested that more organic matter is needed in the system in order to start the process.

4.7 Pipe test experimental results

4.7.1 Hydraulic changes during biofilm growth

Condition 1 - 1.0 m pipe length, no aeration, low shear stress.

The average flow velocity and pipe hydraulic roughness for 1.0 m pipe configuration at non-aeration conditions can be presented in Figure 4.5 (a) and (b). τ_o and S_o values obtained for this condition is summarized by Table 4.7.

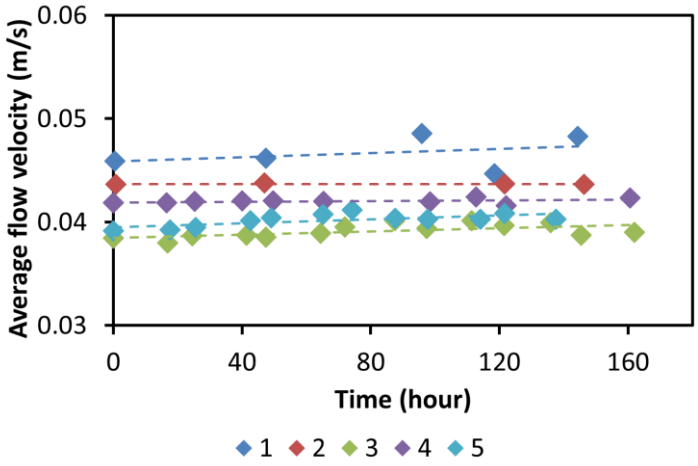
Table 4.7. τ_o and S_o values obtained for 1.0 m pipe long configuration with no aeration condition.

Test number	1	2	3	4	5
τ_o (N/m^2)	0.2842 ± 0.0021	0.2926 ± 0.0038	0.2772 ± 0.0104	0.2719 ± 0.0043	0.2434 ± 0.0043
S_o (m/m)	0.0032	0.0042	0.0040	0.0038	0.0034

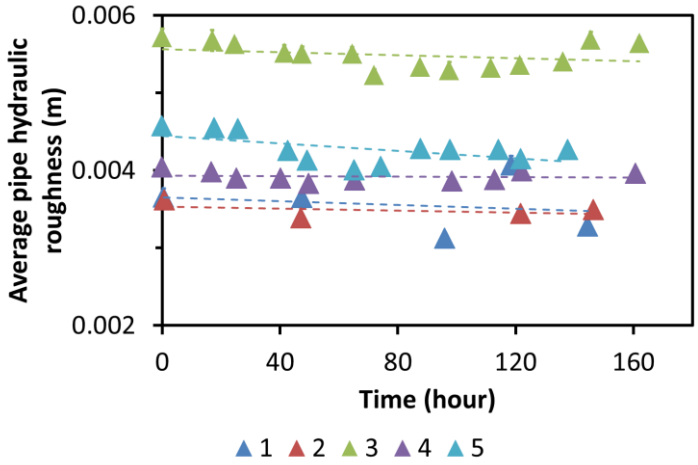
From Figure 4.5 (a), no significant changes in $\bar{u}$ can be observed for Test 2 to Test 5 as the values were fairly constant except for Test 1. Test 1 shows a significant increase in $\bar{u}$ values at T = 90 hours and a sharp decrease at 120 hours.

t test was conducted to compare values of $\bar{u}$ at T = 0 hours and T = 168 hours for all the tests indicates that Test 2 and Test 3 shows no significant difference in $\bar{u}$ values at the start and the end of experiments. Test 1, Test 4 and Test 5 show p values lower than 0.05, which indicated that $\bar{u}$ values experience changes during the tests. Changes in Test 1 can be observed

clearly from Figure 4.5, however, this was not applicable to Test 4 and Test 5. This may cause by very subtle changes in the $\bar{u}$ values which were not shown in the graph.



(a)



(b)

Figure 4.5. Results of (a) average flow velocity, $\bar{u}$ and (b) average pipe hydraulic roughness, $\overline{k_s}$ tests conducted at 1.0 m pipe long configuration with no aeration in the system.

For $\overline{k_s}$ values, Test 2 and Test 4 show almost constant values with time as shown by Figure 4.5 (b). Test 1 experienced a decrease in $\overline{k_s}$ values at approximately 90 hours mark, followed by an increase and ended with a decrease at the end of the test. Test 3 and Test 5 shows a similar trend, as

both tests shows a reduction in the first 80 hours and have a steady increase until the end.

All tests shared a similar trend, where $\overline{k_s}$ values were observed to decrease with biofilm growth. These changes, however, was subtle for several tests, namely Test 2 and Test 4. t test was conducted to compare $\overline{k_s}$ values at T = 0 hours and 168 hours and p values obtained were less than 0.05, which indicated that all tests were statistically significant.

The changes in $\bar{u}$ and k_s was related to each other, as $\overline{k_s}$ was increasing with decreasing $\bar{u}$ as demonstrated clearly by Test 1. These changes were believed to correspond to biofilm growth in the pipe, as biofilm growth may change flow depth thus changing $\bar{u}$ and $\overline{k_s}$ with time.

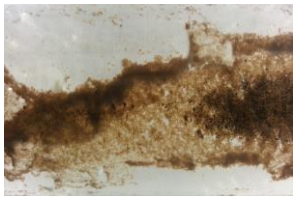
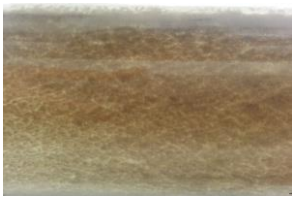
The stages of biofilm growth with time can be summarized in Table 4.8. The table shows images of biofilm that were taken from the area below the pipe at the different time period. All photos were taken at the same pipe section from the same distance, thus, comparison of biofilm growth at the selected pipe section was possible.

Table 4.8 shows uniform biofilm growth for Test 1 to Test 5 at T = 80 hours, which corresponds with decreasing $\overline{k_s}$ values for all tests at the same time period. Detachment was observed in all tests by 168 hours. $\overline{k_s}$ values of Test 1 was observed to be similar to Test 2 at end of the tests although more biofilm was observed in Test 1 as shown by Table 4.8. This may suggest the influence of detached biofilm area coverage was not significant due to calculation of averages of k_s values in the pipe. New biofilm was observed to fill in the detached area for Test 5, which further indicate unlimited nutrient availability in the system although no additional organic matter was added.

Only small amount of biofilm can be observed in for Test 2, which resulting in fairly constant $\bar{u}$ and $\overline{k_s}$ values throughout the tests. This observation was

speculated to be caused by low initial COD condition (approximately 200 mg/L).

Table 4.8. A comparison of biofilm growth for Test 1 to Test 5 at different time period; 0, 80 and 168 hours. Pictures were taken at the same pipe section for all tests (Flow direction to the right, size: 70mm x 50 mm).

Test nu.	T = 0 hours	T = 80 hours	T = 168 hours
1			
2			
3			
4			
5			

All tests produced different biofilm characteristics although they were grown under similar condition. Biofilm was observed to be thick, and uniform in Test 1, patchy and thin in Test 2, non-uniform in Test 3, fluffy and thick in Test 4 and thin and uniform biofilm was observed in Test 5. These findings partially agreed with the available literature, as biofilm grown at low shear stress were

expected to be thicker (Xu *et al*. 2017) and have uniform coverage and growth (Kraigsley *et al.* 1992).

Condition 2 - 1.0 m pipe length, no aeration, high shear stress.

Figure 4.6 shows results obtained for 1.0 m pipe configuration at higher discharge and no aeration condition. τ_o and S_o values obtained in this condition can be summarized by Table 4.9.

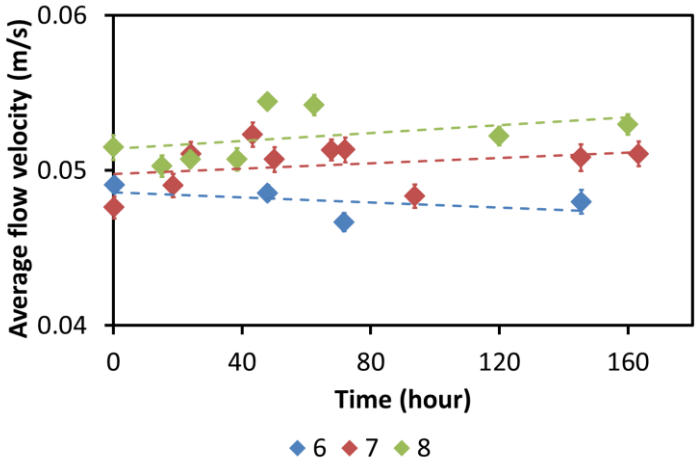
Table 4.9. τ_o and S_o values obtained for tests conducted at this condition.

Test number	6	7	8
τ_o (N/m^2)	0.3531 ± 0.0048	0.3409 ± 0.0074	0.3490 ± 0.0025
S_o (m/m)	0.0033	0.0034	0.0036

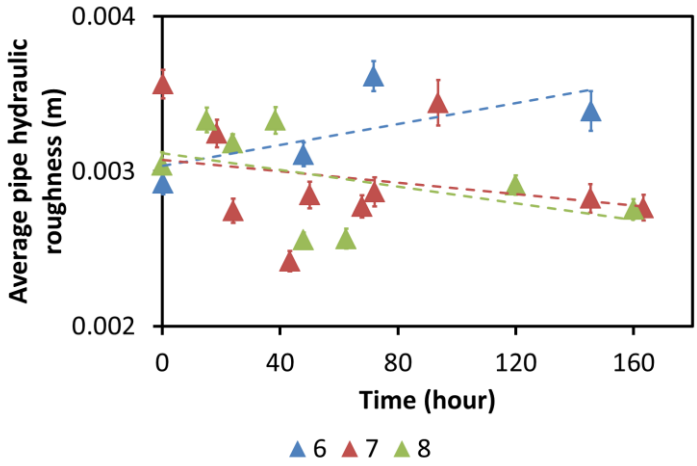
From Figure 4.6 (a), all tests show $\bar{u}$ and $\overline{k_s}$ values were changing with biofilm growth. Test 7 and Test 8 agreed with the findings for tests at Condition 1, as biofilm growth was found to decrease $\overline{k_s}$ values and thus increasing $\bar{u}$ values. Test 6 shows an opposite findings, as biofilm growth was found to increase pipe hydraulic roughness which agrees with study the by Guzmán *et al.* (2007).

Figure 4.6 (a) shows similar trend for $\bar{u}$ values as a slight decrease can be observed to occur at 90 hours. Test 7 and Test 8 show an increase in the first 50 hours into the test while Test 6 shows a decrease in $\bar{u}$ values during the same time period. Other than that, all tests show an increase in $\bar{u}$ values at the end of the tests.

t test conducted on $\bar{u}$ values shows that $\bar{u}$ values had changed significantly from T = 0 hours to T = 168 hours for all tests. This result was consistent with results presented in Figure 4.6.



(a)



(b)

Figure 4.6. Results of (a) average flow velocity, $\bar{u}$ and (b) average pipe hydraulic roughness, $\overline{k_s}$ for 1.0 m pipe configuration without aeration at high shear conditions in the system.

$\overline{k_s}$values were observed to change with time for all tests as demonstrated by Figure 4.6 (b). Both Test 7 and Test 8 show a decrease in $\overline{k_s}$ values at T = 50 hours and have similar $\overline{k_s}$values at T = 168 hours. Test 6 shows an increase of $\overline{k_s}$values until T = 80 hours, where the value seems to be unchanged.

Results from t test obtained for Test 6 was 0.00095, 0.00024 for Test 7 and 0.00019 for Test 8 which concluded that $\overline{k_s}$ values were significantly different at the start of the test and at the end.

These changes in $\overline{k_s}$ values can be further explained by comparing these changes with biofilm growth in the system as shown by Table 4.10. A patchy and thin biofilm was observed for all tests at T = 80 hours, which may have explained changes in $\overline{k_s}$ values of all tests at that time period.

Table 4.10. Biofilm growth at T = 0, 80, 168 hours for Test 6 to Test 8 (Flow direction to the right, size: 70mm x 50 mm).

Test nu.	T = 0 hours	T = 80 hours	T = 168 hours
6			
7			
8			

No biofilm detachment and less biofilm growth were observed for all tests as compared to tests conducted at previous conditions. These findings indicated the effects of shear stress on biofilm growth, as higher shear stress was found to produce thinner (Xu *et al.* 2017), smooth (Liu and Tay, 2001), compact biofilm that has a higher tolerance against detachment (Beyenal and Lewandowski, 2002).

Condition 3 - 1.0 m pipe length, aeration, high shear stress.

Figure 4.7 presents results obtained at 1.0 m long pipe configuration, high shear stress condition with aeration provided in the system. τ_o and S_o values obtained in this tests can be summarized by Table 4.11.

Table 4.11. Bed slopes, S_o and shear stresses, τ_o obtained for 1.0 m pipe configuration, high shear stress with aeration conditions.

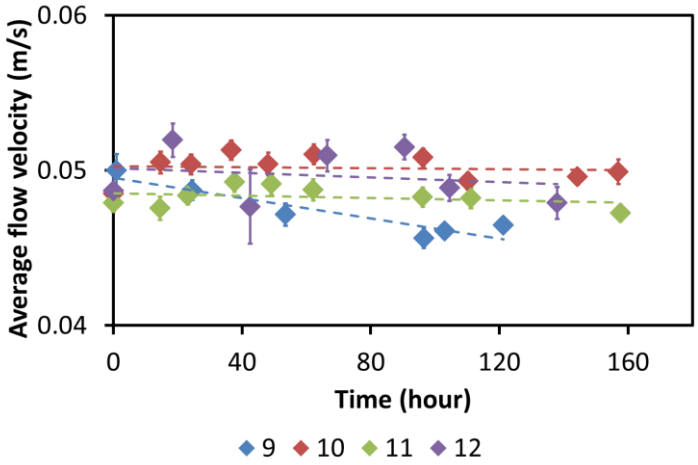
Test number	9	10	11	12
τ_o (N/m^2)	0.3884 ± 0.0072	0.4170 ± 0.0153	0.4020 ± 0.0072	0.3979 ± 0.0325
S_o (m/m)	0.0034	0.0037	0.0041	0.0041

Biofilm was physically visible for all tests during the first 24 hours. Figure 4.7 shows fairly consistent $\bar{u}$ values for Test 10 and Test 11. Test 9 and Test 12 shows a different finding, where $\bar{u}$ values for Test 9 were decreasing with time while Test 12 shows more fluctuations with time.

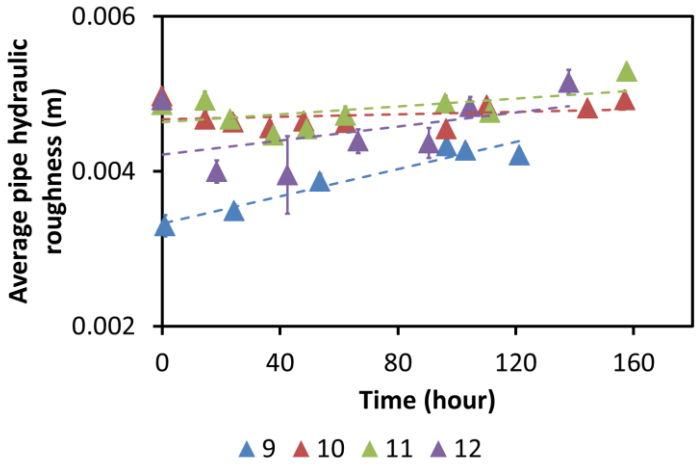
t test conducted on $\bar{u}$ values show p values of 0.048 for Test 9, 0.0521 for Test 10, 0.0552 for Test 11 and 0.032 for Test 12. The results obtained also agreed with Figure 4.7, as, $\bar{u}$ values for Test 10 and Test 11 were observed to be not significantly different during the start and end of test.

$\overline{k_s}$ values for tests at this condition shows subtle changes for Test 10 and Test 11, while Test 12 shows a lot of changes in $\overline{k_s}$ values with time. Test 9 shows a steady increase of $\overline{k_s}$ values with time, which agreed with finding from Test 6 of Condition 2 that demonstrated biofilm growth changes the flow by increasing pipe hydraulic roughness.

Test 10, Test 11, and Test 12 shared a similar pattern as all three tests achieved higher $\overline{k_s}$ values at T = 168 hours. Results for t test analysis gave p values less than 0.05 for all tests, which further proved that $\overline{k_s}$ values obtained at T = 0 hours and 168 hours were significantly different.



(a)

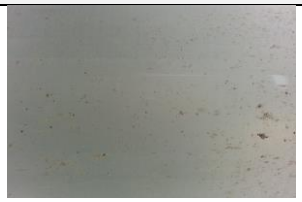
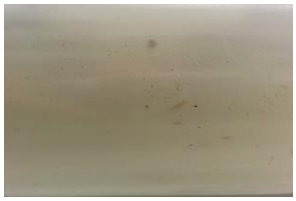
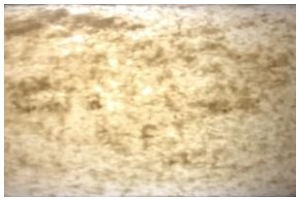


(b)

Figure 4.7. Results obtained for tests conducted at 1.0 m pipe long configurations, high shear stress with aeration provided in the system.

Images of biofilm growth on the pipe can be summarized by Table 4.12. From the table, Test 9 shows more biofilm at T = 168 hours as compared to T = 0 and 80 hours. Test 9 also seemed to have the lowest amount of biofilm as compared to other tests. Biofilm physical characteristics were speculated to be the cause of the increasing $\overline{k_s}$ values for Test 9, as biofilm observed was thin and uniform while all other tests show patchy, thick and fluffier biofilm which may have caused the reduction of $\overline{k_s}$ values for Test 10, Test 11 and Test 12.

Table 4.12. The progress of biofilm growth observed in the pipe at different time period for Test 9 to Test 12 (Flow direction to the right, size: 70 mm x 50 mm).

Test nu.	T = 0 hours	T = 80 hours	T = 168 hours
9			
10			
11			
12			

Test 10 and Test 12 shows larger biofilm detachment in the pipe as compared to Test 9 and Test 11. This can be due to biofilm physical characteristics, as fluffier and thicker biofilm were reported to be more at risk of detachment due to decreasing density of the biofilm with increasing biofilm thickness. These changes further increase biofilm porosity which resulting in a weaker biofilm (Xu *et al.* 2017). No correlations between biofilm growth and high nutrient concentration can be obtained from the results as Test 12 was observed to produce similar biofilm characteristics and quantity as other tests although Test 12 was conducted at high constant COD concentration (800 mg/L). These findings do not agree with a study by Rochex and Lebeault (2007) that reported more biofilm growth was observed at higher nutrient load concentration.

Condition 4 - 1.5 m pipe length, aeration and non-aeration, high shear stress.

Figure 4.8 demonstrates result obtained for 1.5 m long pipe configuration for both non-aerated and aerated condition. S_o and τ_o values obtained under this condition can be summarized by Table 4.13.

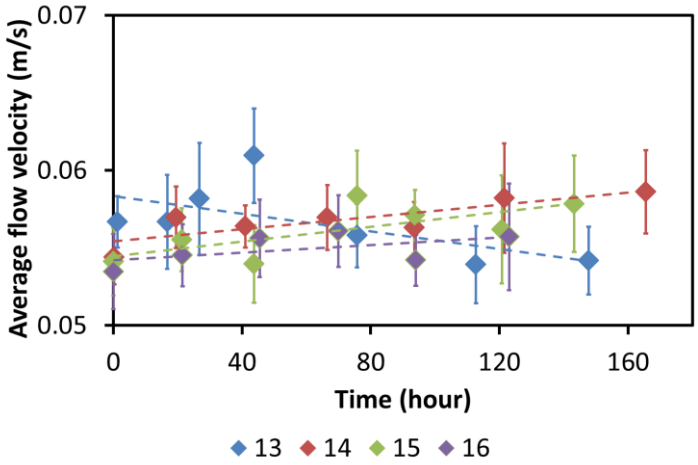
Table 4.13. Results of bed slope and shear stress values obtained for tests conducted at 1.5 m long pipe configuration.

Test number	13	14	15	16
τ_o (N/m^2)	0.4200 ± 0.0149	0.4240 ± 0.0179	0.3970 ± 0.0127	0.4236 ± 0.0109
S_o (m/m)	0.0040	0.0041	0.0034	0.0039

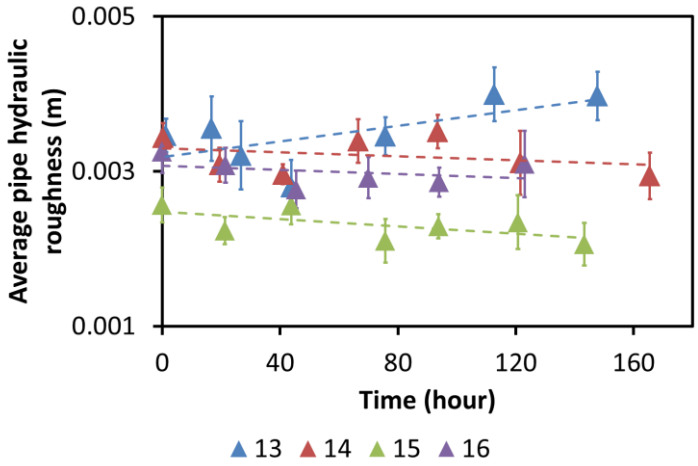
From Figure 4.8, all tests show more significant changes of $\overline{k_s}$ values with time as oppose to $\bar{u}$ values. Test 14 and Test 15 show a fairly similar trend as both have similar $\bar{u}$ values until 40 hours mark and an increase at the end of the test. Test 13 shows more prominent changes in the $\bar{u}$ values. No explanation can be provided for these results, as Test 13 and Test shares similar biofilm characteristics initial COD concentration values and also oxygen level concentration in the pipe.

No significant differences of $\bar{u}$ values can be observed between test without aeration (Test 13 and Test 14) and test with aeration (Test 15 and Test 16). However, Figure 4.8 suggested that tests with aeration show more stable trend. t test conducted on $\bar{u}$ values for all tests show $p < 0.05$, thus indicate $\bar{u}$ values have undergone changes with time.

$\overline{k_s}$ values for all tests show significant changes with time. Test 13, Test 14 and Test 16 show a drop in the first 40 hours followed by a steady increase until the end. Test 15 shows an increase at the same time period, followed by steady $\overline{k_s}$ values. The results were consistent with previous conditions, where $\overline{k_s}$ values were found to be decreasing and thus increasing $\bar{u}$ values with biofilm growth for most of the tests. These changes can be illustrated by Table 4.14.



(a)

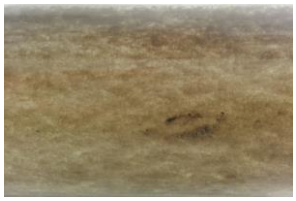
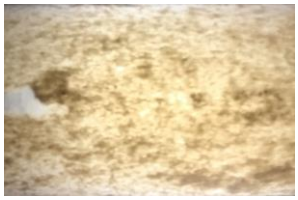


(b)

Figure 4.8. $\bar{u}$ and $\overline{k_s}$ values for 1.5 m pipe configuration at non-aerated condition (Test 13 and Test 14) and aerated condition (Test 15 and Test 16).

In general, biofilm growth was observed in all tests. A Clear difference in biofilm characteristics can be observed for tests conducted with and without aeration. Tests without aeration (Test 13 and Test 14) show thinner, compact, and uniform biofilm. Meanwhile, thicker, fluffy and uniform biofilm was obtained for tests conducted with aeration (Test 15 and Test 16). These significant differences in biofilm characteristics were hypothesized to cause different trend observed in Figure 4.8, where stable trends were obtained for Test 15 and Test 16. Other than that, Test 15 shows the highest amount of biofilm coverage at T = 80 hours which was consistent with lowest $\overline{k_s}$ values from Figure 4.8.

Table 4.14. Images of biofilm growth obtained for tests at 1.5 m long pipe configuration at non-aerated and aerated conditions (Flow direction to the right, size: 70mm x 50 mm).

Test nu.	T = 0 hours	T = 80 hours	T = 168 hours
13			
14			
15			
16			

These findings demonstrated that biofilm obtained from tests with and without aeration have different characteristics even though the oxygen concentration was sufficient for a sustainable aerobic condition to exist in the pipe for tests without aeration. These finding may suggest that some limitation on biological processes may have occurred in the system due to the restrictions provided by the non-aeration condition. Melo *et al.* (1992) have reported an exponential biofilm growth rate with oxygen concentration, where biofilm growth was found to be constant with oxygen concentration of higher than 1 mg/L. The results obtained from this study do not agree with this statement, as biofilm growth was observed to increase over time at minimum oxygen concentration value of 2.8 mg/L.

All tests except Test 15 show an increase in $\overline{k_s}$ values at the end of the test. This observation agreed with the images as biofilm detachment can be seen from the images provided. Test 15 shows a decrease in $\overline{k_s}$ values although the pipe experienced biofilm detachment at T = 168 hours. This suggested that only a small fraction of biofilm was detached in the system thus the influence on pipe hydraulics was not significant.

Table 4.14 also shows that tests without aeration were more inclined to detachment at the end of the tests. This was assumed to be caused by physicochemical stress that the bacteria experienced from the limited oxygen availability which caused the bacteria to detach itself in order to find a better growth environment. This was partially true, as Hunt *et al*. (2004) reported that oxygen limitation triggers biofilm removal of *Shewanella oneidensis* biofilm.

t test on $\overline{k_s}$ values shows that all values obtained at T= 0 hours and 168 hours were statistically significant except for Test 16. p values were 0.0026 for Test 13, 0.0027 for Test 14 and 0.0084 for Test 15. p values on Test 16 yield a value of 0.171, which was higher than set value of 0.05 thus concluded that no significant changes were determined in Test 16.

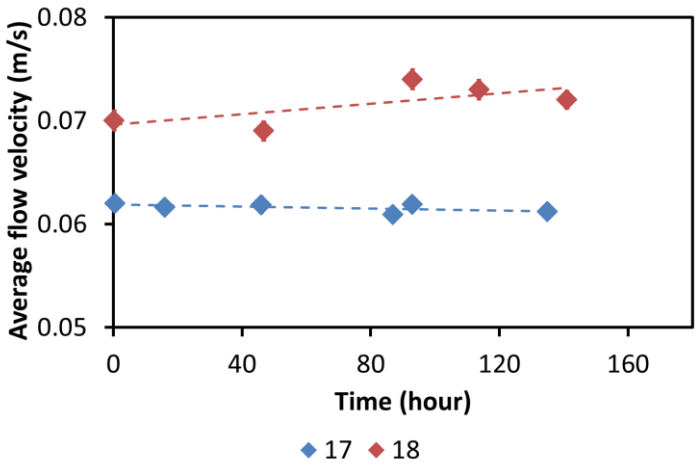
Condition 5 - 1.0 m pipe length, no aeration, 6 mm bed elevations.

Figure 4.9 shows result obtained on tests using 6 mm bed elevation at 1.0 m pipe configuration. S_o and τ_o values obtained under this condition can be summarized by Table 4.15. Biofilm growth was observed on all tests.

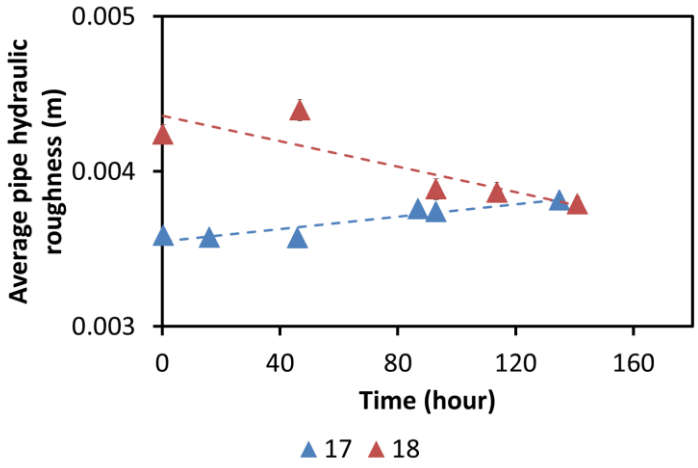
Table 4.15. Bed slope and shear stress values for Test 17 and Test 18.

Test number	17	18
τ_o (N/m^2)	0.5437 ± 0.0093	0.6416 ± 0.0127
S_o (m/m)	0.0067	0.0072

The results show fairly consistent $\bar{u}$ values over time for Test 17 while a small increase was spotted at the 80 hours mark for Test 18. t test results that were conducted to compare the values obtained at the start of the test and at the end shows that Test 17 were not significantly different. t test results for Test 17 were 0.052 and 0.0057 for Test 18 which concluded that changes in $\bar{u}$ values were not significant for Test 17.



(a)



(b)

Figure 4.9. Results of average flow velocity and average pipe hydraulic roughness for Test 17 and Test 18.

The trend observed for $\overline{k_s}$ value was fairly similar to $\bar{u}$ values as shown by Figure 4.9 (b). A significant decrease was observed for Test 18 at 80 hour mark, and t test conducted concluded that this change was significant as p values obtained for Tests 18 were 0.0039. t test performed on Test 17 yield p

values of 0.061, which shows that $\overline{k_s}$ value of Test 17 does not undergone any changes with time.

These changes in $\bar{u}$ and $\overline{k_s}$values can be further understood with reference to Table 4.16. From the table, Test 17 shows similar biofilm characteristics with Test 9 from Condition 2, where thin and uniform biofilm was observed in the pipe. Test 18 shows fluffier and thicker biofilms, with more biofilm was observed in Test 18 at all time period. This may have explained on the significant changes in $\bar{u}$ and $\overline{k_s}$ for Test 18. At T = 168 hours, Test 17 shows evidence of biofilm detachment which agreed with the results shown. Test 18 shows more biofilm growth in the pipe at the same time period, which was consistent with decreasing $\overline{k_s}$ values at the end of the test.

Table 4.16. Biofilm growth observed in Test 17 and Test 18 at different time period. (Flow direction to the right, size: 70mm x 50mm).

Test nu.	T = 0 hours	T = 80 hours	T = 168 hours
17			
18			

Both tests show different results as biofilm was observed to increase pipe hydraulic roughness for Test 17 while Test 18 shows that biofilm growth smoothens pipe surface. Both findings were consistent with tests from previous conditions.

Comparison of the results obtained at different conditions

Both $\bar{u}$ and k_s values for tests conducted at low and high shear stresses has shown to change with time as shown by Figure 4.5 and Figure 4.6. These

changes may depend on different characteristics of biofilm growth at both conditions. At low shear stresses level, more biofilm was observed in the pipe. The biofilm was also more uniform and thicker as compared to biofilm obtained at higher shear stress level which was patchy, more compact and thinner. These finding were partially in agreement with the literature, as smooth (Coufort *et al*. 2007), thin (Xu *et al*. 2017) and non-uniform biofilm growth was obtained at higher shear stress conditions (Kraigsley *et al*. 1992). These differences can be illustrated by tests conducted at Condition 1 and Condition 2 by referring to Table 4.8 and Table 4.10.

To compare influences of aeration in the 1.0 m pipe system, no aeration tests (Condition 2) show fewer changes in the results obtained, as larger changes in $\bar{u}$ and $\overline{k_s}$ values can be observed for tests conducted with aeration (Condition 3) from Figure 4.6 and Figure 4.7. These changes may have associated with biofilm growth in the pipe, as limited oxygen system may have caused limited oxygen diffusion into the biofilms. However, both conditions show fairly similar biofilm pattern as patchy and thin layer of biofilm was obtained under both conditions. More detachment was observed in tests without aeration, and this observation was consistent with the literature as oxygen limitations were reported to cause catastrophic sloughing event for biofilm made of *Pseudomonas putida* (Applegate *et al.* 1991). This finding was supported by Xavier *et al*. (2005) and both study agreed that biofilm with finger-like structure was observed in oxygen limited conditions.

For tests at 1.5 m pipe length configuration (Condition 4), tests with aeration show more stable trend, which may indicate stable growth conditions for the bacteria, thus, allowing the bacteria to survive with minimal efforts. Other than that, for 1.5 m pipe long configuration, thin, compact and uniform biofilm was obtained at non-aerated conditions while fluffy, thicker and uniform biofilm was obtained under aerated conditions. Tests without aeration were also more susceptible to detachment as compared to tests with aeration which consistent with results obtained to 1.0 m pipe length configuration.

Although Condition 2, 3 and 4 are in agreement with the relationship between oxygen concentration and biofilm removal processes, biofilm characteristics observed at each condition were significantly different. Patchy and thin biofilm was observed for tests with and without aeration for 1.0 m pipe length configurations (Condition 2 and Condition 3). Meanwhile, biofilms obtained for tests at 1.5 m pipe lengths (Condition 4) show different biofilm characteristics where thin, and compact biofilm was obtained under non-aeration conditions while thick and fluffy biofilm were obtained under aerated conditions. Tests at 1.5 m pipe length also show more uniform biofilm coverage. This may suggest for a more complex relationship between biofilm characteristics observed with initial COD concentration, shear stress, and oxygen concentration level in the system.

These differences may have suggested that both pipe lengths were able to provide distinct hydraulic conditions for biofilm growth. Since both conditions were conducted with the same volume of wastewater, 1.5 m pipe long setup was assumed to be able to facilitate more biofilm growth in the pipe due to the larger wetted area. Other than that, the flow of 1.5 m pipe length was also observed to be more stable, as the longer length was aiding in maintaining the uniform flow.

There was no significant difference observed for tests conducted at 800 mg/L COD concentration (Test 12 and Test 16) as compared to tests conducted with wastewater without any additional nutrient in the system. The changes in $\bar{u}$ and $\overline{k_s}$ values were similar to the other tests which may have indicated similar biofilm growth and high COD concentration do not guarantee more biofilm growth in the pipe. This findings do not agreed with the literature, as biofilm growth has been reported to increase with increasing nutrient concentration in the system (Peyton, 1996; Rochex and Lebeault, 2007).This disagreement may have been caused by the used of wastewater in the system, which produces different biofilm community and structure as compared to single species biofilm as reported from these studies.

This finding was also indicating that nutrient concentration was not limited in the system. From the observation done the biofilm growth, nutrient and oxygen concentration seems to have a smaller influence on biofilm growth as compared to hydraulic conditions. This was true for comparison conducted on biofilm growth and characteristics obtained for different hydraulic conditions. However, biofilm was observed to have possessed various characteristics under similar hydraulic conditions, and these findings suggested that nutrient and oxygen concentration have a significant influence on the biofilm growth.

Both results from tests done at different bed slope values show no major differences, except for hydraulic conditions in the system. Tests conducted at higher bed slope values were observed to have higher shear stress value as shown in Figure 4.5 and Figure 4.9, which was due to higher flowrate resulting from the higher bed slope values. Biofilm growth obtained at these conditions was partially agreed with the literature, as biofilm was observed to be thick, smooth and uniform in the pipe (Coufort *et al*. 2007; Paul *et al*. 2012).

In summary, both $\bar{u}$ and $\overline{k_s}$ value were changing with time, depending on biofilm growth characteristics in the pipe. The relationship between biofilm, $\bar{u}$ and $\overline{k_s}$ values was biofilm growth decreasing flow depth thus decreasing $\overline{k_s}$ values and increasing $\bar{u}$ values of pipe. 15 out of 18 tests agreed with this relationship, while the rests were showing a conflicting finding. Changes in $\bar{u}$ and $\overline{k_s}$ values over time were exclusive for each tests, although some tests were conducted under the same conditions. The changes were small, as compared to Guzmán *et al*. (2007) who reported an increase of Manning's n coefficient from 0.011 obtained for clean water to 0.014 to 0.043 for biofilm-covered pipe 200 mm pipe diameter at 0.1% slope. For higher slope of 0.5%, the changes were less significant as Manning's n coefficient obtained for biofilm-covered pipe was 0.015 to 0.020. Fewer changes obtained in this study were speculated due to small pipe diameter area and short biofilm growth period as compared to Guzmán *et al*. (2007).

The results show a conflicting finding to study by Guzmán *et al*. (2007), but in agreement to study by Lewandowski *et al.* (1992) and Lewandowski and Beyenal (2005). This can be attributed to similar hydraulic conditions obtained with study by Lewandowski *et al.* (1992), as both studies were conducted at low velocity conditions (unknown Reynolds number for Lewandowski *et al.* (1992), however, Reynolds number obtained for this study was between 1000 to 1400, which was barely in turbulent region for open channel flow). Other than that, this agreement may due to different bacteria population used, as Lewandowski *et al.* (1992) used samples from activated sludge from municipal wastewater treatment plant while Guzmán *et al*. (2007) were using potable water.

This study also agreed with findings by Fang *et al.* (2014), who reported grey coloured biofilm obtained with deionized water while dark brown biofilm obtained user nutrient-rich mixture (results not presented).

Factors such as pipe length and bed slope show no significant influences on biofilm growth as long as the uniform flow was obtained. Low shear stress condition produced more stable biofilm growth in the pipe and thus resulting in a more consistent pattern with time. Aeration was not necessary for the system for the duration proposed, and high nutrient and oxygen concentration in the system do not produce more biofilms in the system.

4.7.2 Biofilm dry mass per wetted area

Figure 4.10 shows results obtained for average biofilm dry mass over the pipe wetted area for all tests. From the graph, various relationships can be observed between initial COD concentration and biofilm obtained in the tests which suggest that initial wastewater conditions do have effects on biofilm growth in the pipe.

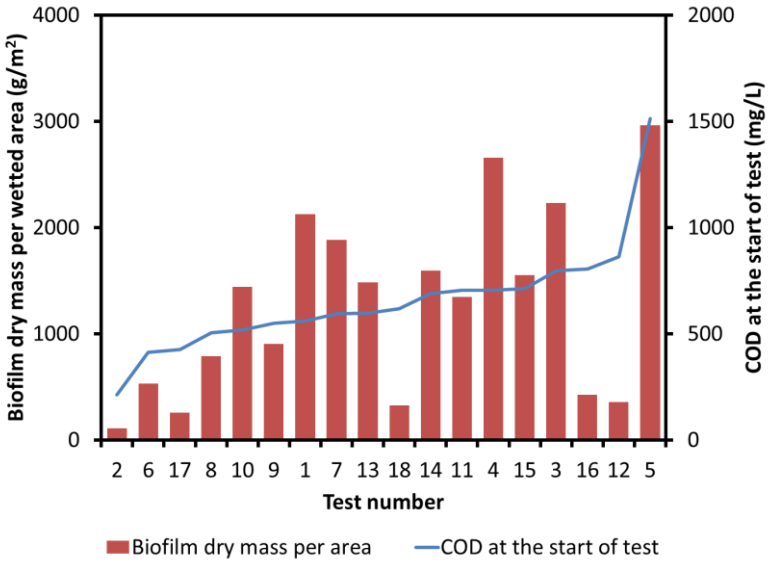


Figure 4.10. Summary of average biofilm dry mass per area obtained for all tests.

Test 2 shows the lowest initial COD values produced the lowest mass of biofilm over the wetted area, while the opposite was true to Test 5. Both tests were conducted under the same conditions, thus show that biofilm growth was influenced by differences in nutrient concentration under similar hydraulic conditions. In general, higher initial COD values produced more biofilm in the pipe. This statement can be used to represents some of the tests, with few exceptions.

Test 17 and Test 18 show that initial COD conditions have less influence on biofilm growth in comparison to hydraulic conditions in the system. Both tests were conducted at high bed slope values which produced the highest shear stresses level. These conditions may have limited biofilm growth in the pipe, as low shear stress level has been demonstrated to produce more biofilm. This finding contradicts with the results obtained by Percival *et al.* (1999) that founds that no significant differences in biofilm dry mass obtained at different flowrates for biofilm grown using potable water.

Test 12 and Test 16 show similar results to Test 17 and Test 18. Test 12 and Test 16 were conducted at a lower shear stress value and at a higher COD

concentration as compared to Test 17 and Test 18. These results may cause by biofilm detachment as shown previously in Table 4.14 and Figure 4.8.

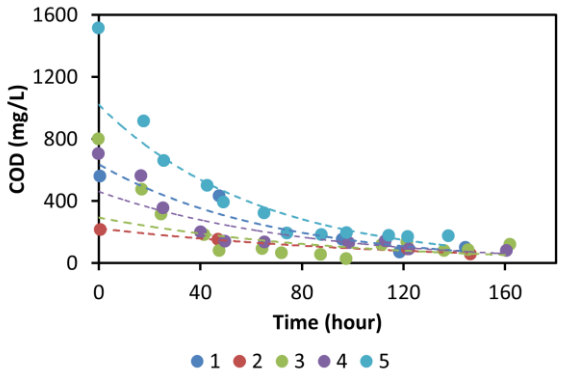
Test 6 to Test 8 yield a similar value of average biofilm dry mass per area compared to Test 9 to Test 12. This observation agreed with previous findings, where no significant differences were obtained in a system with aeration and without aeration at 1.0 m pipe length. A similar average biofilm dry mass per area value was obtained from the comparison of Test 9 to Test 12 with Test 13 to Test 16. This result was consistent with the previous outcome as longer pipe length does not produce more biofilm in the pipe. This was due to lower nutrient concentration in 1.5 m pipe as compared to 1.0 m pipe length, as both were run with the same volume of wastewater.

This method was only able to measure the quantity of biofilm present in the system at different conditions. However, this analysis is limited to the average value of biofilm mass, which means that only biofilm that remains at the end of the test was tested. Mass of the biofilm present at a specific location in the pipe or at specific time period was not able to be determined.

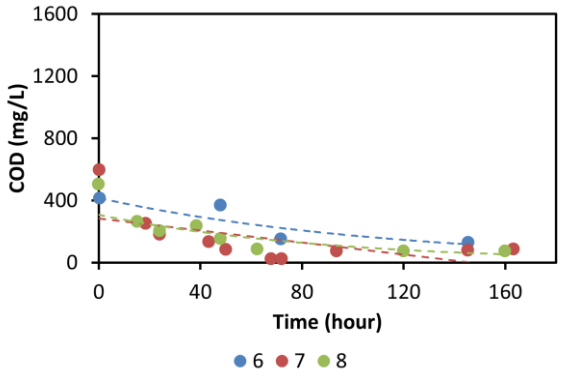
These results can underestimate the actual value by the loss of biofilm during collection procedure or overestimated due to biofilm from tubing and reservoir tank. These values can also be influenced by fine solid samples in the wastewater. However, these influences were deemed insignificant as total solid values obtained for wastewater were very small (0.007 ± 0.002 mg) thus may not significantly change the biofilm dry mass obtained.

4.7.3 Organic matter concentration in the system

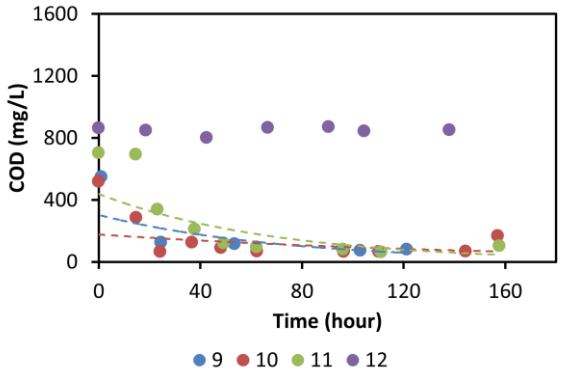
Figure 4.11 demonstrates results of wastewater COD concentration over time. All results show decreasing COD concentration with time except or Test 12 and Test 16 where COD concentration was maintained at 800 mg/L level using a complex organic matter substitute. In general, initial COD concentration values have no influences on the rate of COD consumed in the system.



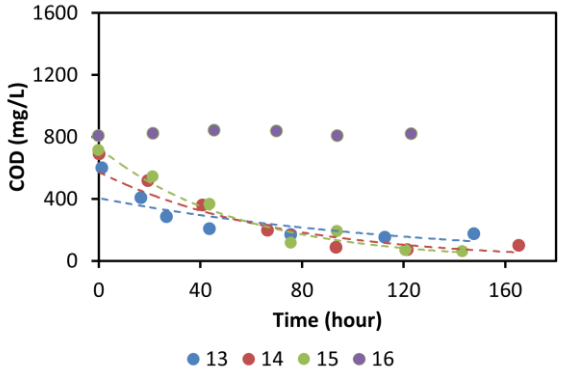
(a)



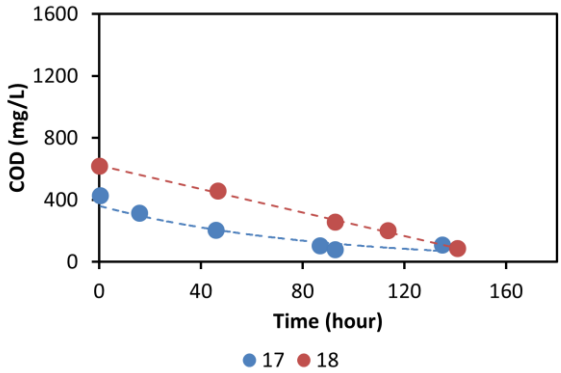
(b)



(c)



(d)



(e)

Figure 4.11. Results of COD concentration of wastewater over time for all tests at different conditions (a) Condition 1 - 1.0 m pipe length, no aeration and low shear stress, (b) Condition 2 - 1.0 m pipe length, no aeration and high shear stress, (c) Condition 3 - 1.0 m pipe length, aeration and high shear stress, (d) Condition 4 - 1.5 m pipe length, both aeration and non-aeration at high shear stress and (e) Condition 5 - 1.0 m pipe length, no aeration and high bed slope values.

pH was observed to be decreasing with time, and ranging from 6.0 to 7.5 for all tests (results not included). This finding was consistent with studies conducted by Szwerinski *et al.* (1986) and Zhang *et al.* (1996). This decrease was speculated due to the production of carbon dioxide during aerobic degradation of organic matter. Carbon dioxide was then hydrolyzed and formed carbonic acid, which is an acidic substance that causes the decrease in pH values.

It was also observed that biofilm detachment occurred after a decrease in pH value for most tests. The pH was observed to decrease by 0.5 at approximately T = 120 hours, and biofilm detachment was observed to occur shortly after. This may suggest that changes in biofilm growth conditions were the cause of biofilm detachment and not by changes in hydraulic conditions. This can be supported by a study by Gerret *et al*. (2008) that reported changes in pH causes biocidal effects on the bacteria.

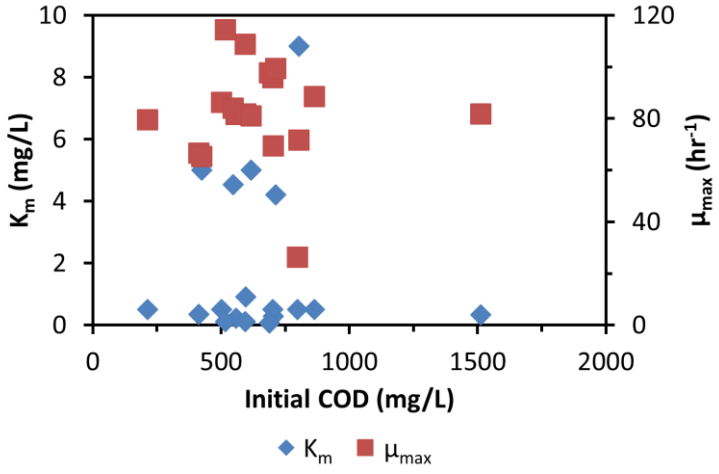
Table 4.17. Summary of K_m and μ_{max} values for all tests.

Test number	K_m (mg/L)	μ_{max} (hr^{-1})
1	0.219	81.524
2	0.500	79.398
3	0.495	26.197
4	0.280	69.273
5	0.328	81.730
6	0.339	66.506
7	0.100	108.737
8	0.500	86.194
9	4.524	83.873
10	0.100	114.200
11	0.500	95.806
13	0.900	81.743
14	0.060	97.652
15	4.201	99.242
17	4.994	65.124
18	4.999	80.853

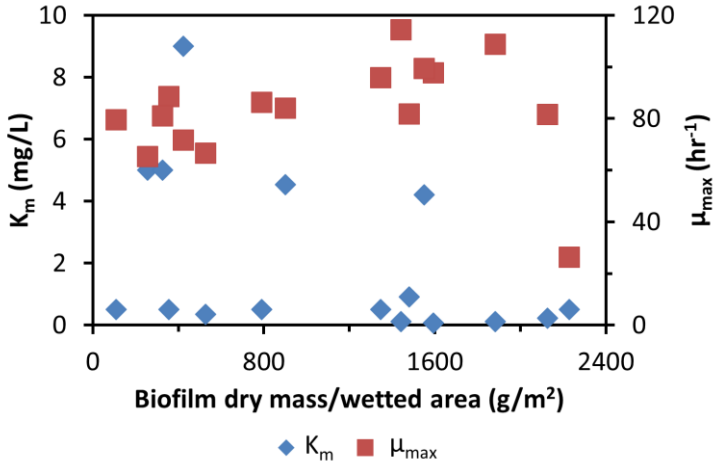
Initial COD concentrations for all tests were varying from 200 to 1600 mg/L. This can be due to the sampling period, as wastewater collected during wet weather period may have lower COD concentration values. Average residual COD concentration at the end of the tests was 105 mg/L, where highest concentration was obtained for Test 5 with a value of 173 mg/L, and lowest concentration was obtained for Test 2 with a value of 56 mg/L. These findings were directly related to initial COD concentration, as Test 5 recorded

the highest initial COD concentration of 1513 mg/L, thus, higher residual COD concentration was obtained at the end of the test. The average COD concentration calculated during biofilm detachment (T = 120 and 144 hours) was 88 mg/L.

Other than that, the half saturation constant, K_m and maximum specific growth rate, μ_{max} were calculated for all tests except for Test 12 and Test 16, following Monod equations. These values were obtained in order to quantify biofilm growth rate relationship with substrate concentration (Kovárová-Kovar *et al*. 1998). The hypothesis of these measurements was to achieve higher μ_{max} values for tests conducted with high COD concentration. The summaries of both values are presented by Table 4.17.



(a)



(b)

Figure 4.12. Plot of K_m and μ_{max} values against (a) initial COD concentration and (b) biofilm dry mass over wetted area values.

From Table 4.17 K_m values obtained were fairly similar for all tests except for Test 9, Test 15, Test 17 and Test 18. μ_{max} values were also comparable for all tests except for Test 3, Test 7, Test 10. To further understanding these results, the values obtained were further compared to initial COD concentration and biofilm dry mass over wetted area values in order to study these abnormalities. A plot of K_m and μ_{max} values with these two factors can be shown in Figure 4.12.

Vast majority of the tests shows similar K_m and μ_{max} values obtained for initial COD concentration between 500 to 1000 mg/L as shown by Figure 4.12 (a). These may suggested that optimum biofilm growth was obtained at these conditions and provide similar biofilm growth rate.

For relationship between K_m and μ_{max} values with biofilm dry mass over wetted area, a more scattered plot was observed, as shown from Figure 4.12 (b). A slight increase of μ_{max} values with increasing biofilm dry mass over wetted area values were also observed, which further indicate that the values obtained were dependent and was not influenced by the initial COD concentration of the wastewater. This finding further suggested that heterotrophic activity in the system was not limited by nutrient concentration level. No explanation can be provided for the drop in μ_{max} values at the higher end of biofilm dry mass over wetted area values.

No correlation between initial COD concentration and biofilm dry mass over wetted area values can be obtained from the findings. These were demonstrated by similar values of K_m and μ_{max} for Test 2 and Test 5, although initial COD values for Test 2 were the lowest at approximately 200 mg/L and initial COD values for Test 5 were the highest at approximately 1600 mg/L. Other than that, Test 2 also shows the lowest biofilm dry mass over wetted area values while Test 5 shows the highest values as shown from Figure 4.10.

Both K_m and μ_{max} values found in this study was comparable with the values reported in the literature. Kommedal (2003) reported K_m values of 4.01 ±

0.09 mg/L and μ_{max} values of 0.51 ± 0.02 hr^{-1} for batch reactors incubated with wastewater collected from primary inlet of a wastewater treatment plant and enriched with phosphate buffer saline. Hunt *et al*. (2004) used K_m values of 0.1 g/m^3 and 0.3 hr^{-1} of μ_{max} values for the modelling of kinetics and solute transport for biofilm made of *Pseudomonas aeruginosa.* Horn *et al*. (2003) obtained higher values of K_m and μ_{max} of 10 g/m^3 and 5 d^{-1} for biofilm growth using primary settle wastewater collected from a wastewater treatment plant in Germany. Mean K_m and μ_{max} values of 9.4 mg/L and 6.1 d^{-1} were reported by Trajanowicz *et al.* (2009) for a study conducted on bacterial growth obtained from a biofilm reactor located in a plant treating petrochemical wastewater.

The results show that microorganisms were not starved during the process even though no additional nutrient was added except for Test 12 and Test 16. This can be proved by more biofilm growth after detachment with time as shown from Test 5 (illustrated by Table 4.8) and Test 10 and Test 11 (shown in Table 4.12). For Test 2, no evidence of nutrient depletion or microbial starvation can be provided, which was initially assumed as the test was conducted with the lowest initial COD concentration.

4.7.4 Results of energy losses for pipe test experiments

Summary of energy losses calculated for the pipe setup can be presented in Table 4.18 and Table 4.19. Table 4.18 shows the values obtained for minor energy losses, namely due to pipe entrance, pipe exit and pipe fittings. In this work, energy losses due to pipe fittings were mainly due to the butterfly valve fitted on the pipe reactor.

From Table 4.18, it can be summarized that energy losses at the pipe entrance and pipe exit (h_i) and (h_o) were very small, as compared to energy losses due to pipe fittings (h_L). These values were expected, as $\bar{u}$ values do not shows any large changes at pipe entrance and exit section at both time periods. All tests show smaller h_i values as compared to h_o values. These

values, however, were very small to have any influence on the tests hydraulic parameters.

Table 4.18. Values of minor losses obtained from calculation following Section 4.5.2.

	T = 0 hours			T = 168 hours		
Test number	h_i(m)	h_o(m)	h_L(m)	h_i(m)	h_o(m)	h_L(m)
1	5.37E-05	1.07E-04	1.16E-03	5.93E-05	1.19E-04	1.28E-03
2	4.85E-05	9.70E-05	3.79E-04	4.85E-05	9.70E-05	3.79E-04
3	3.76E-05	7.52E-05	2.45E-03	3.87E-05	7.75E-05	2.53E-03
4	4.46E-05	8.92E-05	2.91E-03	4.57E-05	9.13E-05	2.98E-03
5	3.90E-05	7.80E-05	8.42E-04	4.13E-05	8.26E-05	8.92E-04
6	6.13E-05	1.23E-04	1.32E-03	5.86E-05	1.17E-04	1.27E-03
7	4.46E-05	8.92E-05	9.63E-04	6.64E-05	1.33E-04	1.43E-03
8	6.76E-05	1.35E-04	1.46E-03	7.15E-05	1.43E-04	1.54E-03
9	6.37E-05	1.27E-04	4.98E-04	5.50E-05	1.10E-04	4.30E-04
10	5.99E-05	1.20E-04	1.29E-03	6.35E-05	1.27E-04	1.37E-03
11	5.84E-05	1.17E-04	1.26E-03	5.69E-05	1.14E-04	1.23E-03
12	6.04E-05	1.21E-04	1.30E-03	5.85E-05	1.17E-04	1.26E-03
13	8.18E-05	1.64E-04	4.19E-02	7.48E-05	1.50E-04	3.83E-02
14	7.54E-05	1.51E-04	3.86E-02	8.76E-05	1.75E-04	4.48E-02
15	7.46E-05	1.49E-04	3.82E-02	8.52E-05	1.70E-04	4.36E-02
16	7.29E-05	1.46E-04	4.75E-03	7.91E-05	1.58E-04	5.16E-03
17	9.79E-05	1.96E-04	6.38E-03	9.55E-05	1.91E-04	6.22E-03
18	1.25E-04	2.50E-04	2.70E-03	1.32E-04	2.64E-04	2.85E-03

From Table 4.19, only 10 tests have been observed to show an increase in the h_f values (as shown in bold), while the remaining tests show a conflicting result. As previously discussed, 15 out of 18 tests have shown that biofilm growth decrease $\overline{k_s}$ values and thus increase $\bar{u}$ values. From this statement, an increase of h_f values from T = 0 hours to T = 168 hours were initially expected. This is due to decreasing $\overline{k_s}$ values of the pipe which will reduce the pipe flow resistance and increasing $\bar{u}$ values and thus increasing the h_f values.

This finding was speculated to be caused by different characteristics and the coverage area of the biofilm obtained, as h_f values calculated at T = 168 hours were based on calculated average flow velocity values at for pipe covered biofilm. Biofilm growth was generally found to decrease flow depth, however, biofilm coverage and thickness were not measured thus the changes in h_f values obtained were considered as unclear.

Table 4.19. Results for pipe energy losses due to friction for all tests conducted with wastewater.

Test number	$\boldsymbol{h_f}$ at T = 0 hours (m)	$\boldsymbol{h_f}$ at T = 168 hours (m)	% changes of $\boldsymbol{h_f}$ values
1	8.80E-04	8.75E-04	-0.603
2	1.15E-03	1.15E-03	-0.009
3	**1.17E-03**	**1.17E-03**	**0.023**
4	**1.10E-03**	**1.10E-03**	**0.008**
5	1.05E-03	1.05E-03	-0.006
6	8.75E-04	8.75E-04	0.019
7	**7.53E-04**	**9.75E-04**	**29.501**
8	1.05E-03	1.05E-03	-0.006
9	**1.00E-03**	**1.00E-03**	**-0.033**
10	**1.30E-03**	**1.30E-03**	**0.011**
11	1.18E-03	1.09E-03	-6.989
12	1.23E-03	1.23E-03	-0.001
13	**1.91E-03**	**1.92E-03**	**0.068**
14	**1.88E-03**	**1.88E-03**	**0.161**
15	**1.54E-03**	**1.54E-03**	**0.169**
16	**1.73E-03**	**1.73E-03**	**0.275**
17	2.01E-03	2.02E-03	0.220
18	**2.54E-03**	**2.93E-03**	**15.092**

Since the tests were conducted in a relatively short pipe, minor energy losses values were observed to be higher than the major energy loss due to pipe friction values. These findings can be improved by using a longer pipe in the future.

4.8 Summary of key findings for pipe test experiments

Key findings obtained from all tests conducted with wastewater can be presented by Table 4.20. Biofilm characteristics, changes in flow parameters values and COD concentration were included in the table

Table 4.20. Summary of key findings for all tests conducted with wastewater.

	Observations		
	Biofilm characteristics	Changes in $\bar{u}$ and $\overline{k_s}$ values	Changes in COD concentration

Condition 1	• Biofilm was observed for all tests.
	• Different biofilm characteristics were observed for each test.
	  • Thick and uniform for Test 1.
	  • Patchy and thin for Test 2
	  • Non-uniform for Test 3.
	  • Fluffy and thick for Test 4.
	  • Thin and uniform for Test 5.
	• Biofilm detachment was observed in all test at T = 168 hours.
		• All tests show decreasing $\overline{k_s}$ values with biofilm growth.
		• The degree of $\overline{k_s}$ values changes depend on the biofilm characteristic of each test.
			• All tests show decreasing COD concentration with time.
			• Low initial COD conditions produced the lowest amount of biofilm in the test (Test 2).
			• High initial COD concentration produced the highest amount of biofilm in the pipe (Test 5).

Condition 2	• Biofilm growth was observed for all tests. • Patchy and thin biofilm was observed for all tests. • No biofilm detachment was observed at T = 168 hours.	• 2 out 3 tests show decreasing $\overline{k_s}$ values with biofilm growth.	• All tests show decreasing COD concentration with time.

	Observations		
	Biofilm characteristics	Changes in $\bar{u}$ and $\overline{k_s}$ values	Changes in COD concentration

Condition 3	• Biofilm growth was observed for all tests.
	• Different biofilm characteristics was observed;
	  • Thin and uniform for Test 9
	  • Patchy, thick and fluffy biofilm for all other tests.
	• Biofilm detachment was observed for 3 out of 4 tests.
		• 3 out of 4 tests show decreasing $\overline{k_s}$ values with biofilm growth.	• All tests show decreasing COD concentration values with time.

	Observations		
	Biofilm characteristics	Changes in $\bar{u}$ and $\overline{k_s}$ values	Changes in COD concentration
Condition 4	• Biofilm growth was observed for all tests. • Different biofilm characteristics were observed for tests with and without aeration; • Thinner, compact and uniform biofilm was observed for tests without aeration. • Thicker, fluffy and uniform biofilm was observed for tests with aeration. • Tests without aeration were more viable for detachment. • Biofilm detachment was observed for all tests except for Test 15.	• All tests show decreasing $\overline{k_s}$ values with biofilm growth. • No significant differences in $\overline{k_s}$ and $\bar{u}$ between tests with and without aeration. • Tests with aeration show more stable trend.	• COD concentration was decreasing with time for all tests.

	Observations		
	Biofilm characteristics	Changes in $\bar{u}$ and $\overline{k_s}$ values	Changes in COD concentration

Condition 5	• Biofilm growth was observed for all tests.
	• Different biofilm characteristics were observed;
	  • Thin and uniform biofilm was observed for Test 17.
	  • Fluffy and thicker biofilm was observed for Test 18.
	• Evidence of biofilm detachment for both tests at T = 168 hours.
		• 1 out of 2 tests shows decreasing $\overline{k_s}$ values with biofilm growth.	• Decreasing COD concentration was observed for all tests.

4.9 Conclusions

This chapter presented a number of controlled experiments conducted to investigate the effects of biofilm growth on pipe hydraulic roughness. A total of 18 tests were conducted at various conditions, namely pipe length, bed slope, dissolved oxygen concentration in the system and wastewater COD initial concentration. Pipe hydraulic roughness was obtained through calculation following the Colebrook-White equations for steady uniform flow.

This work was originally influenced by a study that stated biofilm growth is increasing pipe hydraulic roughness in a system where tap water was enriched with methanol and glucose, and COD was maintained at 800 mg/L (Guzmán *et al.* 2007).

The theory was tested using wastewater and tap water and results obtained show that wastewater-grown biofilm is decreasing hydraulic roughness of the pipe and thus increasing average flow velocities at some timeline during the tests. No significant changes were observed in tests with tap water at the same time period.

More conclusions that are obtained from this work can be presented as below;

- Biofilm growth was observed in all tests with wastewater after 24 hour period, and biofilm detachment mostly occurs after 4 to 5 days.
- Average hydraulic roughness values obtained for background tests using tap water were constant with time as there was no no biofilm growth in the system.
- 15 out of 18 tests conducted with wastewater show that average pipe hydraulic roughness values were decreasing thus increasing average flow velocities with biofilm growth. These findings were related to biofilm growth characteristics in the pipe under different conditions. Low shear stresses show more changes in these values as compared

to higher shear stresses, and tests with aeration show a more stable trend. Pipe length does not have a major influence on the results.

- pH was decreasing with time for all tests, which indicate aerobic degradation occurrence in the pipe. The changes in pH values were also observed to be an indication of biofilm detachment in the pipe. However, there is still a need for further clarification of these findings.
- COD concentration was decreasing with time for all tests. High initial COD values produced more biofilm in the system. However, this finding is only applicable for biofilm grown on the same shear stresses level.
- Constant and high COD concentration does not promote more biofilm growth in the system. This may also suggest that hydraulic conditions have more controlled on biofilm growth as compared to nutrient concentration.

These studies demonstrate that biofilm growth have a significant influence on pipe flow behaviour and needs to be included in sewer networks modelling. The relationship is complex, as the biofilm growth characteristics rely on initial flow profile. Other than that, this study also proves that wastewater-grown biofilm produced a different set of results as compared to tests conducted with tap water by Guzmán *et al.* (2007) which further implies the importance of using wastewater in representing in- sewer processes.

The work conducted has shown high consistency in the results obtained thus provides a good level of confidence in the outcomes generated. However, there are still limitations in the study that needs to be considered. First, the potential for scaling up the experiments is interesting, for example, a setup consists of larger and longer pipe configurations are recommended in order to obtained flow with higher Reynold Numbers as compared to the tests have obtained. This change would mean a gradual approach to mimic the conditions of flow in the sewer and thus produces data that are more relevant for full scale sewer application processes. This upscaling will require the use of larger volumes of wastewater in a laboratory setting.

No analysis of the microbial community composition was conducted in this study due to time and cost limitation. The addition of this analysis was assumed to be able to provide information on bacterial communities in the wastewater, and the influences it has on the differences in biofilm characteristics obtained under similar hydraulic conditions can be determined. In addition to that, obtaining flow depth from measured hydraulic parameters has shown reliable and consistent results. This method, however, was very subjective and the use of an advanced instrument such as surface roughness measurement instrument is recommended for this shortcoming. Using instruments may help to increase the accuracy of the parameters obtained thus conducting tests using different materials such as sewer wall is made possible. Other than that, it is more universal, thus, allowing for an easier knowledge transfer with another researcher.

Lastly, biofilm visualisation was not conducted due to the reactor configuration that leaves no room for a visualizing device such as a camera to be fitted on the reactor. By having a larger reactor configuration, this limitation can be overcome, and information such as biofilm physical characteristics at microscale level can be obtained. This will provide more evidence on changes of biofilm physical characteristics due to biofilm growth condition and its contribution to changes in flow velocity profile.

This study has shown that the hydraulic conditions have more influence on biofilm growth compared to biofilm growth condition. Information on changes of in sewer capacity with and without biofilm growth is scarcely available and the findings obtained from this study could be applied to real sewers.

Chapter 5 Effects of biofilm growth on sewer sediment deposits

The previous chapter has demonstrated that in-sewer biofilm growth on pipe walls can impact on the hydraulic resistance. Sediment deposits can also occur in the sewers. The objective of the study reported in this chapter is to achieve a better understanding of how biofilm growth may impact on sewer sediment deposits under different environmental conditions encountered within sewers. Sewers sediment can have widely varying characteristics depending on local hydraulic conditions and sediment sources (Ashley *et al*. 2004), therefore in these tests, it was decided to use a mix of inorganic and organic sediments to represent the two components often found within in-sewer sediments. The main concept of these tests is to grow biofilm on such surrogate sediments particles under controlled conditions in the presence of water and wastewater. The influence of biofilm growth on the bed sediment strength was observed under increasing shear stress by determining the bed erosion rate. Organic matter; protein and reducing sugar were quantified and used as an indicator of biofilm growth in the system. This study will provide valuable insight into the changes in the sediment stability due to biofilm formation, which will help further understanding of the role of biofilms on sewer sediment behaviour during flow variations in a sewer.

5.1 Erosion tests experimental setup

All tests were carried out using a pre-calibrated device known as erosionmeter, which was originally described by Liam *et al.* (1997). The device consists of a clear, cylindrical Perspex column with a diameter of 100 mm and a sample container that can be inserted into the bottom of the column to holds sediment sample as shown in Figure 5.1.

The sediment sample had an exposed area of 7853 mm^2 and was subjected to a uniform shear stress by a 50 mm propeller that was placed 30 mm above the bed surfaces. Five baffles of 0.2 mm thickness were fitted perpendicularly in the column to promote homogeneous mixing in the column by preventing circulating flow caused by the propeller. Seven vertically spaced outlets of 6 mm external diameter were integrated along the column which allows suspended sediments to be collected during the tests.

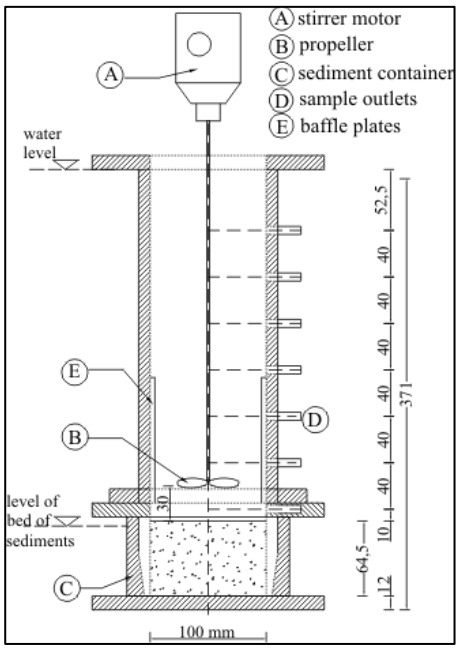


Figure 5.1. A systematic diagram of an erosionmeter (adapted from *Seco et al*. 2014).

Two erosionmeters were run simultaneously for these tests. Both erosionmeters used a different motor to operate the propeller; an Ika Laboratechnik, Eurostar 40 digital, with a speed range of 30 to 2000 rpm and an Ika Laboratechnik, RW-20.n motor, dual speed, with two different speed range; speed range I, 60 to 500 rpm and speed range II, 240 to 2000 rpm. Both motors have been calibrated before the tests started following Camuffo (2001) and van Rijn (1984) in order to obtain estimated values of shear stress produced by the propeller at a certain motor speed.

5.2 Experimental procedures for erosion test

5.2.1 Sediment bed preparation

The test consists of two parts; consolidation phase and erosion phase. The consolidation phase is defined as a period of time where the bed is undisturbed and exposed to a constant level of shear stress. This phase is used to demonstrate the bed behaviour during dry weather period (Seco *et a*l. 2014). The erosion phase is defined as a period when the bed was exposed to increasing shear stress which simulating the start of storm events (Seco *et a*l. 2014) and the bed eroded during the process was studied.

For the experimental works, a homogenous sediment mixture was prepared by mixing 80% of clean sand and 20% of crushed olivestone by dry mass.This was due to the established knowledge that the solids in sewers are 80 to 90% dominated by inorganic materials (Arthur *et al*. 1999; Ashley *et al*. 2004). Total dry mass of sediment for each test was 560 g. The sand was used to represent inorganic fraction that presents in the sewer sediment, and crushed olivestone was used as a substitution for the main source of easily biodegradable organic matter in the system. Both materials provide similar particle size fraction that was found in real sediment as fine sediments (<100 μm) has been reported to dominate the suspended solid phase (Ashley *et al*. 2004).

5% diluted wastewater by volume (5% of wastewater was diluted with 95% of tap water) was used in the experiments. The addition of wastewater in the system provides microorganisms needed to start any biological activities in the system. 5% concentration was used to simulate the conditions commonly found in marine and river system, as reported by Seco *et al*. (2016).

The device was half filled with diluted wastewater and was allowed to mix at high speed (700 RPM) for 3 minutes. After the mixing, sediment mixture was poured from the top in a quick manner to avoid any loss and to promote a homogeneous mixture of the sediment in the column. Any excess sediment

during pouring step was cleaned using distilled water and was added to the column. The column was then filled with more 5% diluted wastewater until full.

The motor speed was reduced gradually from the highest speed of 700 RPM until it arrived at the bed shear stress established for the consolidation phase which was at 0.15 N/m^2 that corresponds to the motor speed of 150 RPM. The motor speed was reduced following a step reduction from 700 RPM, 600 RPM, 500 RPM, 350 RPM and 150 RPM, with 3 minutes was allocated for each change. A cling film was used to cover the top of the column to avoid any materials loss due to the constant aeration in the system. This procedure can be summarized in Figure 5.2.

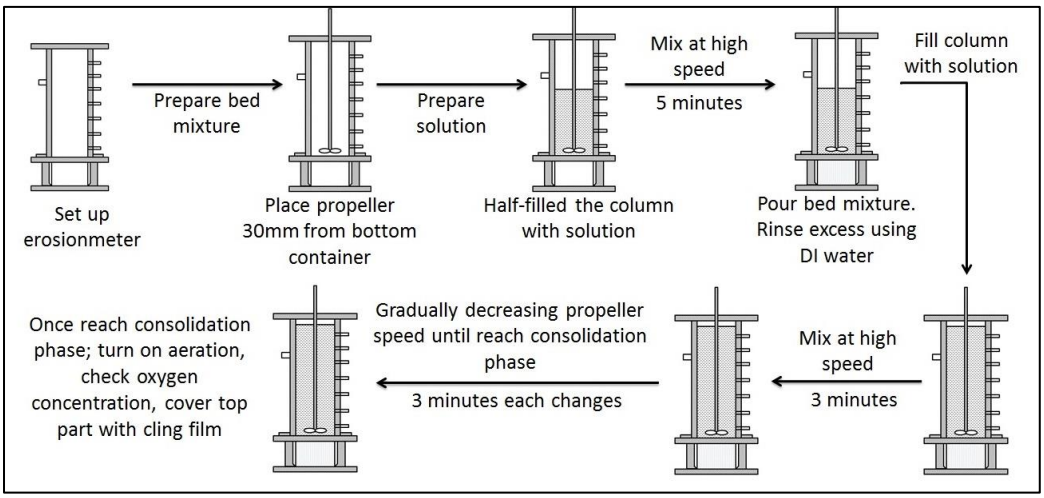


Figure 5.2. A summary of erosionmeter set up protocols.

All tests were conducted in aerobic conditions as aeration was provided in the column using an aquarium pump attached to aeration stone and dissolved oxygen concentration was kept at 80 to 90% air saturation at all times. The aerobic condition was maintained in order to provide sufficient oxygen concentration to penetrate into the sediment. All the tests were carried out in a temperature-controlled laboratory at 20 ºC ± 1 ºC. Temperature and dissolved oxygen concentration were regularly monitored during each test.

5.2.2 Bed sampling during consolidation period

During the consolidation phase, bed samples were taken regularly for further analysis of the organic matter, namely protein and reducing sugar. Bed samples were collected to gather evidence of biofilm growth in the system during consolidation phase. There has not been any bed sampling reported by other researchers, thus, sampling of the bed in a running erosionmeter test was a novel idea to determine and observe biofilm growth on the bed surfaces and changes of organic matter concentration of the bed.

The first method sampling was using a long pipette as shown in Figure 5.3. Samples were collected from a designated sampling point, where the accessible area of the column was measured and divided into six equal points to obtain the same distance between each sampling points as shown in Figure 5.4. Due to the angle of which the erosionmeter was set up, Erosionmeter 1 has 80 mm distance from each sampling point while Erosionmeter 2 has 88 mm distance from each sampling point. The sampling points were located near the wall, in order to avoid the propeller during the procedure. The tip of the pipette had a diameter of 2 mm, thus bed surface area collected for one sampling point was 3.142 mm^2. The sampling was deemed representative of the whole bed, as it covers different locations of the bed.

The sampling was done alternately, namely sampling point 1, 2 and 3 for the first sampling session and sampling point 4,5 and 6 for the next. 4 mL of samples were collected at each sampling point, which was then diluted twice (dilution factor of 3). 12 mL of 100% concentration of fresh wastewater was added to both erosionmeters after the sampling to replenish the nutrient in the system and to maintain the same water volume. Samples were taken once every day for 66 hours consolidation period tests and once every other day for other duration of the consolidation period.

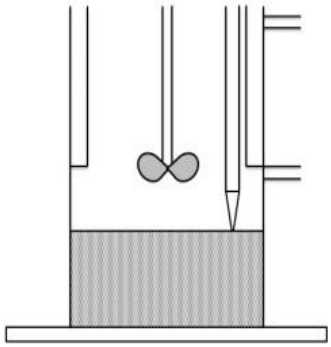
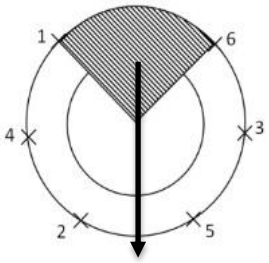


Figure 5.3. Sampling method using long pipette at 6 different sampling point.



Motor subject area that cannot be accessed

Figure 5.4. Six sampling points as viewed from the top of the column.

This method proved to be disruptive to the bed as it created large holes on the bed surfaces which disrupts the bed surface and may also destroy any biofilm on the bed surfaces as shown in Figure 5.5.

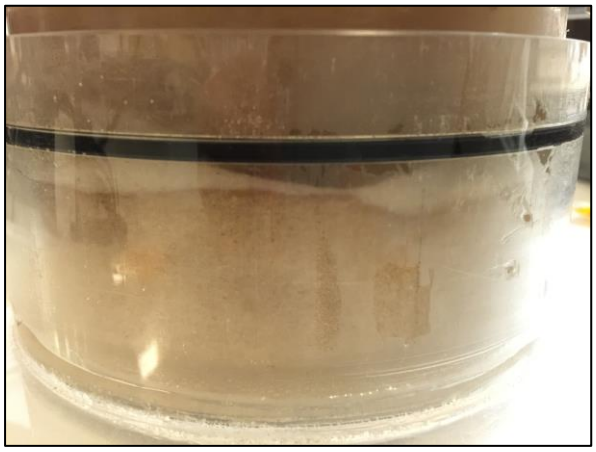


Figure 5.5. The hole created during bed sampling.

A less intrusive sampling method was developed to address this issue. The sampling was done using the same device (long pipette) but was only alternate between two sampling points at opposite end to minimise any damage to bed surfaces. Fresh wastewater was added to replace extracted samples. However, this method was also evaluated to disrupt the bed from large hole observed after the procedure, and thus, another method was developed.

The third bed sampling method involves of slowly draining the erosionmeter and scoped out the samples from the bed using a spatula. The water was then poured back into the erosionmeter in a very slow and careful manner. This method was able to obtain the bed samples without creating any large holes on the bed surfaces, but, the action of draining and pouring the suspended liquid from and into the erosionmeter meter has higher risks of disrupting the bed and also the loss of materials during the process.

As all the bed sampling method proved to do more harm than good to the bed, thus, the procedure was stopped entirely after a few trials. The rest of the sampling was taken from the suspended solid phase, at vertical sampling point number 4, as it is approximately the middle point of the column and thus allowing the assumption that the sample taken could represent the whole system.

5.2.3 Erosion phase

Once the consolidation phase ended, erosion phase was started immediately. For the erosion phase, the erosionmeter was drained, and the suspended liquid was kept for further analysis. The erosionmeter was then filled very slowly with tap water until the fourth vertical sampling point mark and was then drained again. This process was repeated for four times to ensure that there were no more suspended solids in the column. The column was then filled with tap water, and shear stress was increased by increasing the motor speed.

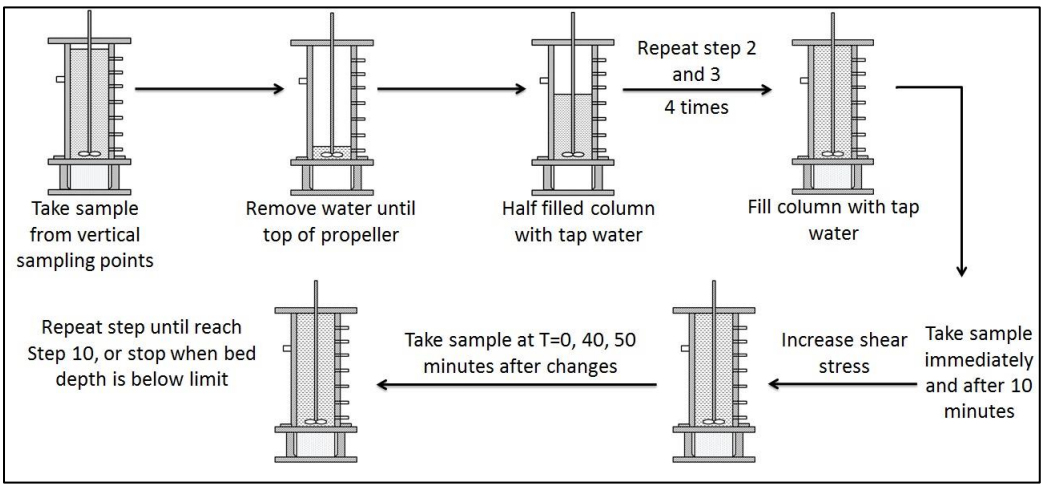


Figure 5.6. The summary of erosion phase experimental procedures.

During this phase, the propeller speed was increased in a stepwise manner and suspended sediment samples were collected from vertical sampling ports for further analysis. Nine steps were introduced in the system as shown in Table 5.1. Each step lasts approximately for 50 minutes, where suspended sediment was collected at every 5, 40 and 50 minutes after each change. 50 minutes were deemed sufficient to allow homogenous sediment concentration in the water column for a representative sampling of the eroded bed (Seco *et al*. 2016; Tait *et al.* 2003b). These procedures can be summarized in Figure 5.6 and Figure 5.7. The duration of the time set ensured that the system attained a steady concentration at the end of each step.

Table 5.1. Shear stress step increase applied during erosion phase.

Shear stress steps (N/m^2)									
1 (consolidation phase)	2	3	4	5	6	7	8	9	10
0.15	0.34	0.45	0.58	0.78	1.00	1.30	1.50	1.70	1.89

50 mL of samples were collected at 5 and 50 minutes and were analysed for TSS and VSS following Section 3.2.6 while 70 mL of suspended sediment was collected at 40 minutes and were analysed for TSS and VSS (50 mL), protein and reducing sugar (10 mL, 5 mL for each) and particle size analysis (10 mL). Samples collected at 40 and 50 minutes were assumed to be homogeneously mixed as suspended solids were allowed to mix for long period of time after the changes in propeller rotation was made.

The volume of suspended sediment collected at vertical sampling point differs from each point as it depends on the distance between the vertical sampling points to the bed surfaces. This means that only a small volume of suspended sediment was collected from vertical sampling point closest to the bed while the larger volume of suspended sediment was required from the farthest vertical sampling point. Table 5.2 outlines the details of the sampling.

Table 5.2. Volumes of suspended sediment collected at vertical sampling points. The vertical sampling points were numbered from the bottom (vertical sampling number 1 was the bottom, and vertical number 7 was at the top).

Vertical sampling point	1	2	3	4	5	6	7
Volume collected for 50 mL total sample (mL)[1] for T = 5 and 50 minutes.	4.9	6.6	6.6	6.6	6.6	6.6	12.0
Volume collected for 70 mL total sample (mL)[2] for T = 40 minutes.	6.8	9.3	9.3	9.3	9.3	9.3	16.8

[1] Analysis for TSS and VSS.

[2] Analysis for TSS, VSS, protein, reducing sugar and particle size analysis.

The suspended sediments collected from each sampling point was homogeneously mixed and regarded as one sample that represents the whole system. After samples were collected, tap water was added to the column to replace the volume of liquid taken. Dilution factor, F_D of the water was then calculated using Equation 5.1. F_D was used to calculate suspended sediments concentration of diluted samples, $C_{SS,i}$ using Equation 5.2.

$$F_D = \frac{V_{cum+} V_i}{Vw} \quad \text{(Equation 5.1)}$$

Where; V_{cum} is cumulative volume of water extracted (L), V_i is sample volume collected at step i (L) and V_w is water volume in the column (L).

$$C_{SS,i} = C_{SS} * F_D \quad \text{(Equation 5.2)}$$

Where; C_{SS} is suspended sediment concentration before dilution (g/L) and $C_{SS,i}$ is suspended sediments concentration of diluted samples at step i (g/L).

Once the phase ended, the erosionmeter was emptied. The sediment surface left was observed and analysed. Suspended sediment drained was kept at 4^{o}C conditions for a week to allow suspended sediments to settle before further analysis. This test can be simplified by Figure 5.7.

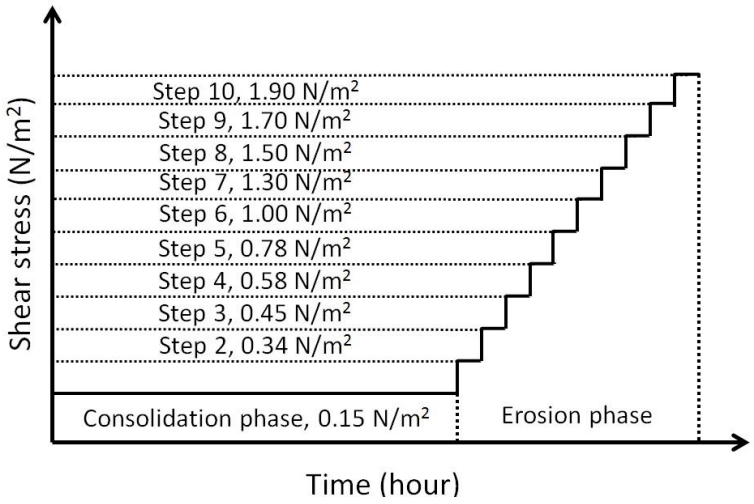


Figure 5.7. A summary of erosion tests conducted. Consolidation phase runs for a designated period of times followed by erosion phase, where the bed was subjected to increasing shear stress steps.

Average erosion rate, q_i during each step was calculated using Equation 5.3, following Seco *et al*. (2014).

$$q_i = \left(C_{SS,i+1} - C_{SS,i}\right)\frac{V_w}{A_b * \Delta t} \qquad \text{(Equation 5.3)}$$

where; q_i is average erosion rate during time step i (g/m^2/s), $\left(C_{SS,i+1} - C_{SS,i}\right)$ is suspended sediment concentration difference between sample i+1 and i (g/L), A_b is area of the sediment bed (m^2) and Δt is duration of the time step (s).

Eroded bed thickness at step i, e_i was obtained using Equation 5.4 as shown below;

$$e_i = \frac{\left(C_{ss,i+1} - C_{ss}\right)(V_w)}{\frac{1-p}{\frac{\rho_b}{A_b}}} \qquad \text{(Equation 5.4)}$$

Where; p is bed porosity (-) and $\rho_b$is bed density (kg/m^3).

Next, value of cumulative eroded bed thickness, e_{cum} was determined by Equation 5.5;

$$e_{cum} = e_i + e_{i+1} + \cdots + e_{i+n} \qquad \text{(Equation 5.5)}$$

The erosion phase was conducted as a simulation of flow behaviour towards bed sediment at the start of storm events (Seco *et al*. 2014) .

5.3 Erosion tests experimental conditions

Different periods of consolidation phase were used in this study to simulate various periods of dry weather that have been reported in the literature (Seco *et al*. 2014; Tait *et al*. 2003b). Five different consolidation phases were used; 66, 118, 166, 312 and 380 hours. Each consolidation phase except for 118 hours had tests that were conducted with 5% diluted wastewater and tap water to compare the results obtained for systems with and without biofilm presence. Other than that, some consolidation phase also has tests that were conducted using sterilised materials, to understand whether sterilising materials have any effects on the results and whether it is necessary for this study. Table 5.3 will further summarize all tests that have been conducted.

Table 5.3. Summary of all tests that have been conducted.

Test number	Consolidation phase (hour)	Column composition	Bed sampling method
1	66 (2.75 days)	20% olivestone 80% clean sand 5% diluted wastewater	6 points sampling method
2			
3			
4		20% olivestone 80% clean sand Tap water	No bed sampling, samples obtained from suspended solids phase
5		20% olivestone 80% clean sand Sterilised tap water	
6		20% sterilised olivestone 80% clean sand Tap water	
7	118 (4.92 days)	20% olivestone 80% clean sand 5% diluted wastewater	6 points sampling method
8			
9			

10	166 (6.92 days)	20% olivestone 80% clean sand 5% diluted wastewater	2 points sampling method
11			
12			Draining method
13		20% olivestone 80% clean sand Tap water	No bed sampling, samples obtained from suspended solids phase
14		20% olivestone 80% clean sand Sterilised tap water	
15		20% sterilised olivestone 80% clean sand Tap water	
16	312 (13.0 days)	20% olivestone 80% clean sand 5% diluted wastewater	
17		20% olivestone 80% clean sand Tap water	
18	380 (15.83 days)	100% sand 50% diluted wastewater	2 points sampling method
19		100% sand 50% diluted wastewater	

5.4 Disruptive sampling of sediment bed

As bed sampling has been proven to be disturbing the bed during the test, another test was developed to try and address this issue. The main concept for these tests is to grow biofilm in a similarly controlled condition as the erosion test during consolidation phase. Six smaller scale reactors were run using a pre-calibrated flocculator for 312 hours, and one reactor will be taken after some period of time for further analysis of biofilm growth on the bed surfaces. These tests will be used to find evidence of biofilm growth on sediment deposit surfaces when the bed was consolidated.

5.4.1 Experimental setup

The main device used in this test was a pre-calibrated flocculator (Fisher Scientific, SW6, USA). The device comes with six propellers (L 63.4mm, H 25.0 mm, W 1.5mm), and has motor speed ranging from 25 to 250 RPM. Six tall beakers were used, each with heights of 180 mm and outside diameter of 95 mm.

The surface area of the bed for each beaker was 6362 mm^2, which corresponds to approximately 80% of the total surface area obtained from erosionmeter tests. Six equally spaced baffles of 0.2 mm thickness were fitted vertically in the beaker to reduce radial flow and promote homogenous mixing of materials. One beaker was provided for each propeller, and all six propellers were controlled by a motor. An aquarium pump with aeration stone was also installed on each beaker to provide aeration in the system. Figure 5.8 illustrates the setup for these tests.

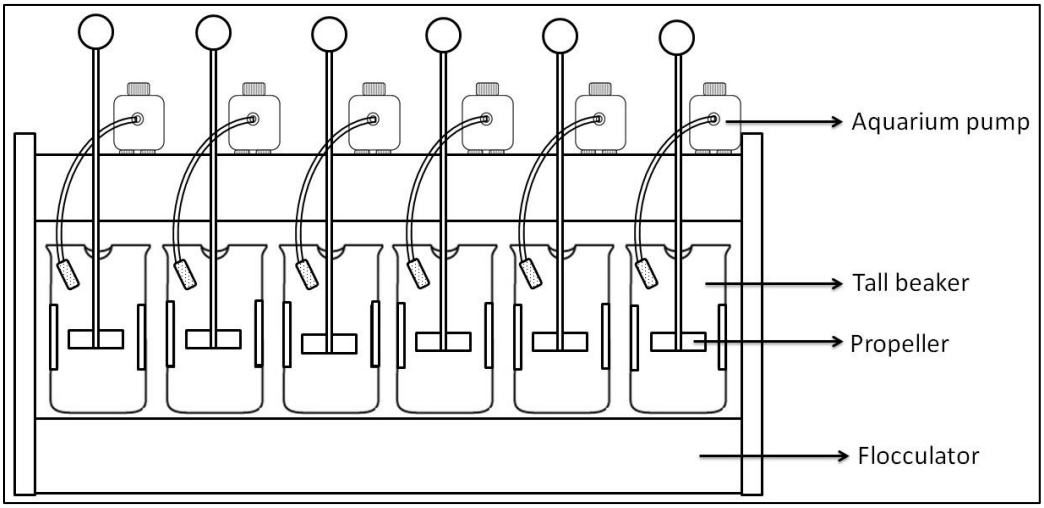


Figure 5.8. Experimental setup for disruptive sampling of biofilm tests.

5.4.2 Flocculator calibration

The flocculator was calibrated to determine shear stress values desired for the bed from known speed of the propeller, which will be referred as the angular velocity of the propeller from now on. The calibration was carried out using ten different sizes fraction of homogenised sand particle as shown in

Table 5.4, where d_{min} is minimum particle diameter, d_{max} is maximum particle diameter and d_{av} is referring to average particle diameter.

Table 5.4. Grain size fractions used in the calibration procedure.

Sieve size (mm)		
d_{min}	d_{max}	d_{av}
0.090	0.150	0.120
0.150	0.300	0.225
0.212	0.355	0.284
0.500	0.600	0.550
0.600	0.710	0.655
0.710	0.850	0.780
0.850	1.000	0.925
1.180	1.400	1.290
1.400	1.700	1.550
1.700	2.000	1.850

These samples were made of a homogenous non-cohesive material to provide a different particle parameter to each size fraction used. This sedimentological particle diameter, D_f is calculated using Equation 5.6, following Camuffo (2001).

$$D_f = d_{av}\left(\frac{\rho_r g}{v^2}\right)^{1/3} \quad \text{(Equation 5.6)}$$

Where; d_{av} is average particle diameter (m), ρ_r is relative density of grain density over water density (-), g is acceleration due to gravity (m/s^2), and v is water kinematic density (m^2/s).

Grain density was assumed to be 2650 kg/m^3 as the material was mostly made of quartz and silicate (Camuffo, 2001). This value was also obtained when the density was measured using density meter (Deante, ES-120D, China).

Each beaker was filled with each different sizes of sediment fraction until 20 mm mark. The sediment was then pushed together using a spatula to create a bed with an even surface. Tap water was then poured slowly along the wall

to avoid any disturbance to the bed. The propeller was fixed 30 mm from bed surfaces.

The angular velocity of the propeller was started at the lowest motor speed settings, and the speed was gradually and slowly increased until the moment when the sand particle was observed to experience a continuous movement on the bed surfaces. The continuous movement is defined when 5% of the top layer of the sediment bed is moving under constant shear stress by rolling, sliding and salting for one-minute duration. Salting is characterised when the particle experience jumping motion on the bed surfaces. These movements can be observed physically during the test.

Once continuous movement of the bed particles was detected, the dial readings of the propeller were taken using tachometer, and critical shear stress value was then calculated using modified Shield's criterion following van Rijn (1984). Three tests carried out for each ten sediment samples for each beaker. Each test was carried out by five different personnel with three independent observations to avoid bias in determining the threshold of sediment movement.

The critical Froude Number, F_{cr} was calculated based on D_f value obtained following different sets of empirical equations following van Rijn (1984);

$D_f \leq 4,\ F_{cr} = 0.109 D_f^{-1}$ (Equation 5.7)

$4 < D_f \leq 10,\ F_{cr} = 0.140 D_f^{-0.64}$ (Equation 5.8)

$10 < D_f \leq 20,\ F_{cr} = 0.040 D_f^{-0.1}$ (Equation 5.9)

$20 < D_f \leq 150,\ F_{cr} = 0.013 D_f^{0.29}$ (Equation 5.10)

$D_f > 150,\ F_{cr} = 0.055$ (Equation 5.11)

Each one of these equations corresponds to a different regime in which the sediment movement started to occur. These five van Rijn equations represent laminar, transition and turbulence flow of the motion. Critical Froude number played an important role in this calibration, as sediment

movement only started when the critical value was achieved. Once critical Froude number values were calculated, critical shear stresses τ_{cr} was calculated using Equation 5.12.

$\tau_{cr} = F_{cr}\rho_r g d_{ch}\rho_f$ (Equation 5.12)

Where; ρ_f is fluid density (kg/m^3)

A plot of critical shear stress against average dial reading was created to show the relationship obtained between these two parameters.

5.4.3 Disruptive tests experimental procedures

The bed composition used in this study was the same as the erosion test; 20 % of crushed olivestone and 80% of cleaned sand by dry mass. Total dry mass of mixed bed sediment for each beaker was 200 g, which corresponds to a bed height of 20 mm. Each propeller was fixed 30 mm from the bed surface.

The device was half filled with 5% diluted wastewater by volume and was mixed at high propeller speed (250 RPM) for 3 minutes. Sediment mixture was then poured from the top and was allowed to settle. Excess sediment during pouring process was rinsed using distilled water and was added to the beaker. Each beaker was then filled with 5% diluted wastewater by volume.

The motor speed was then reduced gradually, with 50 RPM reduction applied after 3 minutes mixing time. Aeration was started when the motor speed reached the desired bed shear stress for the bacterial growth, which was at 0.15 N/m^2 that corresponds to the motor speed of 60 RPM. A clear film covered each beaker to avoid any loss of material due to aeration.

Aeration was provided near the water surface, with the assumption that the condition was aerobic throughout the entire beaker due to its small volume and homogenous mixed of flow by the propeller. All tests were carried out in a temperature-controlled laboratory, 20 ºC ± 1 ºC. Set up procedure for this test can be illustrated in Figure 5.9.

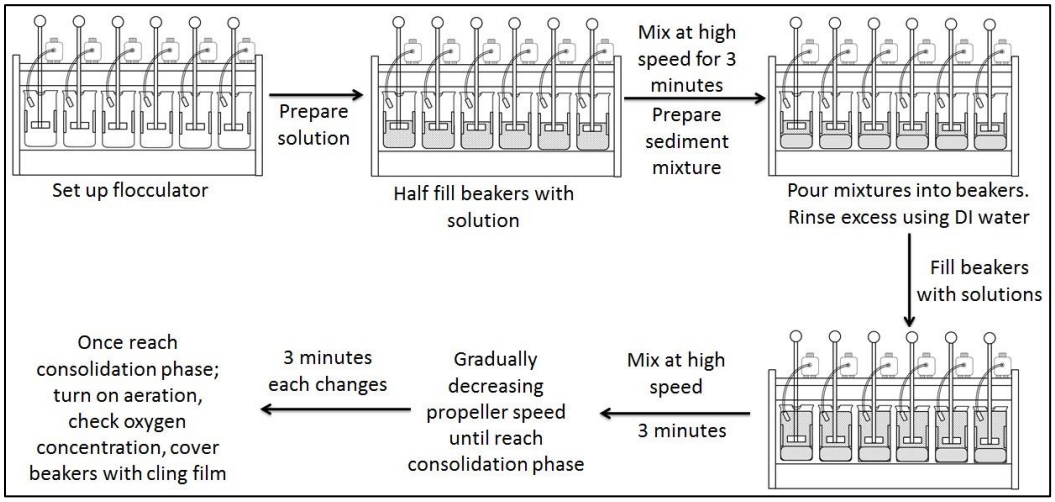


Figure 5.9. Summary of the procedure for disruptive sampling of biofilm test.

The first sample was obtained from the first beaker, which was taken at time 29 hours after the test was started. This was due to previous knowledge where the biofilm was visually visible in the pipe test after 24 hours period. 20 mL of suspended sediment sample was taken using a pipette for further analysis of particle size analysis. Another 10 mL of suspended sediment sample was also collected for protein and reducing sugar analysis.

Once suspended samples were taken, extra suspended sediment in the beaker was collected using a large syringe. The suspended sediment was removed until water level reached below propeller. Aeration was stopped, and the propeller was raised. The beaker was then removed from the flocculator onto a flat surface where leftover suspended sediment was removed using a pipette until bed surfaces were visible. This was done in a very careful manner as to avoid any disturbance to the bed. Suspended sediment collected was kept in 4^{o}C condition for sample preservation before further analysis of TSS and VSS, following Standard Method (APHA *et al*. 1999).

Once all suspended sediment was removed, bed surfaces were observed and visually inspected for any evidence of biofilm growth. The bed sample was collected using a clear glass tube with inner diameter of 9.5 mm.

Sampling was done at three different sampling points as illustrated in Figure 5.10.

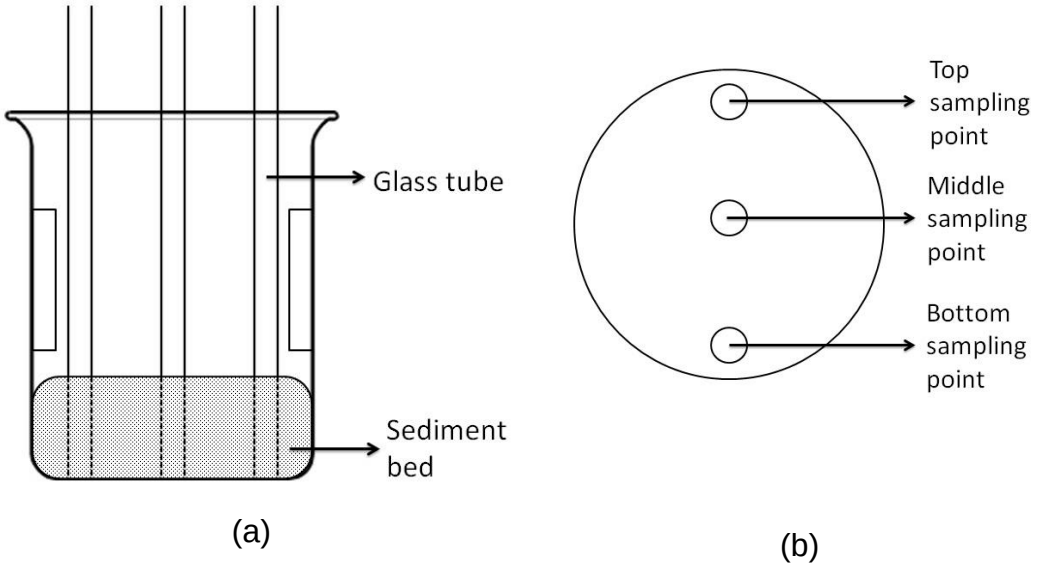


Figure 5.10. Bed sampling method using clear tubes with 9.5 mm inner diameter (a) and sampling site for the test, as viewed from above (b).

The bed samples collected were then diluted using distilled water resulting in 15 mL sample volume for further analysis of protein and reducing sugar of the bed.

10 mL of suspended sample was taken from the rest of the beaker and was replaced with 10 mL of fresh wastewater to replenish nutrient in the system and maintaining the same water volume. The samples were collected for further analysis of organic matter concentration and particle sizes. The dial reading of each propeller was taken using tachometer, and the water temperature was also monitored. The next samples were taken at time 70 hours, 142 hours, 214 hours, 262 hours, and 312 hours. These sampling protocols can be summarized in Figure 5.11.

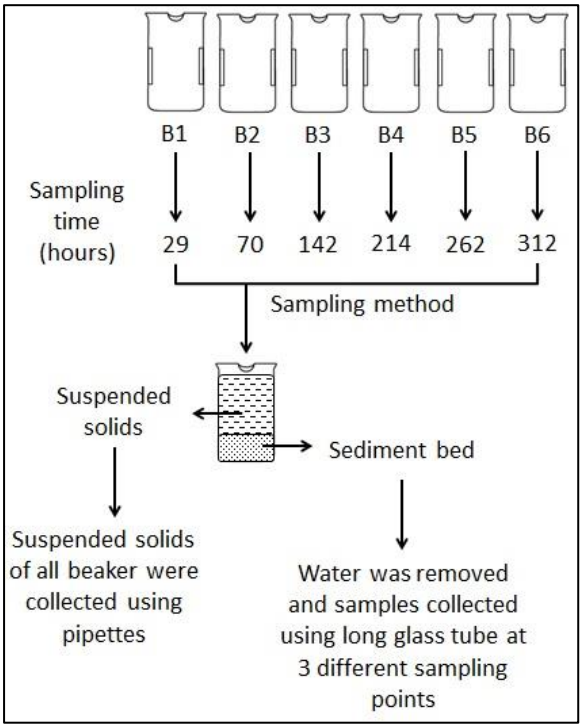


Figure 5.11. Sampling procedure for the disruptive sampling of biofilm tests.

These sampling procedures were conducted on tests with 5% diluted wastewater while only 2 beakers were used for tests with tap water. This was due to limited availability of crushed olivestone. For tests with tap water, suspended solids sample were obtained once every day from both beakers and bed sample for beaker 1 was obtained at T = 150 hours while bed samples for beaker 2 were obtained at T = 312 hours.

5.4.4 Experimental conditions

To understand biofilm growth under two different conditions, two tests were conducted in this study. Both tests were run for 312 hours, where one test was conducted using 5% diluted wastewater while the second one was conducted using tap water. These two tests were conducted to compare bacterial growth under two different conditions where abundant nutrient and microorganisms were provided in the first system for the bacterial growth, and none were provided for the other system.

5.5 Analysis

5.5.1 Sample preparation for organic matter analysis

The samples collected from the bed and suspended solids phase were analysed for protein and reducing sugar as a method to quantify biofilm growth in the system. The sample contains various substances with organic matter from wastewater, tap water and crushed olivestone which may contribute to a higher protein and reducing sugar concentration in the sample.

Samples obtained were not analysed directly for the organic matter and subjected to pre-treatment procedure due to several reasons. First, crushed olivestone and sand may interfere with the absorbance measurement due to its large size particle. Second, biofilm was assumed to grow on the surface and perhaps in the bed thus a method was needed to separate biofilm from the sediment particle. The last reason is to eliminate or minimizing influence from materials other than the biofilm in the sample.

In order to resolve these issues, the sample was prepared using a newly developed sample preparation method for these tests. The method consists of multiple stages of rinsing and bead beating of the sample. All samples were done in triplicate for a better accuracy in the result obtained.

First, 1 mL of homogeneously mixed sample was poured into 3 different microtubes. The samples were then centrifuged (Hettich, D-78532, Germany) at 1000 RPM, or equivalent to 94 G for 5 minutes. The conversion of the microcentrifuge speed in RPM to relative centrifugal force, G was calculated following Equation 5.13;

$$G = (1.18x10^{-5})\, R_c\, S \qquad \text{(Equation 5.13)}$$

Where; R_c is radius of the rotor (cm) and S is centrifuge speed (RPM)

After the centrifuge, supernatants were removed and replaced with distilled water. The sample was mixed gently to allow the protein from possible

biofilm formation to re-suspension. This process was repeated twice to obtain a purer sample. Next, the samples were moved to another tube that was ¾ filled with clean sand with particle sizes from 150 to 300 um.

The mixture was then subjected to bead beating using a vortex genie (Scientific Industries, SI-0236, US) for 15 minutes at 6 RPM. This step was done to rupture bacteria cell walls and to separate biofilm from the bed particles. Sand was selected as the beads as it contains a low concentration of any organic matter as shown previously in Section 3.4. Bead beating was chosen as cell disruption method as it is inexpensive, able to process many samples at the same time with minimal risk of cross-contamination between samples, safe as the method does not release any harmful substances and efficient enough to disrupt a very small volume of sample.

After the bead beating processes, the sample was centrifuged at 94 G for a minute in order to separate the sand from the supernatant. The supernatant was then removed to another microtube and was analysed for protein and reducing sugars.

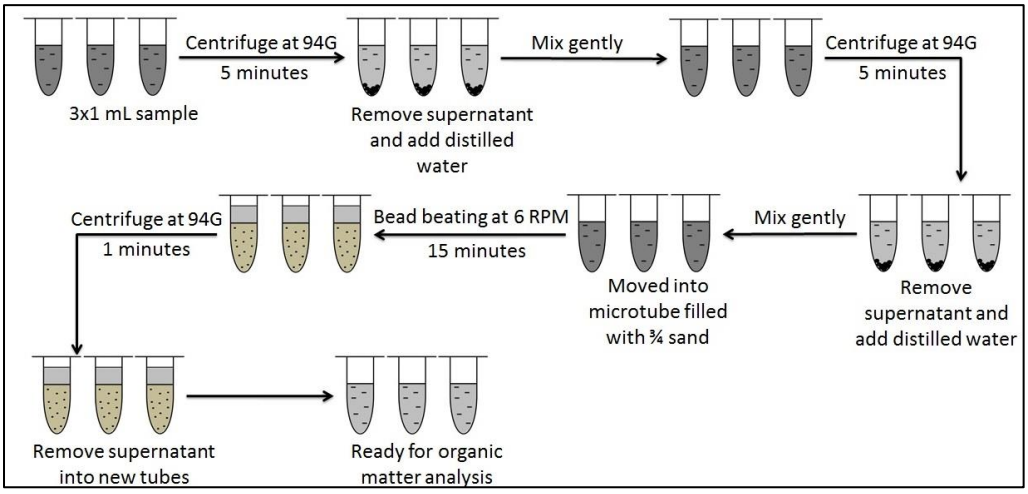


Figure 5.12. Sample preparations method for protein and reducing sugar quantification (Method A).

This method was predicted to be able to quantify all organic matter in the sample with minimal influence from other materials. This procedure can be

demonstrated in Figure 5.12. This method will be referred as Method A in this study.

5.5.2 Development of sample preparation methods

All samples were prepared following the method described in Section 5.5.1. However, the method used provides some concerns that need to be addressed to ensure that the results obtained were reliable. Some issues that have been raised are whether the method was able to eliminate or minimize the influence of crushed olivestone in the sample.

Other than that, bead beating for 15 minutes seems to be too harsh on the sample as protein may overheat and coagulate. Another issue that was raised was the use of the vortex genie for the bead beating. This is due to the movement of the vortex, as vortex genie provides a horizontal movement which resulted in reduce disruptor efficiency as compared to disruptor genie.

To address these issues, two more sample preparation methods were developed and tested. The first one involves filtration using microfibre filter syringe with pore size 0.45 um and diameter of 25 mm (Whatman, 6894-2504, Germany). This method allows complete removal of crushed olivestone and sand in the sample. The sample was then analysed for protein and reducing sugar directly after the filtration treatment. This method will be referred as Method B in this study.

The second method was fairly similar to original method (Method A) as it involves multiple stages of rinsing and bead beating of the sample. This method will now be referred as Method C in this study. The method is summarized in Figure 5.13.

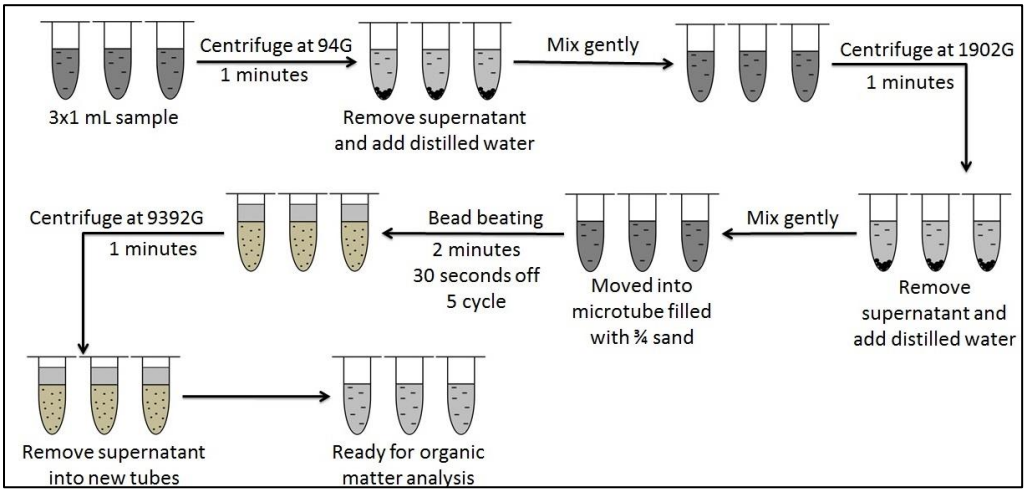


Figure 5.13. Summary of sample preparation method (Method C).

The main difference between Method A and Method C were centrifuge speed, centrifuge time period and usage of disruptor genie (Scientific Industries, US) instead of vortex genie.

The first centrifuge phase was done at the same speed as Method A but with a decrease in centrifuging time as 1 minute was deemed sufficient for a 1 mL of sample. The speed for second centrifuge stage was increased to ensure all suspended organic material in the sample was collected.

Bead beating was done alternately to avoid protein in the sample to coagulate from overheating in the process. Using disruptor genie was also help with the cell wall disruption due to its random movement which increases cell wall rupture efficiency. The last centrifuge phase was conducted at a very high speed to ensure all suspended materials were settled and to easily separate supernatant from the sand particle.

5.5.3 Particle size analysis of samples

For a better understanding of the potential transformation processes of the bed sediment, suspended samples obtained during erosion phase were subjected to particle size analysis. The principle of this method is to measure material particle size using laser diffraction. This analysis will provide an insight into sediment and biofilm growth behaviour when subjected to

different level of shear stresses. This method will also be able to give more information on the layering of the bed sediment which was thought to be mainly due to particle size, density and properties such as the settling velocity of the particles.

The laser diffraction particle size analysis is a method that analysed particle sizes of the sample by measuring variation in light intensity from laser beam passing through dispersed materials. Small particles causing scatter light at large angle as compared to a large particle that scatters lights at a smaller angle as shown in Figure 5.14 and Figure 5.15. The angular scattering data was then used to determine particle sizes that responsible for creating the pattern using Mie theory of light scattering.

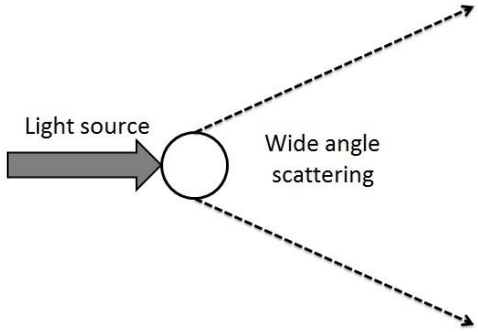


Figure 5.14. Scattering of lights for small particle material.

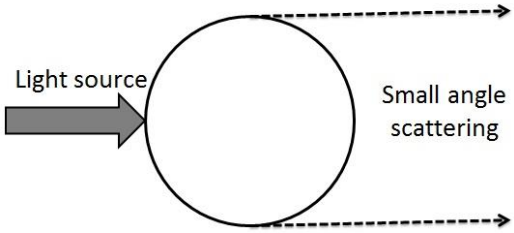


Figure 5.15. Light scattering for large particle material.

Advantages of this method are it was able to measure a large range of particle sizes, from μm to mm size range. The measurement was obtained within a minute, and repeatability of samples was allowed during measurement. The process of particle dispersion can easily be control and monitor by the software provided named 'Mastersizer 3000'. Other than that, calibration is not necessary for this method as it can be done using a standard reference material. This method is covered by ISO 133220 (2009) which further cemented the credibility of this technique.

The downsides of this method are it is expensive, and all the results were automatically calculated by the software thus making it harder to detect if there is any error in the measurement.

The main device used for this method is a particle size analyser (Malvern, Mastersizer 3000, US) as shown in Figure 5.16. The device consists of an optical bench, sample dispersion unit and instrument software. The optical bench houses a series of detectors that measured light intensity scattered by particle for both red and blue light wavelengths at various angles.

Figure 5.16. Main device used in analysing bed particle sizes (taken from Malvern, 2017).

Sample dispersion unit acts as a mixing container that ensures the sample arrives at the optical bench measurement area at a desired concentration and stable. The software was responsible for controlling the system during measurement points and calculating particle size distribution by analysing scattering data obtained from the optical bench.

To start the measurement, the device was first warmed up by changing the water in the sample dispersion unit with distilled water and mixed for 30 seconds. This step was necessary to obtain similar water temperature in the sample dispersion unit and inside the optical bench.

The system was then filled with necessary information of the particles to be analysed in the configuration window. Silica was chosen as particle type as the mixture was mainly made of sand (80%) by mass and the main component of sand was silica and quartz (Camuffo, 2001). Non-spherical shape was selected, and water was chosen as a dispersant.

As this method relied on Mie theory of light scattering, two important optical properties of the sample were needed for the system. Those two optical properties are refractive index (RI) and absorbance index (AI).

RI is defined as the speed of light in vacuum divided by speed of light in the medium or sample. AI is defined as the ability of the sample to absorb light at a specific wavelength (Malvern, 2017). The value of RI used in this study was 1.544, and 0.01 was used for AI, which corresponding to silica material.

There are two types of sample dispersion in this method. The wet dispersion was defined when the individual particle was suspended in a liquid dispersion while dry dispersion was when particles were dispersed in a flowing gas stream. Wet dispersion of sample was used for this method as wetting of the particle will lower the particle surface energy which reduces attraction forces between particles and avoids any coagulation. Other than that, the sand was deemed too heavy to be dispersed using dry dispersion method (Malvern, 2017).

Water was chosen as dispersant due to several reasons; water can provide good wetting of the sample, the sample will not dissolve in water, it does not contain bubbles, and it is transparent and have a different refractive index from the sample and thus will not affect the laser beam.

Background measurement was set for 20 seconds while sample measurement was set at 10 seconds. Background measurement measures any impurities in the distilled water before the addition of sample to ensure that the optical bench was free from any contamination that may alter the composition of materials analysed. This measurement was crucial for the

tests, as each detector was used to determine any impurities in the system which explains the longer period of time is allocated for this step. Triplicate measurement of samples was conducted for each sample.

Obscuration range is defined as the percentage loss of laser light from the materials present in the sample. It is a balancing act between not enough samples that may not representative of the bulk material and causing multiple scattering on the measurement and too many samples in the system that may block the light from passing through the materials. In this analysis, 5 % to 10% obscuration range was chosen due to the sizes of the particles that fall into fine particle category.

The material was homogeneously mixed using a stirrer in the sample dispersion unit. Stirring was needed for wet dispersion to ensure the sample was well mixed and was representative of the materials. The stirrer was set at 800 RPM, which was sufficient to keep the material well mixed without creating any bubbles in the system. This speed was also observed to be sufficient in breaking any aggregates in the sample.

Results obtained were represented as a frequency plot of volume distribution against particle size. From the graph, three main parameters can be obtained; mean, median and mode. The mean is defined as the average size of the materials, the median is material sizes corresponding to 50% of the material population while the mode is the most common particles sizes found in the sample.

The results obtained were then subjected to further analysis in order to obtain a probability density function (PDF) plot for each sample. Changes in the mean, mode, and spread of the PDF of the particle size distribution will provide a further understanding of the bed structure as it was progressively eroded during the test.

5.6 Experimental results

5.6.1 Flocculator calibration test results

Figure 5.17 illustrates result obtained for the calibration of the flocculator that was conducted in order to set up the experiments that aim to test the disruption that may be caused by sampling of the biofilm on the surface of a sediment deposit and to provide evidence of biofilm growth on the bed surfaces. The results show that a small fraction of the smallest particle sizes used in these calibration tests started to move at 45 RPM while the largest particle sizes fraction was observed to start moving at 120 RPM.

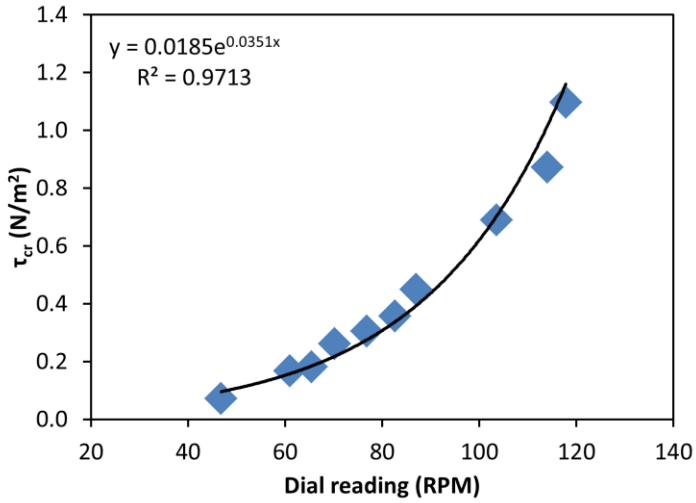


Figure 5.17. Calibration results obtained for flocculator.

An exponential trendline was constructed on the average values of readings of RPM against applied bed shear stress obtained by observation of the initial movement of single size sand particle. Interpolation of the trendline gave the value of 60 RPM for resulting shear stress of 0.15 N/m^2, which is the desired shear stress for the consolidation phase for biofilm growth. This value of shear stress stimulates the dry weather period found in sewers.

5.6.2 Comparison of protein and reducing sugar concentration obtained from different sample preparation methods

All samples presented in the results section were prepared using method A, as previously discussed in Section 5.5.1. However, several samples

collected during the erosion tests were subjected to different samples preparation for organic matter analysis, namely method B and method C from Section 5.5.2 in order to determine the sensitivity and effectiveness of each of the described method. Results of protein and reducing sugar concentration obtained using all three different sample preparation method for erosion phase samples of Test 10 (166 hours consolidation phase) and Test 16 (312 hours consolidation phase) can be illustrated in Figure 5.18. The results show organic matter concentration (mg/L) over TSS concentration (mg/L) thus resulted as a dimensionless final value. Figure 5.18 shows no large variation obtained in the protein and reducing sugar concentration subjected to different sample preparations procedure.

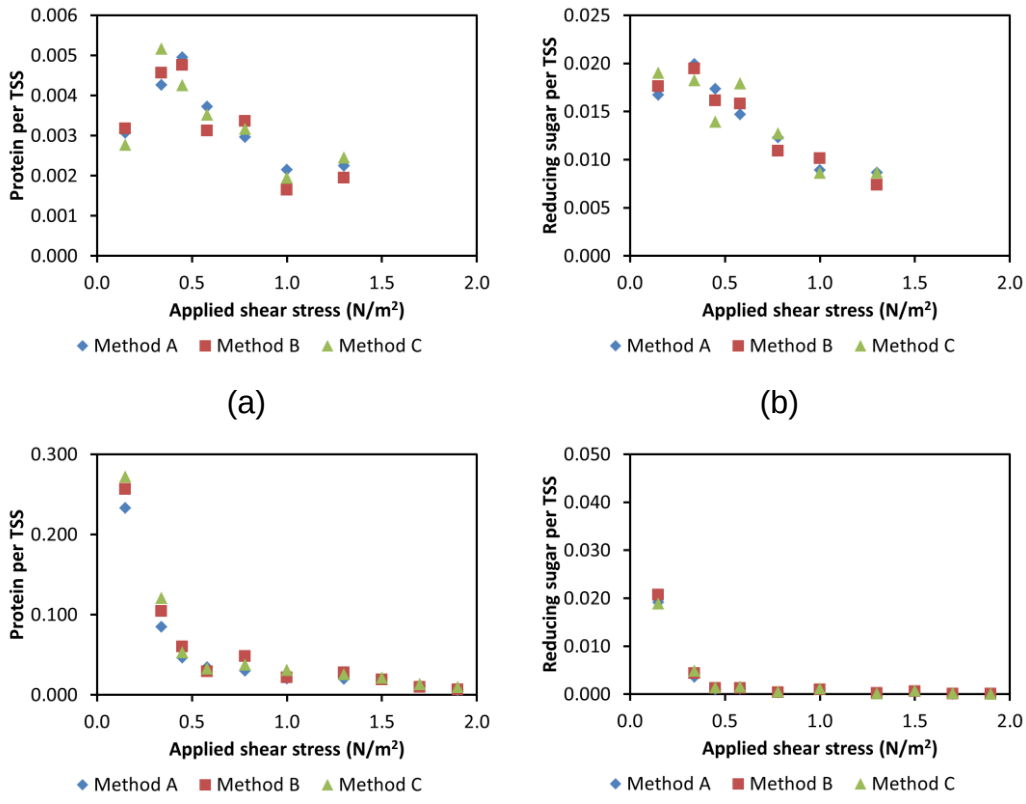


Figure 5.18. Results of protein and reducing sugar concentration for samples obtained from erosion phase of 166 hours (a and b) and 312 hours (c and d).

Results of the t test for protein and reducing sugar concentration over TSS using all three methods of samples preparation can be presented in Table

5.5 and Table 5.6. Independent t test was conducted in order to see the differences in the results yielded by each different method.

Table 5.5. t test results obtained for protein and reducing sugar concentration of Test 10 samples obtained using three different methods.

	Protein per TSS			Reducing sugar per TSS		
	Method A	Method B	Method C	Method A	Method B	Method C
Method A	-	0.8503	0.9805	-	0.9429	0.9761
Method B	0.8503	-	0.8724	0.9429	-	0.9202
Method C	0.9805	0.8724	-	0.9761	0.9202	-

Both tables show that t test values obtained were higher than p = 0.05, which statistically proved that all three methods were able to yield similar results.

Table 5.6. Results for t test conducted on protein and reducing sugar concentration for samples from Test 16 prepared using 3 different methods.

	Protein per TSS			Reducing sugar per TSS		
	Method A	Method B	Method C	Method A	Method B	Method C
Method A	-	0.8130	0.7424	-	0.9195	0.9222
Method B	0.8130	-	0.9233	0.9195	-	0.9934
Method C	0.7424	0.9233	-	0.9222	0.9934	-

In conclusion, all three sample preparation methods were able to generate similar results, which concludes the effectiveness of the methods used. Only results for samples prepared by method A will now be presented in this chapter, as this method has been applied to all the tests, thus, was able to produce a complete set of data for further analysis.

Method B and C was developed in the latter half of the study, in order to satisfy the needs to see whether organic matter concentration of samples changes if samples were subjected to different treatment methods. As these

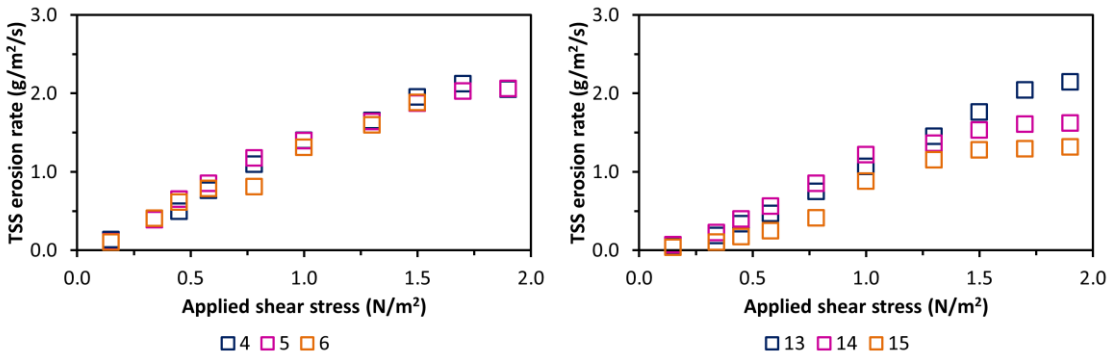
methods produced similar results to Method A, it is deemed unnecessary to include the results obtained from a sample prepared by Method B and C.

5.6.3 Comparison of different sterilising materials

In order to understand the biofilm growth on the bed, another set of tests were conducted using tap water. These tests were first conducted to see whether there are any differences of biofilm growth obtained in tests conducted with wastewater and tap water, due to the differences in microorganisms and nutrient presents for both. However, several tests were conducted with different sterilising materials for tests with tap water in order to see whether sterilising materials have any influences on the result obtained.

The results of these tests can be illustrated by Figure 5.19. The figures show results of TSS erosion rate for tests conducted with tap water with different sterilising materials for 66 and 166 hours consolidation period.

In general, no significant differences were observed between each test, with p values of 0.064 for tests at 66 hours and 0.051 for tests at 166 hours. Tests conducted at 66 hours consolidation phase show more similar TSS erosion values with applied shear stress as compared to values obtained from 166 hours consolidation phase. p values from t test conducted show values higher than 0.05, thus concluded that sterilising different material produces similar results and did not contribute to any changes in the condition of the system.



(a) (b)

Figure 5.19. Results obtained for TSS erosion rate at (a) 66 hours and (b) 166 hours consolidation phase with the different sterilised material; Test 4 and Test 13 conducted using the non-sterilised material, Test 5 and Test 14 conducted using sterilised tap water while sterilised olivestone was used for Test 6 and Test 15. Results shown were obtained from suspended solid samples taken during erosion phase.

To conclude, sterilising materials was not necessary for this work. It is important to note that sterilising olivestone have the risk to cause the material to lose its organic characteristics, and thus not advisable in this study for future references.

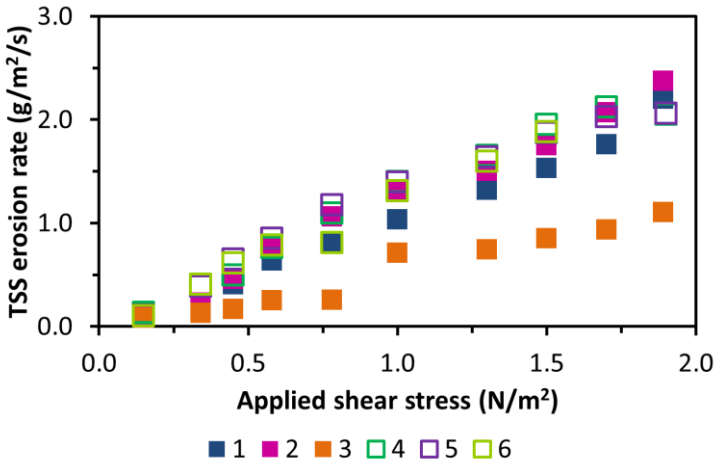
5.6.4 Comparison of results obtained for tests conducted with tap water and wastewater

As previously discussed, each consolidation phase was conducted with two sets of tests; tests with wastewater and tests with tap water. Tests with tap water served as a control, to be used as a comparison for tests with wastewater, where microorganisms and nutrient were provided in the system.

Results obtained during erosion phase for 66 hours and 166 hours consolidation phase will be used as an example to demonstrate the differences of results obtained for both conditions.

Results obtained during erosion tests

Figure 5.20 illustrates results obtained for TSS erosion rates, q_i for tests conducted at 66 and 166 hours consolidation period for both tests conducted with wastewater and tap water. Filled bulled represent tests with wastewater while hollow bullets represent tests conducted with tap water.



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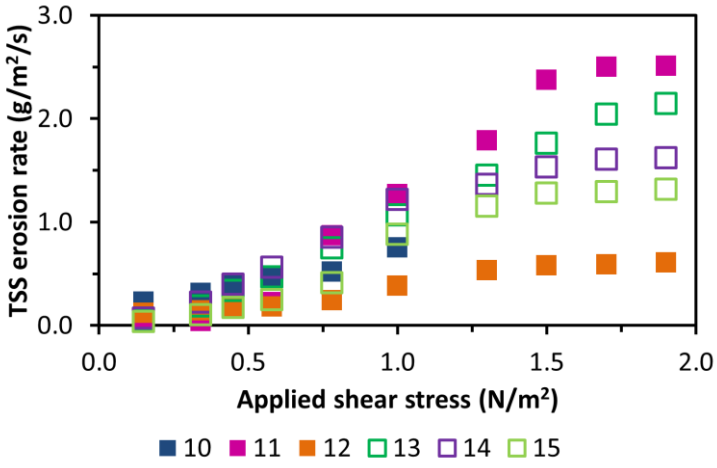


(b)

Figure 5.20. TSS erosion rate values obtained for tests conducted at (a) 66 hours; Test 1 to Test 6 and (b) 166 hours consolidation period; Test 10 to Test 15.

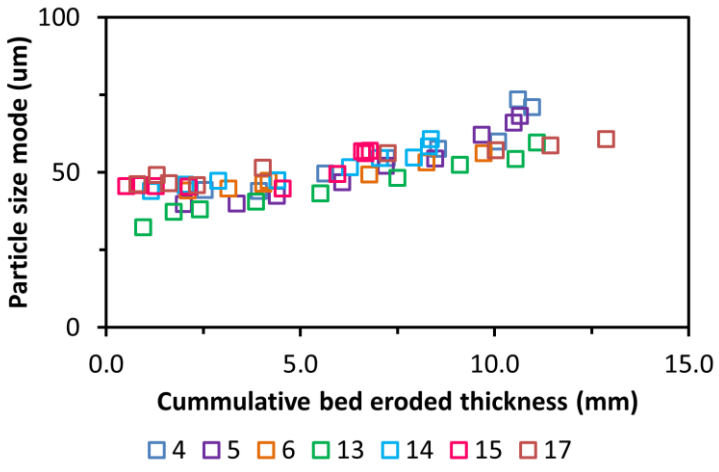
Both graphs show similar q_i values for all tests conducted with tap water while tests conducted with wastewater shows more variation in the results even though all tests was conducted at the same conditions. This may suggest possible biofilm growth in the system with wastewater, that causing more variability in the results. Although tests conducted at 166 hours

consolidation phase shows more changes in q_i values during the part of the tests with higher applied shear stress, this was speculated to be caused by the longer consolidation phase, which will be discussed in the next section of the chapter.

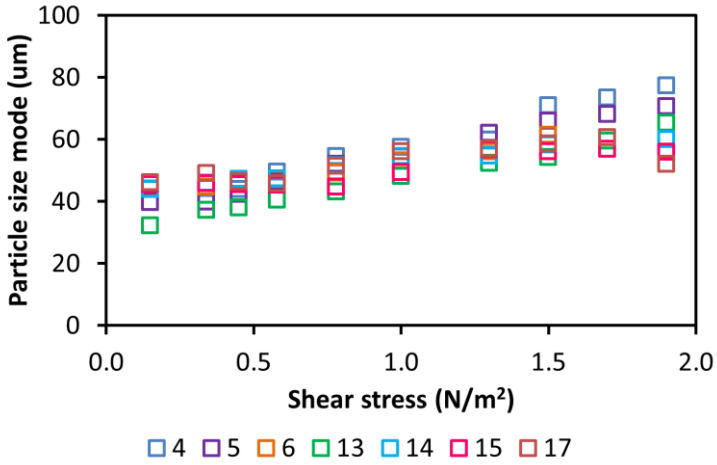
Tests with tap water for 166 hours show more changes at higher shear stress level even though statistical tests indicate that the results were similar. This may provide evidence of more biological activity in the bed at longer consolidation phase.

Results of particle size analysis

Figure 5.21 shows results of particle size mode obtained for tests conducted with tap water at different consolidation phase period. Mode values for tap water ranging from 20 to 80 μm which correspond to particle sizes of crushed olivestone. All tests show increasing modes with bed depth, which suggests that small particles settled at the top layer of the bed and was removed first with the increasing shear stress. The results also show that most of the tests achieved similar e_{cum} values, which further indicate that the bed have similar bed strength and was eroded at a similar rate when subjected to the same shear stress value.



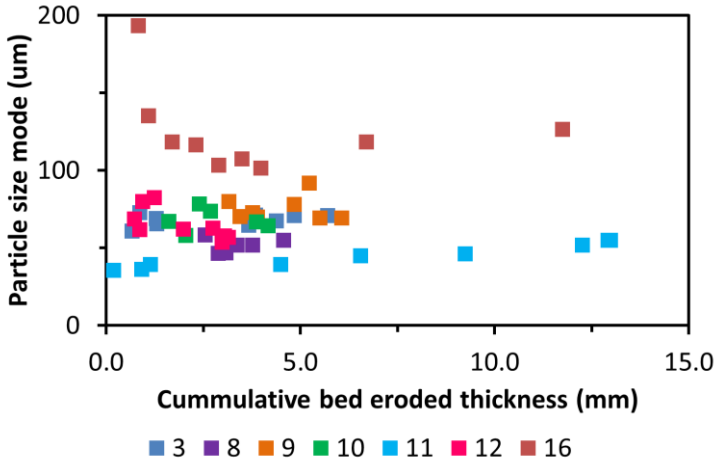
(a)



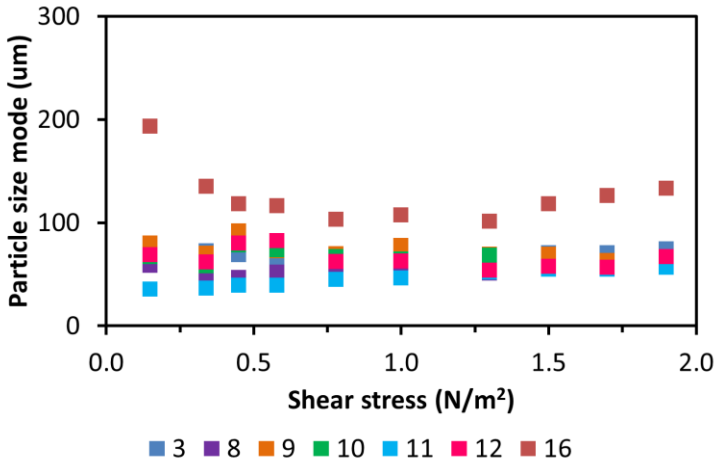
(b)

Figure 5.21.Results for particle size mode obtained for tests conducted using tap water; Test 4, Test 5, and Test 6 (66 hours consolidation phase), Test 13, Test 14, and Test 15 (166 hours consolidation phase) and Test 17 (312 hours consolidation phase).

Results of particle sizes mode for tests with wastewater can be demonstrated by Figure 5.22. From the figure, no significant trend can be observed with increasing shear stress. All tests show more variation within each test as compared to results obtained from tests with tap water. The mode values were also observed to have larger size particle as compared to results from tap water, which suggested either the erosion of larger particles in the tests conducted with wastewater or that the particle that was eroded were able to flocculate very quickly once released into the erosionmeter.



(a)



(b)

Figure 5.22. Particle size modes for tests conducted with wastewater; Test 3 (66 hours consolidation phase), Test 8 and Test 9 (118 hours consolidation phase), Test 10, Test 11 and Test 12 (166 hours consolidation phase) and Test 16 (312 hours consolidation phase).

Concluding remarks

The results obtained show that there is a significant difference observed for tests conducted with wastewater and tap water. Tests with wastewater show more variation in their results, which were speculated to be due to more biological activity occurrences in the system. Tests with wastewater were closer in representing processes occur in a sewer, and it is possible to demonstrate biofilm growth influence on sediment bed by differences in the sediment concentration in the column and possible changes in the mode values of the recovered samples.

5.6.5 Comparing results for tests conducted with wastewater at different consolidation phases

In this section, results will be presented according to the period of the consolidation phase. For each period, different results are offered for each consolidation phase and erosion phase.

For the consolidation phase, results of bed protein and reducing sugar concentration over time are presented in order to understand changes in bed organic matter concentration and to provide evidence of biofilm growth on the bed surfaces. The results were presented as a mass of protein or reducing sugar over the volume of samples collected (g/mL of sample).

For the erosion phase, three groups of results will be presented. The first one consists of the bed erosion rate, q_i plotted against applied shear stress, τ, and cumulative bed eroded thickness, e_{cum} that was plotted against q_i. These results will be used to investigate bed erosion processes when subjected to increasing shear stress. Low values of q_i and e_{cum} with increasing τ indicate stronger bed, as less bed particles were eroded with at higher τ values.

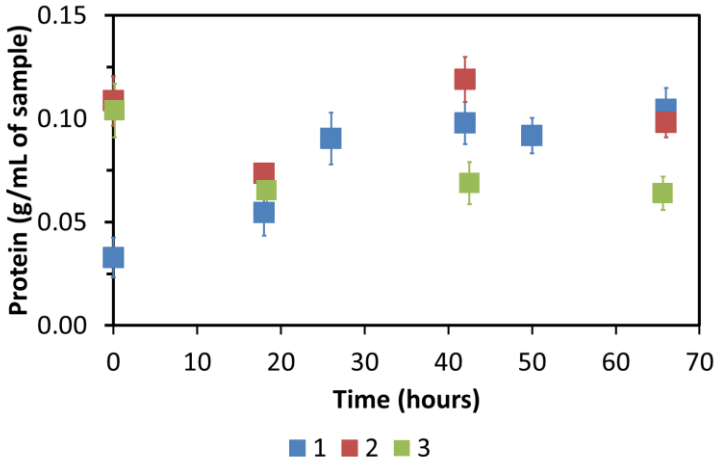
The second results consist of suspended solids protein and reducing sugar concentration over TSS values with increasing τ. These results will give more information on the evidence of the amount of biofilm growth on the bed, and probably within the bed itself as sediment is eroded.

The third results presented for erosion phase are particle size modes obtained and its corresponding e_{cum} values. These results will provide a further understanding on the bed layering characteristics, which is important in understanding bed erosion processes in a sewer.

66 hours consolidation phase

Figure 5.23 shows results of protein and reducing sugar concentration obtained for tests conducted on 66 hours consolidation period. From the results, protein concentration was observed to be significantly higher than reducing sugar concentration, which indicates that protein quantification was more sensitive in determining biofilm growth in a system.

For protein concentration, it can be observed that Test 1 experiencing steady increase while Test 2 experienced an increase in protein concentration values after 30 hours of the consolidation phase. Protein concentration for Test 3, however, was decreasing with time. These results may suggest steady biofilm growth on Test 1 and Test 2.



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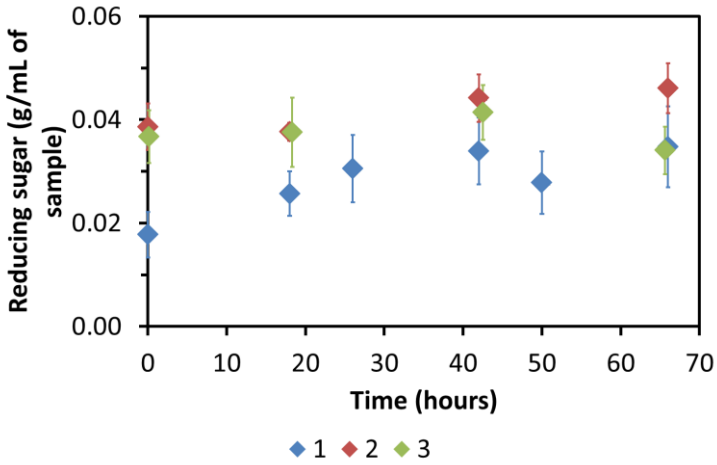


(b)

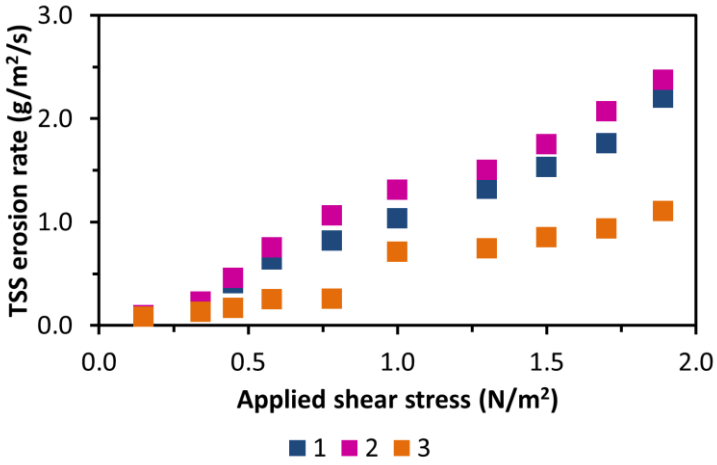
Figure 5.23. Protein and reducing sugar concentration obtained from suspended solids sample during 66 hours consolidation period.

As samples for Test 1, Test 2 and Test 3 were taken alternately between 6 sampling points using a long pipette (Section 5.2.2), the bed condition after sampling action needs to be taken into consideration. During the sampling, possible biofilm growth and the bed were taken and created a hole in the bed. New biofilm layer may grow in this hole thus resulting in high organic matter concentration as speculated for Test 1 and Test 2. However, the hole may also be covered by olivestone instead of biofilm, and thus produced a decreasing trend as observed by Test 3.

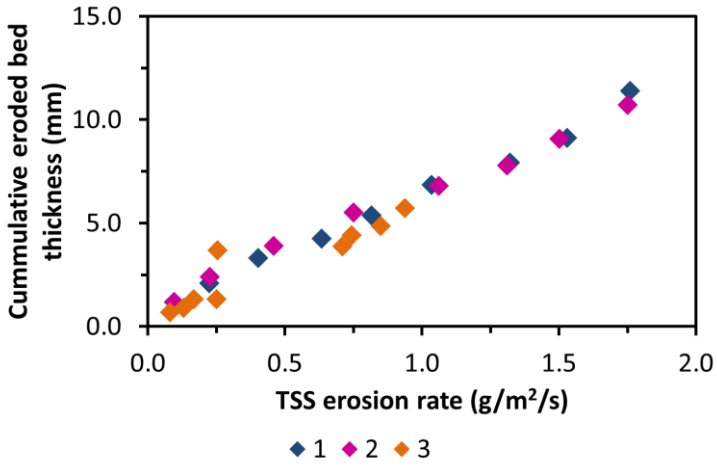
No significant changes were observed in reducing sugar concentration for all tests, although a small increase was obtained at T = 50 hours for Test 1. This small increase corresponds to an increase of protein concentration values at the same period.

Figure 5.24 presented results of q_i and e_{cum} for tests conducted with wastewater at 66 hours consolidation phase. Figure 5.24 (a) shows that q_i values were increasing with increasing , while Figure 5.24 (b) illustrates that e_{cum} values was increasing with increasing q_i for all tests. These results shows that more bed particles were eroded when subjected to increasing shear stress level thus yield higher values of total bed eroded thickness.

q_i values were observed to increasing in a linear manner for all tests until τ = 0.78 N/m^2 were the trend was observed to have a sudden increase. This increase occurred at e_{cum} values of 5.36 mm for Test 1, 6.78 mm for Test 2 and 3.67 mm for Test 3. This observation suggests that the bed may consists of two layer, where stronger top layer shown to have more resistance to shear stress as compared to bottom layer of the bed.



(a)



(b)

Figure 5.24. Results of (a) TSS erosion rate and (b) cumulative bed eroded thickness values for tests conducted at 66 hours consolidation periods.

Test 1 and Test 2 were observed to have higher values of q_i and e_{cum} as compared to Test 3, which may indicate less bed strength in Test 1 and Test 2. For more understanding of these findings, the results were compared with protein concentration obtained during consolidation phase. From the comparison, higher protein concentration obtained during consolidation phase links with higher values of q_i and e_{cum} values, which suggests that the biofilm growth on the bed surfaces may have weaken the bed stability at 66 hours consolidation phase.

Unfortunately, no organic matter quantification during erosion tests was conducted at this stage of the study, thus, the information of organic matter

concentration of the bed during increasing shear stress level was not able to be obtained.

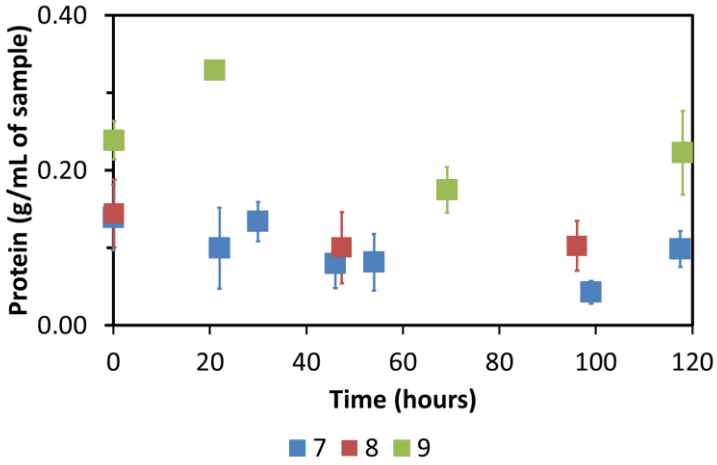
118 hours consolidation phase

Figure 5.25 shows the results for protein and reducing sugar concentration of the during consolidation phase for all tests conducted with wastewater at 118 hours consolidation period.

The results agreed with results from 66 hours consolidation period as protein concentrations obtained were significantly higher than reducing sugar concentration. Both protein and reducing concentration shows two times higher values as compared to results obtained in 66 hours consolidation period. This suggests that longer period of time is necessary in order to achieve more biofilm growth on the bed under these experimental conditions (5% diluted wastewater).

Protein concentration was observed to slowly decrease with time, as shown from Test 7 while Test 8 and Test 9 show more variation with time. These results were similar to 66 hours consolidation phase, as no established trend can be observed from the tests. This finding further suggested that more variation was obtained in tests with wastewater, due to biological activity present in the system.

Other than that, the protein concentration of Test 7 and Test 9 was shown to increase at the end of the 118 hours period, which provides evidence that more biofilm growth was obtained with longer consolidation phase period. No trend was detected for reducing sugar concentration of Test 7 and Test 8. Test 9 shows slight changes which correspond to changes in bed protein concentration.



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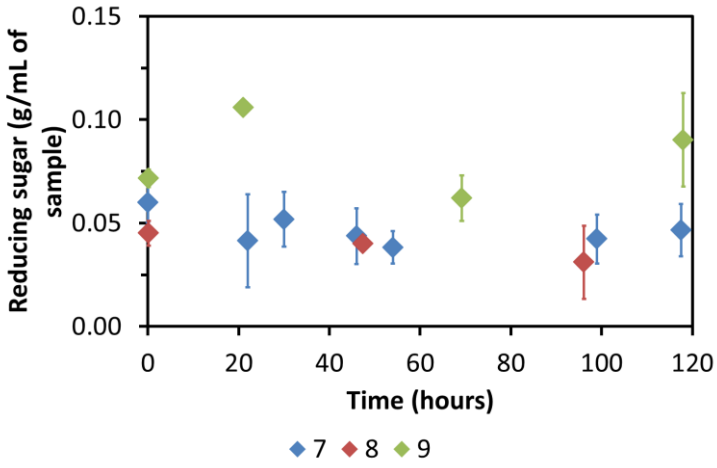


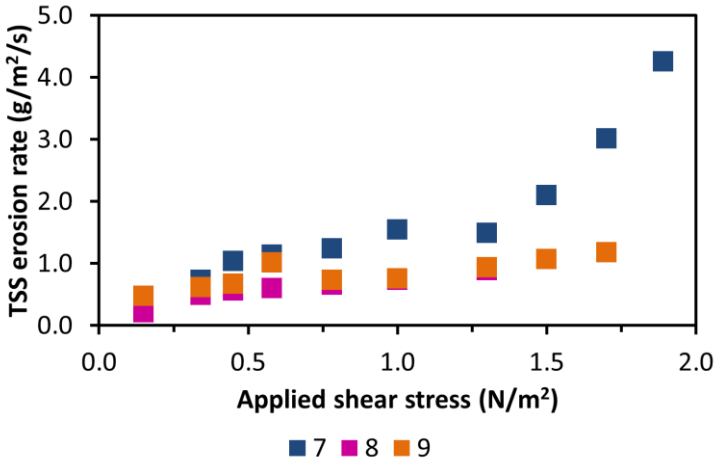
(b)

Figure 5.25. Bed protein and reducing sugar concentration for tests conducted at 118 hours consolidation period.

Figure 5.26 illustrates values of q_i and e_{cum} values obtained for tests conducted at 118 hours consolidation period. All tests show similar trend as results from 66 hours consolidation period, as both values were increasing with increasing τ. In general, overall q_i and e_{cum} values obtained were lower as compared to values obtained for 66 hours consolidation period. This indicates that 118 hours consolidation phase had stronger beds than 66 hours consolidation phase as less bed particle was eroded at the same shear stress level.

This finding suggests that more biofilm was produced at longer consolidation phase and thus increases bed stability and resistance to higher shear stress,

and the bed was more also stronger due to compression from physical self-weight of the bed. No explanation can be provided for an exceptionally high value of q_i and e_{cum} for Test 7.



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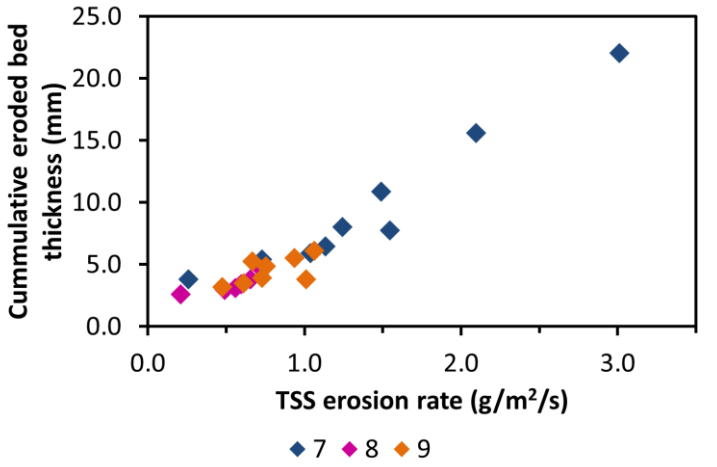


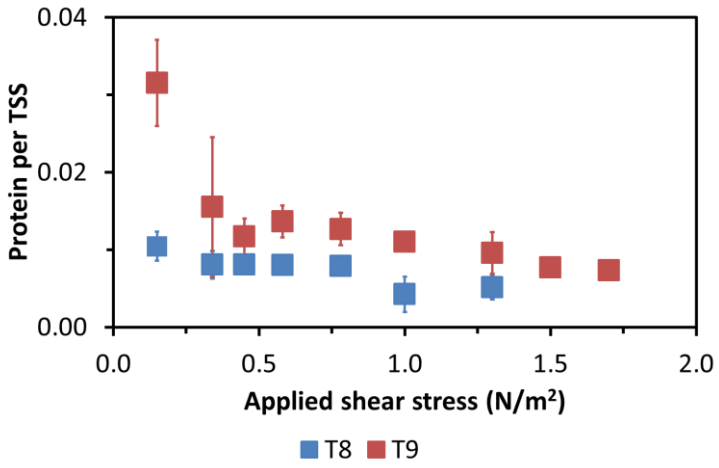
(b)

Figure 5.26. Results obtained for tests conducted at 118 hours consolidation period.

Other than that, the results show similar trend for q_i and e_{cum} values as 66 hours consolidation period, where an almost linear trend was observed until a small increase at τ = 1.0 N/m^2, causing the pattern to change afterwards. These changes occurred at e_{cum} values of 7.70 mm for Test 7, 4.57 mm for Test 8 and 4.86 mm for Test 9.

However, no significant increase in q_i and e_{cum} values were observed for Test 8 and Test 9 as compared to results from 66 hours consolidation phase,

which may indicate that stronger bottom layer was obtained at longer consolidation phase period. This may have been due to compression or the bed may have stabilised and more homogenously mixed during the long consolidation phase.



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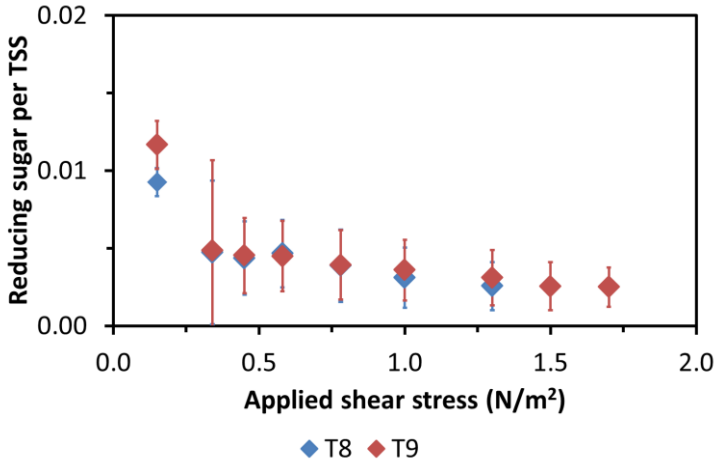


(b)

Figure 5.27. Protein and reducing sugar concentration obtained during erosion phase for tests conducted at 118 hours consolidation phase.

Protein concentration during consolidation phase seems to have little influence on q_i and e_{cum} values, as Test 9 obtained a similar q_i and e_{cum} values as Test 8 even though Test 9 recorded the highest protein concentration during consolidation phase. These may suggest that the influence of consolidation period on the bed can be observed for consolidation phase longer than 66 hours.

Figure 5.27 shows protein and reducing sugar concentration obtained during erosion phase for the test conducted at 118 hours consolidation period. Both figures show similar trends, as protein and reducing sugar concentration over TSS were observed to decrease with increasing τ. These changes were not significant for reducing sugar as compared to protein concentration.

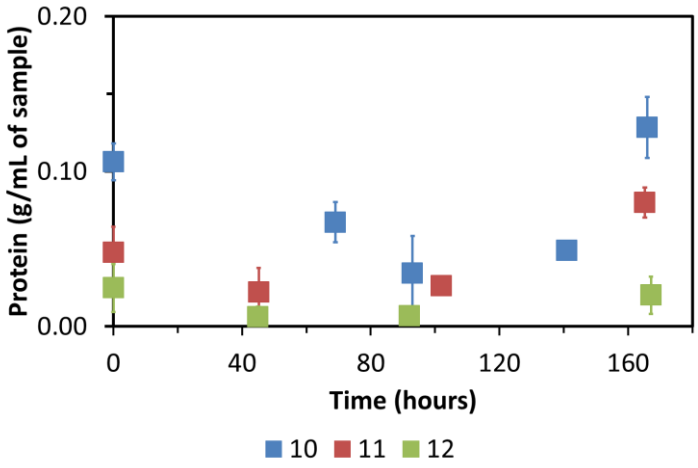
These findings suggest that highest organic matter concentration was obtained at the top layer of the bed and the concentration was decreasing with bed thickness. This may have been caused by biofilm growth on the bed layer, thus producing higher organic matter concentration during the start of the erosion phase.

166 hours consolidation phase

Figure 5.28 demonstrates results obtained for protein and reducing sugar concentration obtained from tests conducted at 166 hours consolidation period. In general, the values obtained were similar to results from 66 hours consolidation period. Protein and reducing sugar concentration was observed to have a similar concentration for Test 11 and Test 12 while reducing sugar obtained for Test 10 was significantly higher than protein concentration. This interesting finding may have indicated changes in biofilm characteristics at consolidation phase by 166 hours.

The organic matter concentration values obtained were similar to results from 66 hours tests, which suggest that the idea of longer consolidation phase produce more biofilm and yield higher protein concentration is not necessarily true. However, evidence of biofilm growth with longer consolidation phase was demonstrated as all the tests show increasing protein concentration after T = 110 hours. This observation was similarly obtained in tests conducted at 118 hours consolidation period, thus indicating that organic matter presents in the system was sufficient to facilitate the biological activity for long period of time.

Other than that, Test 12 shows the lowest bed protein concentration when compared with all tests. This may due to draining bed sampling method which may have destroyed the biofilm growth on the bed surfaces.



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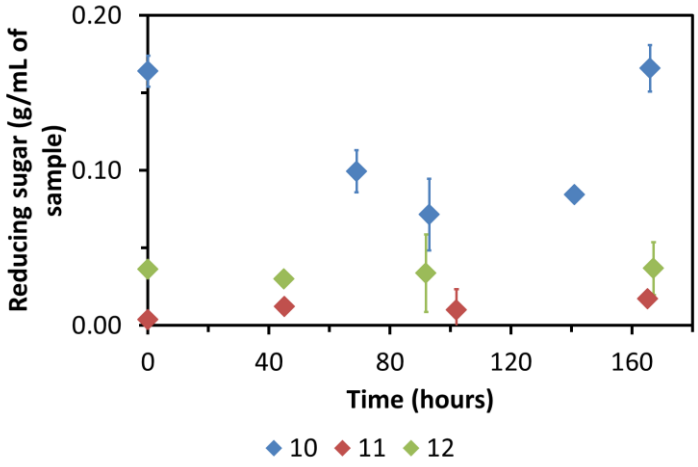
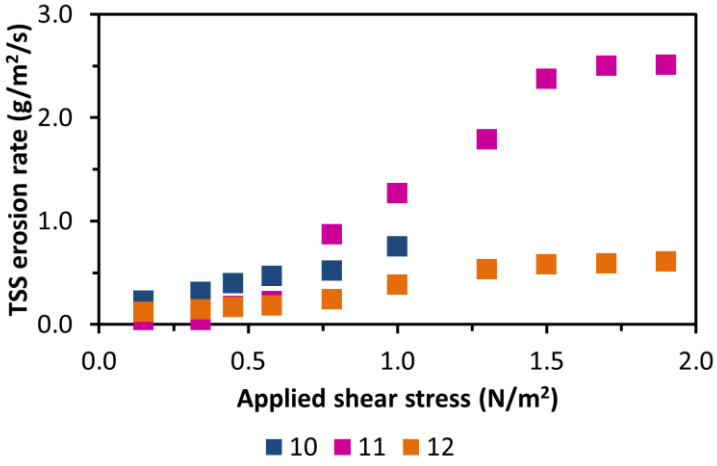


(b)

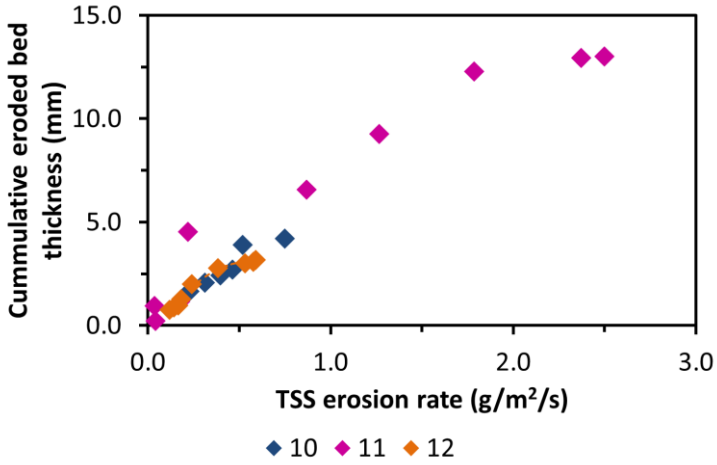
Figure 5.28. Protein (a) and reducing sugar (b) concentration obtained for bed samples of 166 hours consolidation phase period.

Figure 5.29 illustrated q_i and e_{cum} values obtained during erosion tests for bed consolidated at 166 hours. The figures share the same trend as two previous consolidation phases, as both q_i and e_{cum} values were observed to be increasing with increasing τ. With exception of Test 11, the results show lower overall q_i and e_{cum} values as compared to results from 66 and 118 hours consolidation phase. These findings were consistent with previous observation at 118 hours, as q_i and e_{cum} values are decreasing with increasing consolidation phase period, which indicate stronger bed was

obtained at longer consolidation phase. These finding were speculated to be caused by biofilm growth on the bed, and bed consolidation effect.



(a)



(b)

Figure 5.29. Results of (a) TSS erosion rate (b) Cumulative eroded bed thickness for tests conducted at 166 hours consolidation period.

Other than that, lower q_i and e_{cum} values were observed at τ higher 1.0 N/m^2 in these tests as compared to 66 and 118 hours consolidation period. This may indicate stronger bottom layer of the bed, which further suggests long consolidation period may facilitates biofilm growth within the bed, thus reducing bed particle eroded when subjected to high shear stress.

The occurrence of a small increase in q_i and e_{cum} values were observed at τ = 1.3 N/m^2, as compared to 0.78 N/m^2 for 66 hours and 1.0 N/m^2 for 118

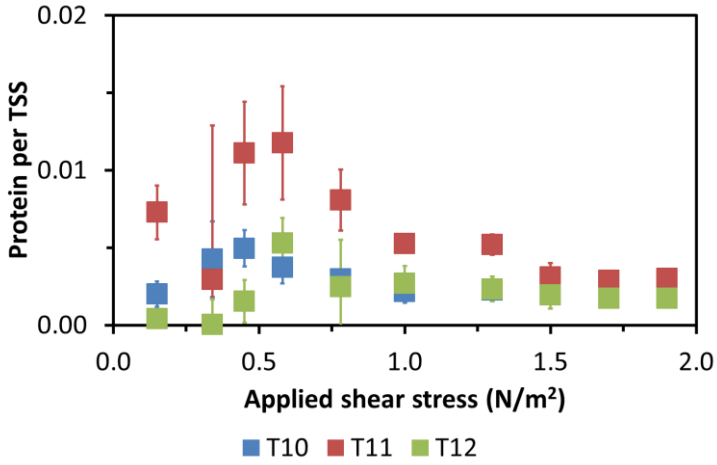
hours consolidation phase. This increase in τ values indicate that the bed top layer was getting stronger with longer consolidation phase, as the top bed layer was able to withstand higher shear stress value before eventually eroded.

The result obtained also show similar values of Test 11 and Test 12, which imply that bed sampling method used in Test 12 was only causing a disturbance on the biofilm growth, but no such effects on the bed were demonstrated from the figures.

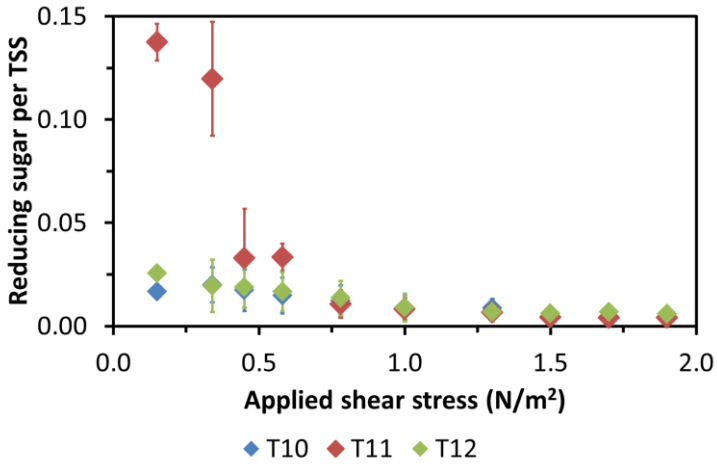
No relationship can be obtained between protein concentration obtained during consolidation phase and q_i and e_{cum} values. Test 10 yield similar q_i and e_{cum} values as Test 12, even though Test 10 shows the highest bed protein concentration while Test 12 has the lowest bed protein concentration. These findings were consistent with results from 118 hours tests.

Figure 5.30 shows result for protein and reducing sugar concentration obtained during erosion phase for 166 hours consolidation phase. The results show higher reducing sugar over TSS values as compared to protein per TSS for all tests. In general, protein concentration obtained was lower compared to 118 hours tests, while reducing sugar concentration was similar to that of 118 hours tests.

No significant trend was observed for Test 10 and Test 12 as the changes were very subtle. Test 11, however, shows significant decreasing in reducing sugar values with increasing τ while more variation of protein concentration was observed. The protein concentration shows a decrease at τ = 0.45 N/m^2, followed by an increase at τ = 0.58 N/m^2 and τ = 0.78 N/m^2 before gradually decreasing until the end of erosion phase. This result provides an evidence of biofilm growth in the bed at 166 hours.



(a)



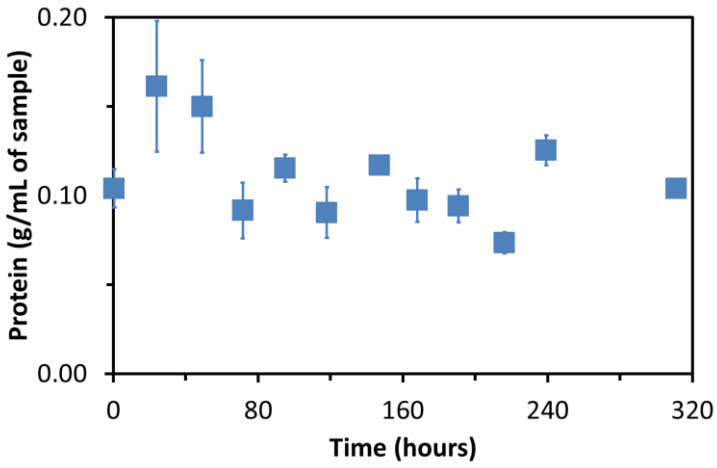
(b)

Figure 5.30. Results of protein and reducing sugar concentration per TSS for tests conducted at 166 hours consolidation phase.

Comparing the results obtained with tests conducted with tap water at the same consolidation period shows that tests with tap water produced more bed erosion at the same shear stress level. These findings suggested that tests with tap water have weaker beds due to limited biological activity on the bed. Bed erosion obtained at 166 hours was also observed to be lower than the values obtained at 66 hours consolidation phase, which further indicate the bed consolidation effects were more prominent at longer consolidation phase.

312 hours consolidation phase

Figure 5.31 shows results of protein and reducing sugar concentration obtained for tests conducted at 312 hours consolidation phase. In general, the results supported previous findings from 66 and 118 hours consolidation phase, where protein concentration was observed to be higher than reducing sugar concentration.



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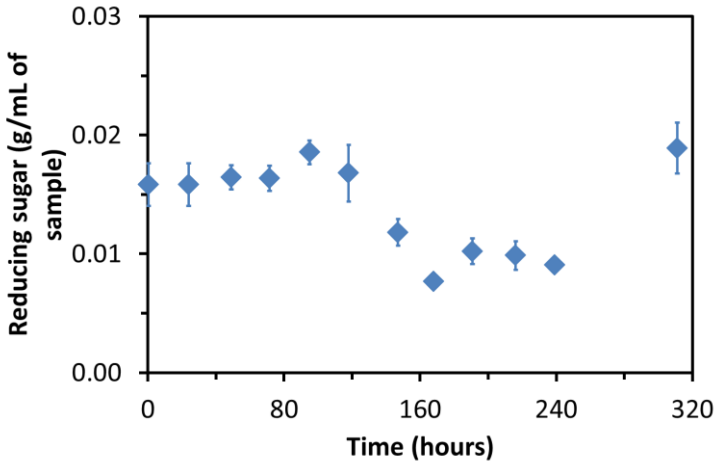


(b)

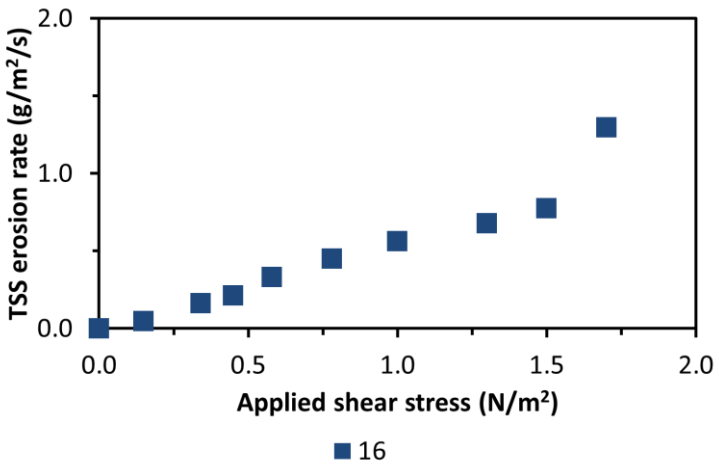
Figure 5.31. Results of (a) protein and (b) reducing sugar concentration obtained from suspended solids samples of tests conducted at 312 hours (Test 16).

The results show higher protein concentration and lower reducing sugar concentration as compared to results from 166 hours consolidation period. No established pattern can be observed from the result of protein

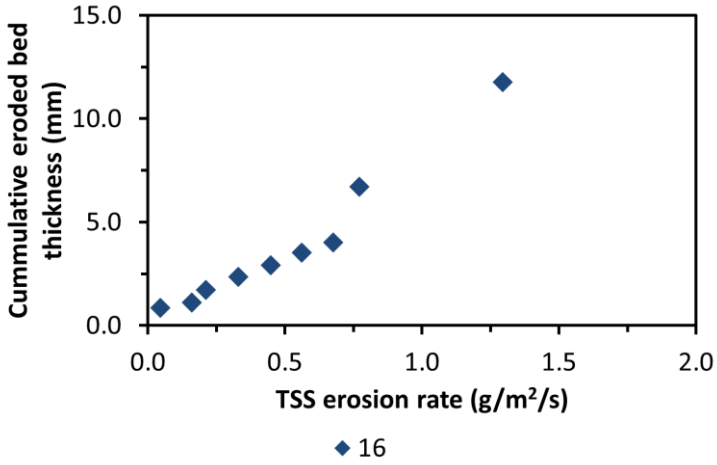
concentration, and no significant changes were observed in reducing sugar concentration, which was consistent with previous findings at 66 and 118 hours consolidation phase.

The protein concentration shows multiple changes during the duration of the tests, which was speculated to be caused by biofilm detachment and loss of biofilm from sampling and growth processes. However, it is important to note that an increase of protein concentration was observed at T = 240 hours, which consistent with previous findings at 118 and 166 hours, where more biofilm growth was observed near the end of each consolidation phase. This finding further indicates there is abundant of organic matter in the system to support microbial activity at 312 consolidation phase.

Figure 5.32 shows q_i and e_{cum} values obtained during erosion tests. The results were consistent with tests at previous consolidation phase, as q_i and e_{cum} values were increasing with increasing τ. Both values obtained were generally similar to values obtained at 118 and 166 hours consolidation period. An increase of shear stress and changes in trend was observed at τ = 1.3 N/m^2, which was similar to results from 166 hours consolidation period.



(a)

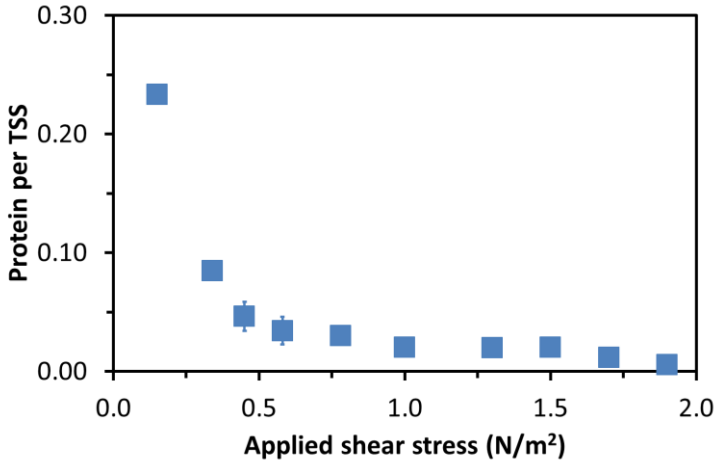


(b)

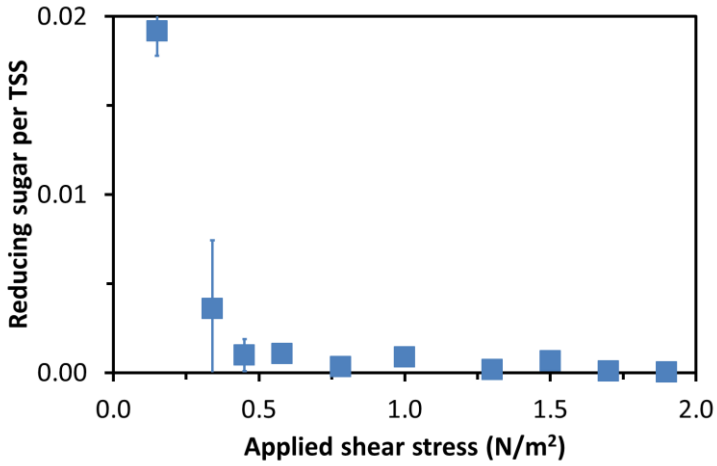
Figure 5.32. Results obtained for Test 16 (312 hours consolidation phase period).

This may indicate 166 hours consolidation phase was long enough to observe effects of biofilm growth on the bed deposits, however, 312 hours was deemed as too long, as biofilm influences on the bed were less significant in comparison to the effects of the long consolidation phase has on the bed.

Figure 5.33 illustrates result obtained for protein and reducing sugar concentration obtained during erosion phase. From the figure, protein concentration over TSS values was significantly higher than reducing sugar over TSS. Both figures show similar trends, as protein and reducing sugar concentration over TSS were observed to decrease with increasing τ. These values indicate that highest protein and reducing sugar was obtained on the bed top layer, and the concentration was decreasing with increasing bed depth. This finding does not agree with results obtained at 166 hours, where it was speculated that biofilm may grow within the bed at long consolidation phase.



(a)



(b)

Figure 5.33. Protein and reducing sugar concentration over TSS values for the sample obtained during erosion phase of tests conducted at 312 hours consolidation period.

Results of particle size mode for tests conducted with wastewater

Bed particle eroded during erosion tests was subjected to particle size analysis in order to determine the size of particles eroded when subjected to increasing shear stress level.

As previously discussed, the results of tests with wastewater at different consolidation phase may indicate that the bed consists of two or more different layer. Tests conducted at 66 hours show a small increase of q_i and e_{cum} values with τ until 0.78 N/m^2 where a sudden increase was spotted. The same trend was present in all tests, however, the sudden increase was

shifted to 1.0 N/m^2 for 118 hours consolidation period and 1.3 N/m^2 for both 166 and 312 hours consolidation period.

Table 5.7. Summary of TSS erosion rates and cumulative bed eroded thickness for tests conducted with wastewater at various consolidation phases.

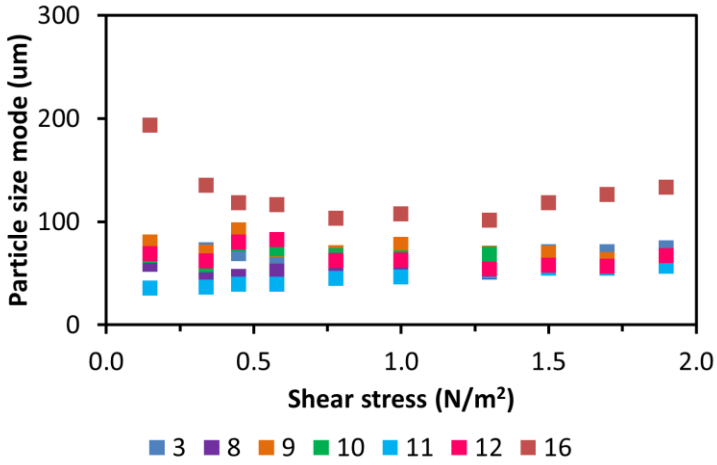
	Consolidation phase (hour)							
	66	118			166			312
Test number	3	7	8	9	10	11	12	16
τ (N/m^2)	0.78	1.00	1.00	1.00	1.30	1.30	1.30	1.30
q_i (g/m^2/s)	0.26	1.55	0.73	0.75	0.81	1.79	0.58	0.68
e_{cum} (mm)	5.36	7.71	4.57	4.86	4.17	12.28	3.04	3.99

The sudden increase in q_i and e_{cum} values were observed to occur at similar bed depth, which can be summarized by Table 5.7. Except for a few anomaly (Test 7 and Test 11), the table shows that the changes occurred on average bed depths of 4.40 mm.

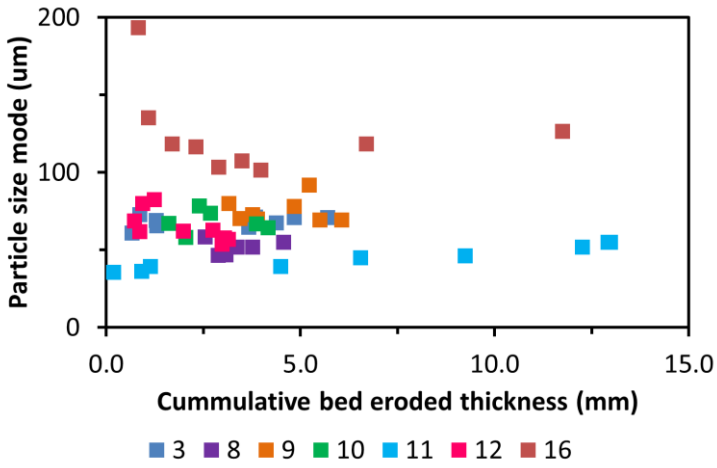
This may have explained the increasing of shifted shear stress values with longer consolidation phase period. This finding shows that bed top layer was getting stronger with consolidation phase thus requires higher shear stress in order to erode the bed. As mentioned before, longer consolidation phase may have provided more time for biofilm to grow and mature in the system. It also causes the bed to be more compressed and compact, and thus made the bed more resistant to higher shear stress. In addition to that, longer consolidation phase may have helped the bed to be more homogenously mixed and stabilised with time thus more resistance to shear stress.

Figure 5.34 shows particle size mode values obtained for the test conducted with wastewater at various consolidation phase periods. In general, all tests

obtained similar particle size mode values except for Test 16 (312 hours consolidation phase).



(a)



(b)

Figure 5.34. Particle size mode results obtained for Test 3 (66 hours consolidation phase), Test 8 and Test 9 (118 hours consolidation phase), Test 10, Test 11 and Test 12 (166 hours consolidation phase) and Test 16 (312 hours consolidation period phase).

Particle size mode values against τ were similar for all tests except for Test 16. Observation on Test 16 shows that larger particle was eroded at the start of erosion phase and followed by smaller particles being eroded at the higher shear stress value. This may suggest that at 312 hours consolidation phase, top layer of the bed was changing physically, possibly from biofilm growth that glued particles together and forming a larger particle. For the bottom layer of the bed, the particle was larger, thus more difficult to move. No

significant trend can be observed for the rest of the tests, as mode values obtained were similar and agreed with size particle of olivestones used in the test.

Concluding remarks

Several conclusions can be made from the results presented. The vast majority of the tests show higher protein concentration values as compared to reducing sugar concentration for both consolidation and erosion phase. This result suggests that that protein quantification was more sensitive and reliable in determining changes of organic matter concentration in the system. Organic matter concentrations during consolidation phase have shown to have random influence on q_i and e_{cum} values obtained during erosion phase.

Highest protein concentration during consolation phase was obtained for 118 hours tests, followed by 312 hours, 166 hours and 66 hours tests. From observations, longer consolidation phase does not facilitate more biofilm growth in the system. This can be due to the biofilm detachment or from limited availability of organic matter at longer consolidation period to produce more biofilm in the system. Unfortunately, there have been no studies on organic matter concentration during consolidation phase can be found in the literature, thus, no comparison can be conducted for these findings.

All tests show an increase of q_i and e_{cum} values with τ. This finding agrees with Seco *et al.* 2014 and 2006 and Tait *et al.* 2003a and 2003b. These values were observed to be decreasing at longer consolidation phase which indicate the bed was stronger and more resistant towards high shear stress (Tait *et al.* 2003b). These changes can be attributed to physical consolidation of the bed and from biofilm growth. However, the changes were inconclusive, as several studies reported weaker bed while others claimed otherwise.

Black *et al*. (2002), Gerbersdorf *et al*. (2008a) and (2008b), Huang *et al*. (2012), Righetti and Lucarelli (2007), Seco *et al*. (2014), and Tait *et al*. (2003b) have reported stronger beds obtained after the consolidation period.

As previously discussed, the changes in the bed were caused by bed consolidation and biofilm formation on the bed. These were influenced by the period of consolidation and biological activity in the system (Xu *et al*. 2017). The consolidation causes a reduction in bed voids, thus producing more compact and fewer voids in the bed (Arthur *et al.* 1999). Consolidation has also been reported to cause structural changes in the sediment, as the surface layer and bottom bed layer was separated (Xu *et al*. 2017). The surface layer was under aerobic conditions, which further promotes biological growth. Biofilm growth or microbial activity changes the bed strength by enhancing sediment stability (Fang *et al*. 2014) by increasing particle interlocking from agglutination and cementation effects between the bed and organic substances (Arthur *et al.* 1999). This was in agreement with results observed for tests conducted for consolidation phase of longer than 66 hours, as biofilm growth was found to increase bed strength for the vast majority of the tests.

Mermillod-Blondin and Rosenberg (2006), Le Hir *et al*. (2007), Schellart *et al*. (2005), Seco *et al.* (2016), Sakrabani (2004) and Tait *et al*. (2003a) have reported the weakening of the bed which is in agreement with the findings found in this study for 66 hours consolidation phase periods. The bed was weakened during 66 hours tests, and these findings were speculated to be caused by the growth of young biofilm on the bed, which have not been able to form strong bonds with the bed particles due to the short time period. Other than that, these changes were also speculated due to bubble formation from biofilm formation processes.

Stronger influence of biofilm growth over bed physical consolidation was observed for tests at 66 and 118 hours, while tests at 166 and 312 hours was found to be more affected by bed consolidation. This was speculated from the observation between tests at 118, 166 and 312 hours tests. 166 hours tests were observed to have lower organic matter concentration values as compared to 118 hours tests, however, 166 hours shows less bed eroded valued. A similar trend was observed between 166 and 312 hours test. 312 hours test shows higher organic matter concentration values, however, the

bed was found to have similar strength as 166 hours tests as they were eroded at a similar rate. Other than that, two-layer bed configuration for observed for all the tests, which in agreement with Tait *et al*. (2003a). The average bed depth of this observation was approximately at 4.40 mm.

Longer consolidation phase has shown to facilitate more biological activities in the bed. These findings were supported by results obtained from tests at 118, 166 and 312 hours consolidation phase that shows an increase of protein concentration by the end of the tests.

Protein per TSS shows a decreasing trend with τ, while no such development was noted in reducing sugar per TSS values. This findings suggested that biofilm was mostly found on the bed surfaces, although, tests at 166 hours shows an increase of the protein concentration in the bed and biofilm growth in the bed was considered. No such findings were found for tests at 312 hours. Higher organic matter per TSS values was observed for longer consolidation phase period.

In general, large variation was observed in the results obtained for tests with wastewater conducted under the same conditions as that of tap water tests. This may cause by various elements, for example, initial nutrient concentration in the wastewater and also biofilm ability to grow under these conditions. None of the previous studies was found to provide any evidence of biofilm growth, thus, no comparison or references can be made regarding these findings.

5.6.6 Results for disruptive sampling of biofilm

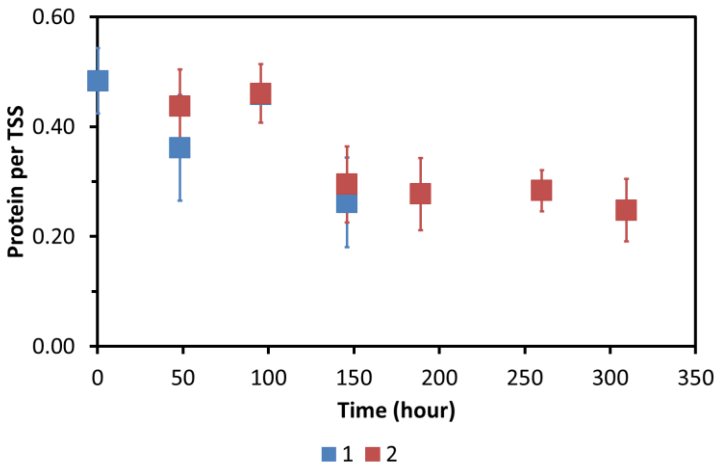
These tests were conducted in order to understand the disturbance the bed may have experienced during sampling session of erosion tests. Other than that, these tests also aim to provide evidence of biofilm growth on bed surfaces with time during consolidation phase of erosion tests. Test with tap water was conducted using 2 beakers, where the beakers were eliminated at T = 150 and 312 hours. Tests with wastewater were conducted with 6

beakers, where each beaker was eliminated at T = 29, 70, 142, 214, 262 and 312 hours. Suspended solid samples were collected before the beaker was eliminated followed by a collection of the bed for further analysis.

Results for test conducted with tap water

Figure 5.35 (a) and (b) shows results obtained for suspended solids sample during consolidation phase for both beakers; Beaker 1 shows results up to T = 150 hours as the beaker was then eliminated in order to obtain bed sample, while, Beaker 2 shows results for the whole duration of tests. Both beakers provide consistent results.

From the results, it can be observed that protein concentrations were significantly higher than reducing sugar concentration. This finding was consistent with results obtained for the suspended sediment samples obtained in the erosion tests for 312 hours consolidation phase. Both concentrations show a similar pattern, as the protein and reducing sugar concentration was observed to be decreasing with time. This may suggest a continued usage of any available nutrient in the system from biological activity that occurred in the suspended solids phase.



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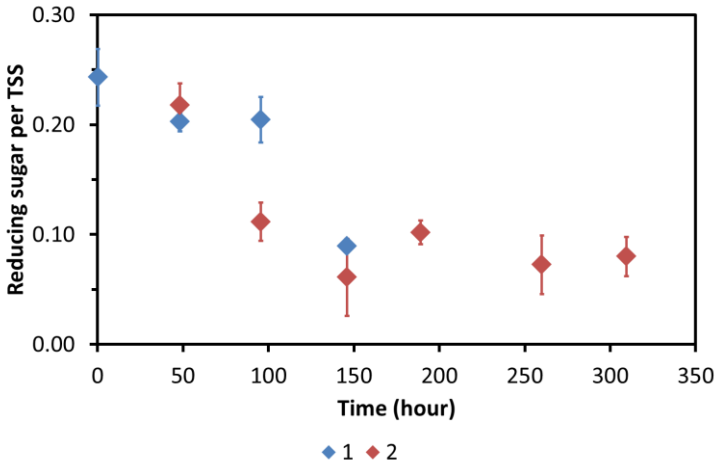
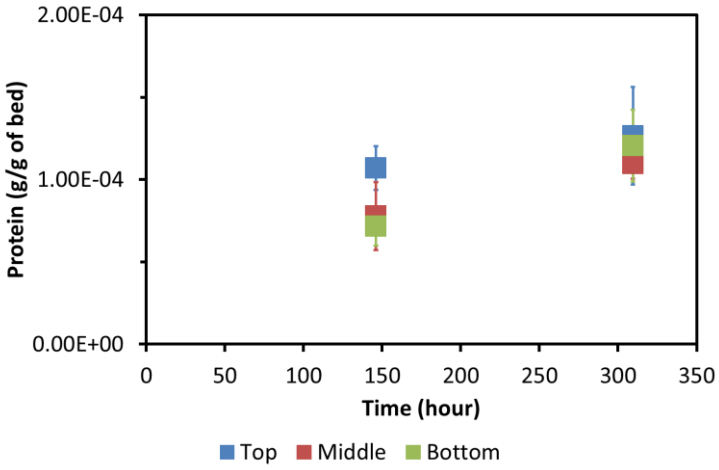


(b)

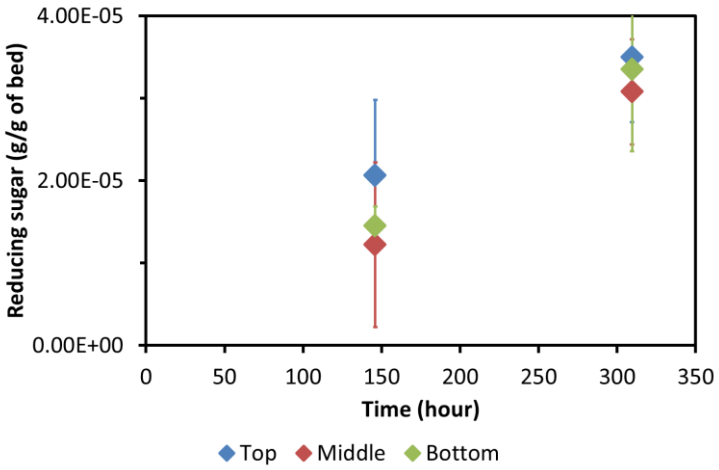
Figure 5.35. Suspended solids protein and reducing sugar concentration for tests conducted with tap water.

Figure 5.36 shows protein and reducing sugar concentration obtained from bed sample collected for tests conducted with tap water at 312 hours consolidation period. The results show that bed protein concentration obtained was significantly higher than bed reducing sugar, which agreed with results obtained from suspended solids phase.

Both concentrations show a small increase with time, which may indicate biofilm growth was obtained on the bed surfaces at the end of 312 hours consolidation phase. The result supports previous findings from erosion tests with tap water, which suggested that lower biological activity takes place in tests with tap water due to the low concentration of nutrient and microorganisms available. Other than that, the results further indicate that biofilm growth was possible to occur in low nutrient and microorganism concentration at long duration consolidation phases.



(a)



(b)

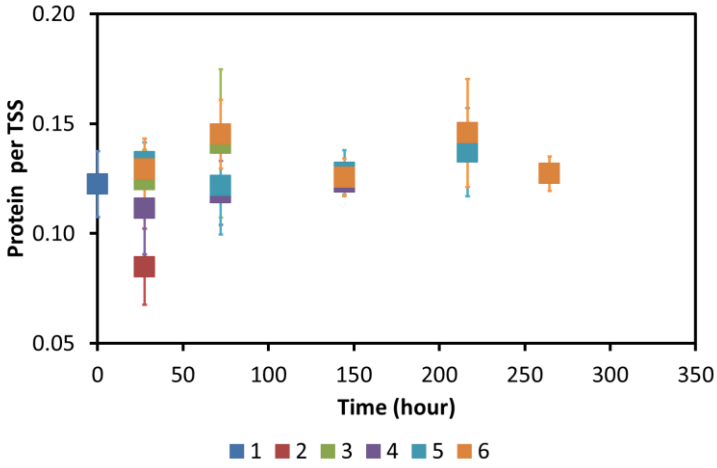
Figure 5.36. Results of protein and reducing sugar concentration obtained from bed samples for tests conducted with tap water. Top, middle and bottom represent bed sampling points as previously discussed in Section 5.4.3.

Similar bed protein and reducing sugar concentrations values were observed at the different sampling points, which may indicate that the bed shares similar conditions.

Results for test conducted with wastewater

Values of protein and reducing sugar concentration obtained from suspended solids phase for the test with wastewater can be illustrated by Figure 5.37. From the figures, protein concentration was observed to be significantly higher than reducing sugar concentration, which agreed with tests conducted with tap water.

Both protein and reducing sugar concentration shows fairly constant values over time. Small changes were observed for Beaker 6. However, these changes were very small thus it was deemed insignificant. Constant values of organic matter concentration in suspended solid phase may have implied that no biofilm growth was obtained in the suspended solids phase for all beakers, or biofilm growth was consistent in each beaker with time. The concentration obtained for different beakers at the same time period was also observed to be fairly similar, which proved that hydraulic and biofilm growth conditions were the same for all beakers.



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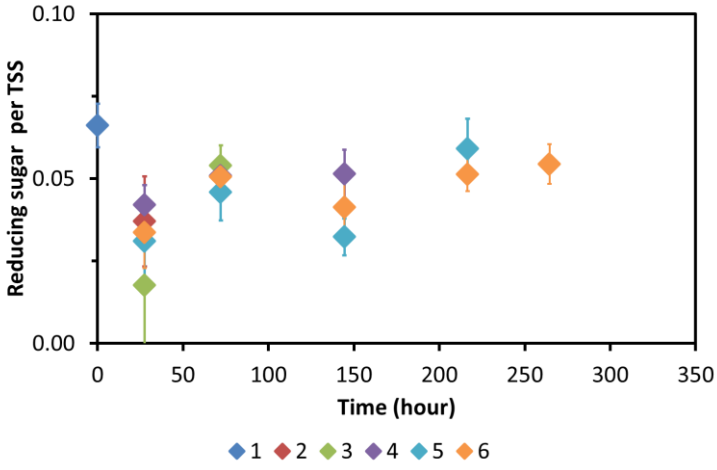
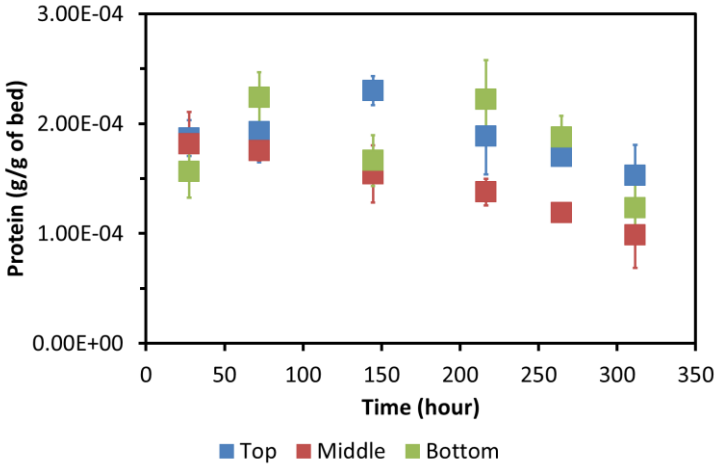


(b)

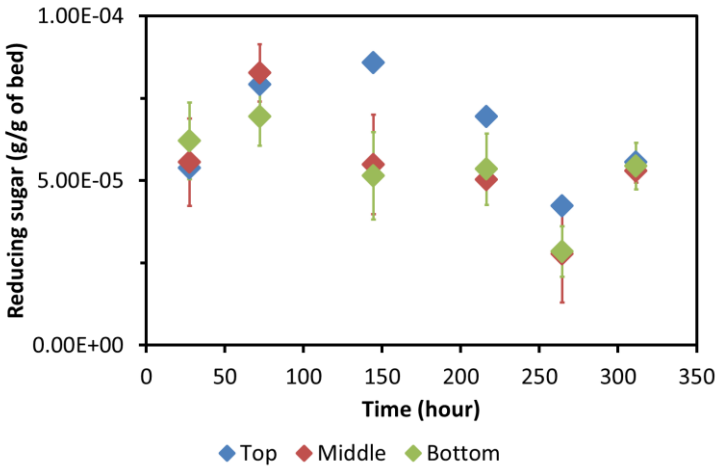
Figure 5.37. The suspended solids concentration of protein and reducing sugar obtained for tests conducted with 5% diluted wastewater.

Figure 5.38 presented results obtained for protein and reducing sugar from bed samples for the test conducted with wastewater. The results were

consistent with results from the test with tap water, as bed reducing sugar concentration was observed to have lower values than bed protein concentration.



(a)



(b)

Figure 5.38. Organic matter concentration obtained from bed samples for test with wastewater.

From the graphs, a clear trend was observed; bed protein and reducing sugar concentration were decreasing with time at all sampling points. This may occur due to loss of biofilm during water removal procedure, or detached biofilm was released to liquid phase during the process. The detached biofilm may have increased the bed protein and reducing sugar concentrations in suspended solids phase. As previously discussed, a decreasing trend was observed for tests with tap water, however, a constant values of organic matter concentrations were obtained for test with

wastewater which further indicates that tests with wastewater obtained some additional protein and reducing sugar from possible biofilm detachment from the bed that contributed to high organic matter concentration of suspended solid samples throughout the tests.

For bed protein concentration, values obtained at T = 20 hours was fairly similar for all three sampling points. The highest bed protein and reducing sugar concentration was observed at T = 150 hours, which may have indicated that biofilm growth was the highest during the first 150 hours consolidation phase. Other than that, values obtained at the top and bottom sampling points show higher values with time in comparison to values found at middle sampling points. This may be caused by the rotating propeller in the setup which formed a bump in the middle section of the bed. This formation may have disrupted any biofilm growth on the affected area. The results also demonstrate that bacteria were not starved at long consolidation period as there was still organic matter available in the system at the end of 312 hours time period.

Results for particle size modes for both tests

Figure 5.39 shows the result of particle size modes obtained for suspended solids sample obtained right before the beaker was eliminated. The analysis was conducted in order to determine changes in particle size of suspended solids over time.

Results for wastewater show a clear increase in particle size modes over time while a stable trend was detected for tests with tap water. Results for tap water also yield smaller particle size modes as compared to the result of the test with wastewater. These results were similar to erosion tests, as little changes were observed to test with tap water while more significant changes were observed in the test with wastewater.

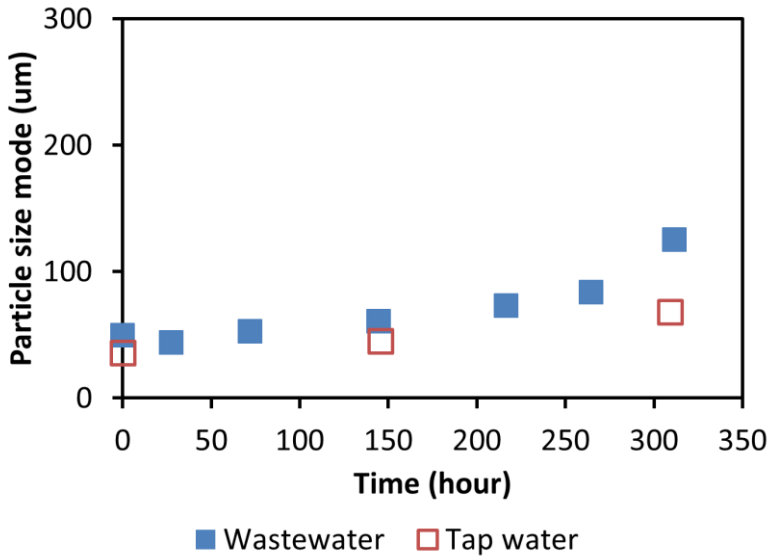


Figure 5.39. Particle size modes obtained for tests with wastewater and tap water in the flocculator tests. Each data points represent beakers used in the tests.

Increasing particle size modes with time show that larger particles were suspended over time. This value, however, was smaller than sand particle size which suggested that olivestone was being aggregated together by biofilm over time. As the values were higher for wastewater tests, these findings further validate the concept that less biological activity was taking place in tap water tests.

Concluding remarks

Comparing results obtained for organic matter concentration obtained from suspended solid phase shows that test with tap water obtained 3 times higher concentration of protein and reducing sugar values. This was due to higher TSS values obtained for the tap water test, which was approximately 5 times higher than TSS values for the tests with wastewater.

Test with tap water also shows decreasing protein and reducing sugar concentration with time as compared to stable and consistent concentrations obtained from the tests with wastewater. This was speculated to be caused by additional organic matter obtained from the tests with wastewater from detached biofilms, which further indicated that no or less biofilm growth

obtained from tap water tests, thus the decreasing trend was due to constant usage of any available nutrient in the system.

For organic matter concentrations obtained from bed samples, wastewater tests show higher values of protein and reducing sugar concentration as compared to tap water tests. Test with wastewater shows more changes with time, while tap water test shows a small increase at the end of the 312 hours consolidation period. These findings provide evidence of biofilm growth and detachment processes on the bed for wastewater test. Wastewater tests were also observed to have the most biofilm at T = 150 hours while highest biofilm obtained for the tests with tap water was observed at 312 hours.

This further indicates that test with wastewater has a higher rate of biofilm growth, which may be due to the high concentration of nutrient available in the system. A small increase of organic matter observed for bed samples from tap water test shows limited biofilm growth in the system. These findings also suggested that biofilm growth was possible under limited nutrient and microorganisms condition. Other than that, longer consolidation phase was demonstrated to cause olivestone to clump together and increasing particle size modes over time.

5.8 Summary of key findings

Key findings of erosion tests were presented in Table 5.8. The table summarizes significant findings obtained during the tests and was a simplification from the discussion (Section 5.6.5). Table 5.9 presents results obtained from disruptive sampling of biofilms, where changes in suspended solids, bed particle and results of the particle size analysis are reported.

Table 5.8. Key findings obtained from erosionmeter tests conducted for all consolidation phase periods

Consolidation phase period (hours)	Key findings
66	• Protein concentration obtained was higher than reducing sugar concentration during consolidation phase for all tests. • q_i and e_{cum} values were observed to increase with τ. • No results of organic matter during erosion phase were conducted. • Highest protein concentration during consolidation phase produced highest values of q_i and e_{cum} • Biofilm growth weakened the bed strength 66 hours is sufficient to observe biofilm growth effects on bed stability.

118	• All tests show higher protein over reducing sugar concentration during consolidation and erosion phase.
	• q_i and e_{cum} values were observed to increase with τ. Bed was stronger than 66 hours tests.
	• Organic matter over TSS values shows decreasing trend with time.
	  • Highest biofilm was obtained on bed surfaces.
	• Highest protein concentration during consolidation phase shows lowest values of bed eroded at high shear stress level.
	  • Biofilm growth increases bed stability and resistance to shear stress.
	Biofilm growth was observed to have more influence on bed stability than bed physical consolidation effect.

166	• 2 out of 3 tests show higher protein over reducing sugar concentration during consolidation and erosion phase. • Changes in biofilm characteristics were considered to occur at longer consolidation period. • q_i and e_{cum} values were observed to increase with τ. Stronger bed was observed as compared to 166 hours tests.

	• Reducing sugar over TSS values show a decreasing trend with time. • Protein over TSS values shows the highest value at the middle section of the bed. • Biofilm growth in the bed was considered possible for 166 hours consolidation phase. • No relationship can be observed between organic matter concentration during consolidation phase to q_i and e_{cum} values. Bed stability was found to be more affected by bed consolidation effects as compared to biofilm growth.

312	• Higher protein over reducing sugar concentration was observed during consolidation and erosion phase for all tests.
	• q_i and e_{cum} values were observed to increase with τ. Bed was observed to be eroded at similar rate as 166 hours tests.
	• Organic matter over TSS values shows decreasing trend with time.
	  • More biofilm growth was observed on bed surfaces.
	• Bed physical consolidation was found to have more influence on bed stability as compared to biofilm growth.
	• Highest mode values were observed for tests at 312 hours.
	  • Olivestone particle on bed top layer was aggregated by biofilm growth thus forming larger particles.

Table 5.9. Key findings for disruptive sampling of biofilm tests.

	Tap water tests	Wastewater tests

Suspended solids	• Organic matter concentration was decreasing with time.
	  • Due to continuation usage of organic matter in the system.
	  • Protein concentration was higher than reducing sugar concentration.
		• Organic matter concentration was fairly constant with time.
		  • Minimal usage of organic matter due to abundant nutrient availability.
		  • Biofilm detachment from the bed may contribute to the organic matter loss due to nutrient usage by microorganisms.
		• Consistent organic matter concentration for all beakers.
		• All beakers achieved similar hydraulics and environmental conditions for biofilm growth.

Bed samples	• Protein concentration shows higher values than reducing sugar.
	• Organic matter concentration was observed to increase with time.
	  • Provide evidence of biofilm growth in the system.
	  • Biofilm growth was very slow and little due to limited availability of nutrient and microorganisms.
	• Samples obtained from all three sampling points show similar results.
		• Higher protein concentration was observed for all tests.
		• Organic matter concentration was observed to decrease with time.
		  • Loss of biofilm to suspended solid phase.
		• Highest organic matter obtained at T = 150 hours.
		  • Biological activity was the most active during the first 150 hours.
		• Middle sampling point shows lowest organic matter concentration.

	• Bed experienced similar biofilm growth and hydraulic conditions.	• Effects of propeller may cause biofilm detachment.
Particle size modes	• Mode values were consistent with time. • Mode values obtained were in agreement with olivestone particle sizes. • No significant changes were noted on suspended solid samples collected.	• Mode values were observed to be increasing with time. • Larger particles were suspended overtime. The particles were aggregated by biofilm growth.

5.9 Conclusions

This chapter describes a number of tests conducted to understand influences of biofilm growth on bed sediment deposits using tap water and wastewater under aerobic conditions. The work demonstrates that biofilm growth increases bed stability for consolidation period of more than 66 hours thus decreasing the quantity of bed eroded at higher shear stress. More specific findings from this work are as below;

- Protein and reducing sugar concentration during consolidation phase for the vast majority of tests show that protein concentration was significantly higher than reducing sugar concentration. The concentration obtained shows variation with time, which can be explained due to bed sampling method implemented. The results also indicate that protein analysis was more sensitive to be used as an indicator for biofilm growth for the tests.
- Most wastewater tests at consolidation phase period of longer than 66 hours show increasing protein and reducing sugar values near the end of the consolidation period. This result implied that abundant organic matter was available to facilitate biological activity in the system for long period of time.
- A clear relationship between protein and reducing sugar concentration during consolidation phase with bed TSS erosion rate and cumulative bed eroded thickness was obtained for 66 hours tests.
- All tests show increasing TSS erosion rate, and cumulative bed eroded thickness values with increasing shear stress. Tests with tap water show higher values as compared to tests with wastewater thus concluded that tap water tests have a weaker bed. Both TSS erosion rate and cumulative bed eroded thickness values were also decreasing with longer consolidation phase, which shows increasing bed stability due to bed physical self-weight, and from possible biofilm growth on the bed.
- Test with tap water shows similar TSS erosion rate and cumulative bed eroded thickness values at 66 and 118 hours consolidation phase, and the values were decreasing for 166 and 312 hours

consolidation phase. These findings suggested that consolidation period longer than 118 hours is necessary in order to observe any changes in bed stability due to bed consolidated processes.

- Results of erosion tests conducted with wastewater show more variation as compared to tests with tap water. This was speculated due to more biological activity occurs in wastewater tests. Similar TSS erosion rate and cumulative bed eroded thickness values were obtained at 166 and 312 hours, which suggest that 166 hours consolidation period was sufficient to allow biofilm growth and to influence bed stability.
- All erosion tests show a possible two-layer bed. TSS erosion rate for wastewater tests was observed to be linear with applied shear stress before a sudden jump at τ = 0.78 N/m^2 for 66 hours consolidation period, τ = 1.00 N/m^2 for 118 hours consolidation period and τ = 1.30 N/m^2 for 166 and 312 hours consolidation period. These findings suggest that top layer of the bed was getting stronger at longer consolidation phase, thus, needs higher level of shear stress to erode. The same trend was observed with tap water, however, the jump was observed at τ = 1.00 N/m^2 for all tests.
- Erosion tests conducted with tap water show particle size modes value from 20 to 80 μm for all tests which correspond to particle size of olivestone. Increasing mode values were observed with bed depth, with similar cumulative bed eroded thickness were obtained for all tests.
- Particle size modes obtained for tests with wastewater were similar to tests with tap water except for wastewater tests conducted at 312 hours that show decreasing mode values with shear stress. This suggests that bed top layer underwent some physical changes, which may have due to biofilm growth that aggregated particles together.
- For disruptive biofilm tests, organic matter concentration in suspended solids was observed to be decreasing with time for tap water tests while a fairly constant trend was obtained for wastewater tests.

- A small increase of bed protein and organic matter concentration was observed for the tap water at T = 312 hours which shows that biofilm growth was possible under low nutrient and microorganisms concentration at sufficiently long period of time. Tests with wastewater show higher bed organic matter concentration which proved the existence of biofilm growth on the bed. The concentration was decreasing with time, which was speculated due to biofilm detachment. Highest biofilm growth was found at T = 150 hours.
- Sterilising materials was deemed unnecessary as organic matter concentration and bed erosion results obtained were not affected.
- Different sampling preparation methods were observed to yield similar protein and reducing concentrations, which suggested that the methods used were effective.

The results obtained demonstrate that bed sediment stability was changing with biofilm growth under the different durations of the consolidation phase. Bed consolidation was shown to have less influence on the bed stability as compared to biofilm growth for 66 and 118 hours consolidation phase. Results for the tests conducted with wastewater were also shown to have more variation than the tests conducted with tapwater, which may indicate that more factors are responsible for these findings. These results are believed to be closer to real sewer conditions as compared to many previous studies conducted in this area of research.

Tests with higher wastewater concentration were proposed as potential works if this study is to be taken one step ahead. Feasibility studies conducted on 50% and 100% wastewater on sand particle for consolidation period of 380 hours show visible biofilm growth in the column. However, TSS values obtained were below detection limit, which suggests that more biofilm growth is possible at a higher nutrient concentration which then increases the bed strength significantly. Overall, these results were important as it displays a clear relationship between biofilm growths with organic matter concentration in the system and bed strength for fine sediment particles after subjected to long consolidation period.

Chapter 6 Conclusions

6.1 Achievements and discussion

The thesis has presented various works conducted for better understanding the impact of biofilm presence in the sewers. Novel methods have been developed to understand i) biofilm growth obtained under different conditions, ii) differences in flow capacity for systems with and without biofilm iii) influences of biofilm growth on the bed stability and iv) organic matter consumption at different conditions.

The results of the pipe experiments concluded that;

i. Biofilm growth is achievable under all conditions.
ii. Hydraulic conditions have a direct influence on the characteristic of biofilm growth.
iii. Initial wastewater concentrations produce more biofilm growth in the system.
iv. Minor energy losses due to pipe fittings were found to be more significant than major energy losses due to friction. The losses were deemed insignificant to the flow profile observed.

The results obtained show that biofilm growth is decreasing flow depth, thus decreasing average pipe hydraulic roughness and increasing average flow velocity. This observation relies on hydraulic conditions of the bed, namely bed slope, shear stress level and discharge flowrate. The changes of flow profile and biofilm growth were observed to depend on the characteristics of the biofilm obtained in the pipe. pH was observed to decrease in all tests, and biofilm detachment was found to occur due to this change.

The series of tests conducted for investigating influence of biofilm growth on bed sediment shows that;

i. Protein analysis was more sensitive in quantifying biofilm growth as compared to reducing sugar analysis.
ii. Bed consolidated for a longer period of time was stronger and was more resistant to erosion at higher shear stress level.
iii. Biofilm growth have more influence on bed stability for 66 and 118 hours consolidation period while for 166 and 312 hours, bed physical consolidation has more effects on the bed stability.
iv. Tests with wastewater show more variation in the result obtained as compared to tests with tap water.

The results show that more biofilm growth was obtained for consolidation phase longer than 118 hours. 66 hours was found to be sufficient for the biofilm to grow and to have effects on bed stability. The bed was weakened after subjected to 66 hours consolidation phase while a stronger bed was observed for bed consolidated at 118, 166 and 312 hours. More bed was eroded when subjected to higher shear stress, however, these values were decreasing with longer consolidation period. The bed has shown to possess two layer properties after the consolidation phase. The strength of the top layer was observed to increase with consolidation period, thus requires higher shear stress in order to initiate the erosion.

6.2 Recommendations for future work

The study has shown to provide an excellent starting point for understanding the impacts of biofilms on the physical transport processes in sewers. However, there are some limitations in the techniques and analysis methods implemented that should be addressed in the future.

Bed sampling methods used in the study of biofilm on bed sediments was a novel method developed for the purpose of estimating biofilm growth on the bed and measurement of its influence on the bed stability. However, the methods were observed to be destructive on the bed structure and biofilm growth. This problem can be overcome by developing an in-situ bed sampling method. This can be interesting for another researcher to look at

too, as it may provide valuable information on the changes in biofilm characteristics with time during the consolidation phase and thus the temporal influence of biofilm on sediment bed can be clarified.

All the works in this study was conducted using laboratory-scale reactors. The setups for each of the reactors were constructed exclusively in order to obtain the aim of the research. However, more comprehensive tests need to be conducted using real sediments in order eliminate the restrictions achieved by using substitute sediments. The substitute sediments used were not able to demonstrate cohesive properties of sediment found in the sewer. The sediment size was also limited and only representing a small fraction of particles size commonly found in sewer networks. Using real sediments will offer a valuable understanding of sediment transport processes.

Data obtained from these laboratory studies have shown to have a high level of consistency and confidence from various control applied while doing the study. However, there is still a need for the data to be fitted into existing sewer models in order to refine the results obtained in terms of how much of these changes can be applied to real sewer applications. This will require a comparison with data collected from sewers, in order to see whether the laboratory studies were comparable to the sewers. Data collection will also help with the modelling works, as more data will produce more reliable and comprehensive models with a high level of confidence.

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